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**Investigation of starch metabolism in  
Cassava (*Manihot esculenta* Crantz)**

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## Table of content

|                                                                                      |            |
|--------------------------------------------------------------------------------------|------------|
| <b>Summary</b> .....                                                                 | <b>I</b>   |
| <b>Zusammenfassung</b> .....                                                         | <b>III</b> |
| <b>Abbreviations</b> .....                                                           | <b>VII</b> |
| <b>1. Introduction</b> .....                                                         | <b>1</b>   |
| 1.1. Cassava – an important starch crop .....                                        | 1          |
| 1.2. Physiology of cassava.....                                                      | 1          |
| 1.3. Starch – an important raw material.....                                         | 2          |
| 1.4. Composition of starch and its architecture .....                                | 3          |
| 1.5. Carbon assimilation and storage: From source to sink tissue .....               | 4          |
| 1.6. Carbon assimilation by photosynthesis .....                                     | 4          |
| 1.7. Starch synthesis in chloroplasts.....                                           | 5          |
| 1.8. Starch degradation in leaves.....                                               | 8          |
| 1.9. Starch phosphorylation.....                                                     | 8          |
| 1.10. Starch hydrolysis in chloroplasts.....                                         | 10         |
| 1.11. Sucrose synthesis .....                                                        | 11         |
| 1.12. Sucrose transport.....                                                         | 12         |
| 1.13. Sucrose metabolism in heterotrophic tissues .....                              | 13         |
| 1.14. Starch synthesis in amyloplasts .....                                          | 14         |
| 1.15. Starch degradation in heterotrophic tissue upon germination and re-growth..... | 14         |
| 1.16. Adding value to cassava as a starch crop.....                                  | 14         |
| 1.17. Scope of the work .....                                                        | 15         |
| <b>2. Material and Method</b> .....                                                  | <b>17</b>  |
| 2.1. Greenhouse grown cassava .....                                                  | 17         |
| 2.2. Growth analysis.....                                                            | 17         |
| 2.2.1. Age determination .....                                                       | 17         |
| 2.3. Photosynthetic capacity.....                                                    | 17         |

|          |                                                                                              |    |
|----------|----------------------------------------------------------------------------------------------|----|
| 2.3.1.   | Photosynthesis at different light intensities .....                                          | 18 |
| 2.3.2.   | Photosynthesis of leaves at different developmental stages .....                             | 18 |
| 2.4.     | Iodine staining, microscopy .....                                                            | 18 |
| 2.4.1.   | Microscopy .....                                                                             | 18 |
| 2.5.     | Carbohydrate extraction .....                                                                | 18 |
| 2.6.     | Carbohydrate measurements .....                                                              | 19 |
| 2.6.1.   | Insoluble carbohydrate determination .....                                                   | 19 |
| 2.6.2.   | Soluble carbohydrate determination .....                                                     | 19 |
| 2.6.2.1. | Enzymatically .....                                                                          | 19 |
| 2.6.2.2. | HPLC-PAD .....                                                                               | 20 |
| 2.7.     | Protein extraction .....                                                                     | 20 |
| 2.7.1.   | Soluble protein extraction for Immunoblot analysis and native PAGE .....                     | 20 |
| 2.7.2.   | Protein amount determination by Bradford .....                                               | 21 |
| 2.8.     | SDS PAGE .....                                                                               | 21 |
| 2.9.     | Western Blot .....                                                                           | 21 |
| 2.10.    | Determination of amylolytic activity .....                                                   | 22 |
| 2.10.1.  | Native PAGE .....                                                                            | 22 |
| 2.11.    | Proteome analysis, sample digestion, mass spectrometry and spectra analysis .....            | 22 |
| 2.11.1.  | Protein preparation .....                                                                    | 22 |
| 2.11.2.  | Mass spectrometry .....                                                                      | 23 |
| 2.11.3.  | Protein identification and label-free peptide quantification .....                           | 23 |
| 2.12.    | Construct design .....                                                                       | 24 |
| 2.12.1.  | Overexpression of potato StGWD in cv. 60444 .....                                            | 24 |
| 2.12.2.  | RNAi: Hairpin design .....                                                                   | 25 |
| 2.12.3.  | <i>Agrobacterium tumefaciens</i> transformation .....                                        | 27 |
| 2.12.4.  | Tissue culture and FEC transformation .....                                                  | 27 |
| 2.13.    | Construct functional analysis .....                                                          | 28 |
| 2.13.1.  | Starch isolation from transgenic cassava storage root and <sup>31</sup> P NMR analysis ..... | 28 |

|           |                                                                                                                                                   |           |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 2.13.2.   | Genomic DNA extraction .....                                                                                                                      | 29        |
| 2.13.3.   | RNA extraction .....                                                                                                                              | 29        |
| <b>3.</b> | <b>Cassava growth carbon assimilation and allocation analysis .....</b>                                                                           | <b>33</b> |
| 3.1.      | Introduction .....                                                                                                                                | 33        |
| 3.2.      | Growth analysis of greenhouse-grown cassava plants.....                                                                                           | 34        |
| 3.2.1.    | Growth analysis of plant batches grown at different time points.....                                                                              | 34        |
| 3.3.      | Photosynthetic capacity measurements.....                                                                                                         | 37        |
| 3.3.1.    | Photosynthetic capacity of cassava leaves in dependence of light and age .....                                                                    | 37        |
| 3.4.      | Carbohydrate accumulation in cassava leaves and stem .....                                                                                        | 39        |
| 3.4.1.    | Carbohydrates in cassava leaves of different developmental stage and time points.....                                                             | 39        |
| 3.4.2.    | Carbohydrates in cassava stem at different developmental stages and time points.....                                                              | 43        |
| 3.5.      | Conclusions drawn from the analysis of carbohydrate assimilation in cassava plants .....                                                          | 48        |
| 3.5.1.    | Analysis of plant batches used for subsequent experiments .....                                                                                   | 48        |
| 3.5.2.    | Capacity of cassava plants to perform photosynthesis and carbon assimilation .....                                                                | 48        |
| <b>4.</b> | <b>Carbohydrate metabolism in Cassava storage roots after induction of sink-to-source transition .....</b>                                        | <b>53</b> |
| 4.1.      | Introduction .....                                                                                                                                | 53        |
| 4.2.      | Remobilization of carbohydrates from storage organs .....                                                                                         | 55        |
| 4.3.      | Cassava storage root proteome comparison: Unravelling enzymes involved in carbohydrate metabolism in storage roots before and after pruning ..... | 58        |
| 4.3.1.    | Investigation of metabolic pathway changes after pruning .....                                                                                    | 60        |
| 4.3.1.1.  | Changes in the abundance of proteins involved in primary carbon metabolism.....                                                                   | 60        |
| 4.3.1.2.  | Changes in different metabolic pathways accompany the metabolic shift from sink-to-source .....                                                   | 66        |
| 4.4.      | Discussion .....                                                                                                                                  | 72        |
| 4.4.1.    | Robustness of proteomics data analysis.....                                                                                                       | 72        |
| 4.4.2.    | Changes in starch metabolism caused by pruning .....                                                                                              | 73        |
| 4.4.3.    | Changes in sucrose metabolism caused by pruning .....                                                                                             | 74        |
| 4.4.4.    | Evidence for sugar signalling in cassava storage roots .....                                                                                      | 75        |

|           |                                                                                                                                     |            |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------|------------|
| 4.4.5.    | General conclusion and outlook .....                                                                                                | 77         |
| <b>5.</b> | <b>Increasing starch bound phosphate level: A transgenic approach.....</b>                                                          | <b>81</b>  |
| 5.1.      | Introduction .....                                                                                                                  | 81         |
| 5.2.      | Transformation and regeneration of cassava .....                                                                                    | 84         |
| 5.2.1.    | Overexpression of potato glucan, water dikinase in cassava tissue culture .....                                                     | 84         |
| 5.2.2.    | Construct description, <i>in vitro</i> analysis.....                                                                                | 84         |
| 5.2.3.    | Growth analysis of transgenic <i>StGWD</i> and <i>StGWD</i> <sub>C1084S</sub> lines compared to wild-type plants                    | 88         |
| 5.2.4.    | Starch visualization in leaf and determination of storage root growth in the transgenic plants.....                                 | 91         |
| 5.2.5.    | Storage starch: <sup>31</sup> P-NMR reveals increased phosphate bound to C6 and C3 position in <i>StGWD</i> <sub>C1084S</sub> ..... | 94         |
| 5.2.6.    | Increase in total starch phosphate: RNAi construct design against <i>MeSEX4</i> and <i>MeLSF2</i> ....                              | 95         |
| 5.2.7.    | RNAi construct design against <i>MeAMY3</i> .....                                                                                   | 98         |
| 5.3.      | Discussion .....                                                                                                                    | 99         |
| 5.3.1.    | Analysis of Cassava plants expressing <i>StGWD</i> and <i>StGWD</i> <sub>C1084S</sub> .....                                         | 99         |
| 5.3.2.    | RNAi-constructs transformed to Cassava cv. 60444 under investigation.....                                                           | 101        |
| 5.3.3.    | Outlook .....                                                                                                                       | 103        |
| <b>6.</b> | <b>General Discussion .....</b>                                                                                                     | <b>105</b> |
| 6.1.      | Integrating Proteomics and carbohydrate metabolism.....                                                                             | 106        |
| 6.2.      | Potential of modified starch in industry .....                                                                                      | 109        |
| <b>7.</b> | <b>References .....</b>                                                                                                             | <b>115</b> |
| <b>8.</b> | <b>Curriculum vitae .....</b>                                                                                                       | <b>127</b> |

## **Summary**

Cassava (*Manihot esculenta* Crantz) is a perennial shrubby plant grown in the tropical and subtropical regions for its starchy roots. In South America and Africa it is mainly grown as a security food and feed stock whereas in Asia the starch industry is the major consumer. In the last decades the economic interest of cassava as a starch crop increased markedly. However, cassava is vegetatively propagated and limited in germplasm.

Starch is the major carbohydrate in plants and an important raw material used for food and non-food industry for mankind. Plants store carbohydrates in form of starch, a polyglucan consisting of linear  $\alpha$ -1,4 linked glucose units with  $\alpha$ -1,6 branch points. The insoluble, semi-crystalline starch granules are either stored transiently in autotrophic (source) leaf tissues or as reserve compound in heterotrophic storage organs (sink; i.e. seeds and tubers). Transitory starch is synthesised during the day in source tissues from photosynthetically assimilated carbon. During the subsequent night transitory starch is degraded to meet the demand for carbohydrates in sink tissues. In most plants carbohydrates are transported through the phloem from source to sink in form of sucrose, a non-reducing disaccharide. In sink tissues sucrose is unloaded and converted to starch and stored as a carbohydrate reserve for long term. In the process of starch biosynthesis multiple enzymes are involved. Starch metabolism in source and sink tissues share some common features, however there are some differences in which enzymes are involved. Differences also occur depending on the botanical source, in respect of starch architecture, granule size and shape. These characteristics define physico-chemical properties. For the diverse industrial applications (i.e. pharmaceuticals, instant food, paper-making) starches of different characteristics are desired.

In order to increase the value of cassava as a starch crop the subject of my thesis was to identify possible key enzymes involved in cassava root starch metabolism. With the help of profound knowledge about starch metabolism and improved biotechnology tools, transgenic cassava plants can be engineered with better starch properties or increased yield.

In my first part of the thesis I have investigated the growth performance of cassava (cv. 60444) grown under defined greenhouse conditions. The interest was to study the photosynthetic capacity and the allocation of assimilated carbon in form of starch and soluble sugars. In the first part the main focus was on leaf and stem tissue at different developmental stages. Hence, photosynthetic capacity and non-structural carbohydrates were visualized and measured from leaf and stem tissue at different developmental stages. Integration of photosynthetic rate and accumulated carbohydrate revealed a high source capacity of cassava leaves. Hence, more carbohydrates are accumulated than needed throughout the day.



In a second part of my thesis I asked the question what key enzymes are involved in remobilizing root starch. Therefore, storage roots, harvested from untreated cassava plants and 10 days after cutting off the aerial plant material were compared. Analysed starch levels and amylolytic enzyme activity revealed a negative correlation. Further, a large scale proteome analysis indicated a metabolic transition from sink to source. This analysis elucidated the involvement of an  $\alpha$ -amylase, AMY3 to be a major enzyme responsible for starch mobilization.

In my third part of the thesis I focused on the attempt to modify starch properties in order to add economic value to cassava starch. Phosphorylated starches, the only naturally occurring modification, are used in paper-making industry to increase paper-strength. Depending on the botanical source the degree of starch-bound phosphate varies from high (i.e. 0.5% in potato) to low (i.e. 0.05 % in cassava). Phosphorylation of starch in plants is executed by a glucan, water dikinase (GWD) and dephosphorylated by two glucan phosphatases (SEX4, LSF2). Activity of GWD is redox regulated. Thus either the potato *StGWD* or the redox-insensitive and constitutively active *StGWD*<sub>C1084S</sub> were transformed into cassava. Preliminary analysis revealed a positive functionality hence, an increase in total phosphate content. Secondly, cassava plants were transformed with an RNAi constructs targeting *SEX4* or *LSF2* transcript. The constructs were specifically expressed in root tissue to avoid manipulation of starch metabolism in other tissues. In order to increase starch yield an RNAi construct was made targeting *AMY3* as I could show that this is the major enzyme involved in starch mobilization.

## Zusammenfassung

Cassava (*Manihot esculenta* Crantz) ist eine ausdauernde, strauchartige Pflanze die besonders in den Tropen und Subtropen für ihre stärkehaltigen Wurzeln angebaut wird. In Südamerika und Afrika wird Cassava als Nahrungs- und Futtermittelsicherheit angebaut während in Asien das Industrielle Interesse im Vordergrund steht. Das wirtschaftliche Interesse an Cassava als Kulturpflanze zur Gewinnung von Stärke ist in den letzten Jahrzehnten markant gestiegen. Durch die vegetative Vermehrung ist die genetische Diversität jedoch limitiert.

Stärke ist eines der wichtigsten Kohlenhydrate in Pflanzen und ein wichtiger Rohstoff für die Nahrungsmittel und nicht-Nahrungsmittel Industrie für die Menschheit. Pflanzen speichern ihre Kohlenhydrate in Form von Stärke, ein Polymer das aus Glucose Einheiten besteht, die linear  $\alpha$ -1,4 zu linearen Ketten verbunden sind mit  $\alpha$ -1,6 Verzweigungen. Die unlöslichen, semi-kristallinen Stärkekörner wird einerseits transient in autotrophen (Ort der Produktion, Source) Blattgewebe, oder als Reserveverbindung in heterotrophen Geweberorganen gespeichert (Ort des Verbrauchs, Sink, bsp. Samen und Knollen). Transiente Stärke wird am Tag aus photosynthetisch assimiliertem Kohlenstoff synthetisiert. Während der folgenden Nacht wird die transiente Stärke wieder abgebaut um den Bedarf an Kohlenhydraten in Sink Gewebe nachzukommen. In den meisten Pflanzen werden die Kohlenhydrate mittels dem Phloem vom Source zum Sink Gewebe transportiert in Form von Saccharose, einem nicht-reduzierenden Zweifachzucker. Im Sink Gewebe wird Saccharose vom Phloem entladen, in Stärke umgewandelt und als Reservekohlenhydrat über längere Zeit gespeichert. An der Stärke Biosynthese ist eine Mehrzahl an Enzymen beteiligt. Der Stärkemetabolismus in Source und Sink Geweben hat einige gemeinsame Eigenschaften wobei es auch Unterschiede bezüglich der Enzyme gibt, die beteiligt sind. Abhängig von der botanischen Herkunft kann es auch zu Unterschieden bezüglich der Stärke Zusammensetzung, Grösse und Form des Stärkekorns kommen. Diese Merkmale definieren die physikalisch-chemischen Eigenschaften. Für die diversen, industriellen Anwendungen (Pharmazeutika, Fertigprodukte und Papierherstellung) sind unterschiedliche Merkmale erwünscht.

Um Cassava als Kulturpflanze einen Mehrwert zu verleihen, war das Thema meine Doktorarbeit die Identifizierung möglicher Schlüsselenzyme die im Stärkemetabolismus von Cassava beteiligt sind. Mit Hilfe von fundiertem Wissen über den Stärkemetabolismus und den verbesserten biotechnologischen Werkzeugen kann eine Wertsteigerung von Cassava als Kulturpflanze für Stärke erreicht werden.

In meinem ersten Teil der Dissertation habe ich das Wachstumsverhalten von Cassava (cv. 60444) Pflanzen untersucht, die bei definierten Gewächshaus Bedingungen angezogen wurden. Das

Interesse lag in der Kapazität für Photosynthese und der Verteilung des assimilierten Kohlenstoffs in Form von Stärke und löslichen Zuckern. Der erste Fokus lag auf dem Blatt- und Stammgewebe zu unterschiedlichen Entwicklungsstadien. Dabei wurden die photosynthetische Kapazität und die nicht-strukturelle Kohlenhydrate gemessen und visualisiert von Blatt- und Stammgewebe zu unterschiedlichen Entwicklungsstadien. Das Vergleichen der Photosyntheserate mit der Menge an assimilierten Kohlenhydraten zeigte eine hohe Source Kapazität in den Cassava Blätter. Demzufolge werden mehr Kohlenhydrate synthetisiert während des Tages als verbraucht während der Nacht.

In einem zweiten Teil meiner Abhandlung habe ich die Frage gestellt welche Schlüsselenzyme benötigt werden um die Wurzelstärke zu mobilisieren. Dafür wurden Speicherwurzeln von Cassava Pflanzen verglichen 10 Tage nach Entblättern mit unbehandelten Kontrollpflanzen. Die analysierte Menge an Stärke und amylolytische Enzym Aktivität zeigte eine negative Korrelation. Des Weiteren hat eine umfangreiche Proteome Analyse auf einen Wechsel von Sink zu Source Metabolismus hingewiesen. Die Auswertung hat eine Beteiligung von  $\alpha$ -amylase, AMY3 als wichtiges Enzym der Stärke Mobilisierung aufgezeigt.

Im dritten Teil meiner Doktorarbeit habe ich mich damit beschäftigt in Cassava Stärke mit modifizierten Eigenschaften herzustellen um der Stärke aus Cassava einen wirtschaftlich höheren Wert zu verleihen. Phosphorylierte Stärke – die einzige natürlich vorkommende Modifizierung, wird in der Papierherstellung gebraucht um das Papier zu stärken. Abhängig von der botanischen Quelle kann der Grad von Stärkegebundenem Phosphat von hoch (Bsp. 0.5% in Kartoffeln) und niedrig (Bsp. 0.05%, in Cassava) variieren. Die Pflanzenstärke wird durch die glucan, water dikinase (GWD) phosphoryliert und durch zwei glucan phosphatasen (SEX4, LSF2) dephosphoryliert. Die Aktivität von GWD ist Redox reguliert. So wurden das Kartoffel StGWD Protein oder die redox-insensitive und konstitutive aktive StGWD<sub>C1084S</sub> Form in Cassava transformiert. Erste Ergebnisse zeigen eine positive Funktionalität und somit einen erhöhten Phosphatgehalt. Weiter, Cassava Pflanzen konnten mit einem RNAi Konstrukt gegen die Transkripte von SEX4 und LSF2 transformiert werden. Die Konstrukte wurden wurzelspezifisch exprimiert um das Verändern auf den Stärkemetabolismus in anderen Geweben zu verhindern. In einem dritten Ansatz mit dem Ziel den Stärkeertrag zu erhöhen habe ich ein RNAi Konstrukt entwickelt gegen das AMY3 Transkript, da ich zeigen konnte das dieses ein wichtiges Enzym ist um gespeicherte Stärke abzubauen.

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## Abbreviations

|                |                                                  |
|----------------|--------------------------------------------------|
| AGPase         | ADPglucose pyrophosphorylase                     |
| AMY3           | $\alpha$ -amylase 3                              |
| ANOVA          | analysis of variance                             |
| ATP            | adenosine triphosphate                           |
| BAP            | 6-benzylaminopurine                              |
| BCA            | bicinchoninic acid                               |
| BE             | branching enzyme                                 |
| Bp             | base pairs                                       |
| BSA            | bovine serum albumin                             |
| CBM            | carbohydrate-binding module                      |
| DEPC           | diethylpyrocarbonate                             |
| DHAP           | dihydroxyacetonephosphate                        |
| DP             | degree of polymerization                         |
| DTT            | dithiothreitol                                   |
| EDTA           | ethylenediaminetetraacetic acid                  |
| EoD            | end of day                                       |
| EoN            | end of night                                     |
| EtOH           | ethanol                                          |
| Expt           | experiment                                       |
| F1,6BPase      | fructose-1,6-bisphosphatase                      |
| F6P            | fructose-6-phosphate                             |
| FBA            | fructose-1,6-bisphosphate aldolase               |
| FEC            | friable embryonic callus                         |
| FK             | fructokinase                                     |
| FR             | fibrous root                                     |
| Fru            | fructose                                         |
| FW             | fresh weight                                     |
| G1P            | glucose 1-phosphate                              |
| G6P            | glucose 6-phosphate                              |
| G6PDH          | glucose-6-phosphate dehydrogenase                |
| GAP            | glyceraldehyde-3-phosphate                       |
| GBSS           | granule bound starch synthase                    |
| Glc            | glucose                                          |
| GPT1           | plastidial hexose-phosphate translocater         |
| GWD            | glucan, water dikinase                           |
| H <sup>+</sup> | proton                                           |
| HXK            | hexokinase                                       |
| HPAEC          | high pH anion exchange chromatography            |
| IITA           | International Institute for Tropical Agriculture |
| Int            | internodium                                      |
| INV            | invertase                                        |
| IRGA           | infrared gas analyser                            |
| ISA            | Isoamylase                                       |
| LC             | liquid chromatography                            |
| LDA            | limit-dextrinase (pullulanase)                   |
| LSF2           | like sex4 2                                      |

## Abbreviation

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|                        |                                          |
|------------------------|------------------------------------------|
| Mal                    | maltose                                  |
| MeOH                   | methanol                                 |
| Mops                   | 3-(N-morpholino)propanesulfonic acid     |
| MS                     | mass spectrometry                        |
| NAD <sup>+</sup> /NADH | nicotinamide adenine dinucleotide        |
| NaOAc                  | sodium acetate                           |
| Nos                    | nopaline synthase                        |
| OPPP                   | oxidative pentose phosphate pathway      |
| PAD                    | pulsed amperometric detection            |
| PAGE                   | polyacrylamide gel electrophoresis       |
| PAR                    | photosynthetically active radiation      |
| PCA                    | principle component analysis             |
| PCR                    | polymerase chain reaction                |
| PGI                    | phosphoglucosomerase                     |
| PGM                    | phosphoglucomutase                       |
| PPD                    | post-harvest physiological deterioration |
| PPI                    | inorganic pyrophosphate                  |
| PWD                    | phosphoglucan water dikinase             |
| PVPP                   | polyvinylpolypyrrolidon                  |
| SBE                    | branching enzymes                        |
| SD                     | standard deviation                       |
| SDS                    | sodium dodecyl sulfate                   |
| SEX                    | starch excess                            |
| SEX4                   | starch excess 4                          |
| SnRK1                  | Snf1-protein kinase                      |
| SPS                    | sucrose phosphate synthase               |
| SR                     | storage root                             |
| SS1                    | starch synthase                          |
| Suc                    | sucrose                                  |
| SUS                    | sucrose synthase                         |
| T6P                    | trehalose-6-phosphate                    |
| TCA                    | trichloroacetic acid                     |
| TCA cycle              | tricarboxylic acid cycle                 |
| TDP                    | ten days of pruning                      |
| TEMED                  | tetramethylethylenediamine               |
| TPS                    | trehalose 6-phosphate                    |
| Tris                   | tris(hydroxymethyl)aminomethane          |
| TTBS                   | tris-buffered saline with tween-20       |
| ZDP                    | zero days of pruning                     |

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## 1. Introduction

### 1.1. Cassava – an important starch crop

Cassava (*Manihot esculenta* Crantz) belongs to the five important starch crops beside, rice, wheat, potato and maize. In recent years, its commercialization increased markedly. World market assessment revealed a 9% increase in cassava productivity within the years 2006-2010 ([www.fao.org/giews](http://www.fao.org/giews); Food outlook 2008 and 2012). While in Africa cassava is mainly a strategic crop in terms of food security and poverty reduction, an increased industrial interest is observed on the Asian market. Especially in Thailand, one of the leaders for cassava starch production, this market is supported by the government ([www.fao.org/giews](http://www.fao.org/giews); Food outlook May 2012). Hence, the interest in cassava biology in respect of starch production increased during the last decades. One main focus in cassava research is the improvement of cassava as a starch crop by investigating storage root production, starch properties and to increase yield in order to attribute more value to this crop.

### 1.2. Physiology of cassava

*Manihot esculenta* Crantz or cassava is perennial, shrubby crop that belongs to the family of Euphorbiaceae. It originates from South America, most likely from Brazil, and is grown for its starchy roots in tropical and subtropical regions as a food and feed crop. As a crop it shows several good characteristics like stress tolerance, tolerance for limited soil-nutrient and, as it is a perennial crop, there is no defined harvesting time. Thus, in South America and Africa, cassava is grown by small-scale farmers as a security food crop along with other crops in intercropping systems. Cassava is vegetatively propagated from stem cuttings. Older parts of mother plants are cut into 20-30 cm sticks which are transplanted to soil. The performance of a new plant depends on the fitness of the mother plant. Harvesting of storage root is performed between seven and twelve months after planting. Cassava roots can be harvested when needed. However, fresh roots need to be used immediately or further processed due to rapid post-harvest physiological deterioration (PPD). This process happens within 24-72h where after storage root become unpalatable (Sanchez et al., 2006). With respect to its economic use, PPD is a major drawback as harvested roots have a short shelf live. Hence, harvested storage roots are often further processed to chips or flour.

Cassava synthesizes cyanogenic glycosides (CG), linamarin and lotaustralin in all plant tissues. Cyanogenic glycosides are involved in herbivour defence, where tissue damage brings together CG with specific enzymes. In subsequent enzymatic reactions linamarin and lotaustralin are converted to the neurotoxic cyanide (Mcmahon et al., 1995; Du et al., 1995). Hence, before consumption cassava tissues need to be prepared to detoxify them. Depending on the CG content cassava varieties are

broadly categorized into being sweet (low CG content) or bitter (high CG content) (Ceballos et al., 2004).

Cassava has two types of roots; fibrous roots involved in nutrient uptake and thick storage roots containing high starch levels (up to 80% of dry weight). Fibrous roots are built at the lateral side of stem cuttings. During growth some fibrous roots undergo a developmental transition to form starchy storage roots. This occurs by radial thickening and starch deposition in the phloem and xylem parenchyma cells (Teerawanichpan et al., 2008).

### **1.3. Starch – an important raw material**

For the diverse applications in the starch industry, a raw material is needed that shows a number of specific properties. Knowledge about starch metabolism is growing, through many ground-breaking studies performed on model plants like *Arabidopsis thaliana*. Thanks to such studies, many of the enzymes involved and the regulatory mechanisms controlling them have been described. This knowledge comes from leaf tissue which synthesises starch during the day and degrades during the following night to meet the metabolic needs of the plant and fuel growth in the dark (reviewed in Streb and Zeeman, 2012; Stitt and Zeeman, 2012; Smith and Stitt, 2007; Zeeman et al., 2010).

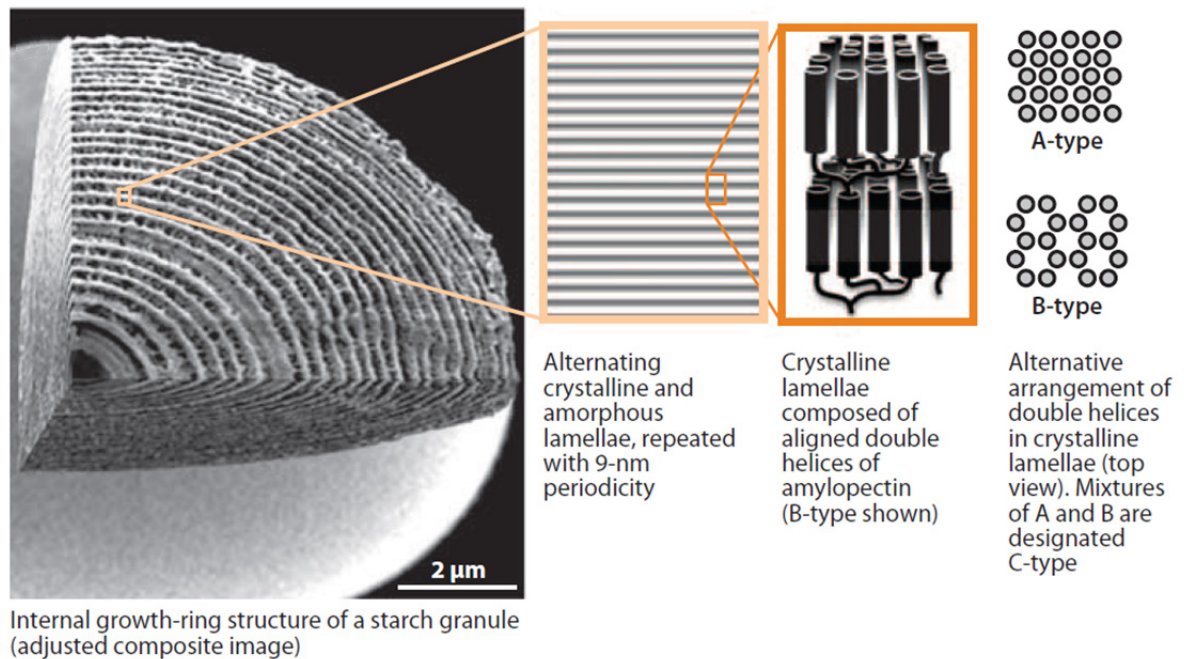
Two kinds of starches can be distinguished in plants which fulfil different storage requirements. Transitory starch in photosynthetic tissues like *Arabidopsis* leaves undergoes a diurnal cycle of synthesis and degradation. In non-photosynthetic tissues (i.e. potato tubers, cassava storage roots, maize kernels) carbohydrate is translocated from the photosynthetic tissue, imported into the amyloplast and converted to starch. Starch in amyloplasts is built-up and stored over a long-term period (Geigenberger, 2003; Sonnewald and Kossmann, 2013). Cassava produces both transient and storage starch, but neither process has been studied in depth at the molecular genetic level in this species.

Starch originating from different botanical sources behave in a different physico-chemical way. The tissue as well as the species it is extracted from defines the properties of starch. The granule size and the amylose to amylopectin ratio both contribute to defining the starch properties. In pharmaceutical industries, starch is used as a filling material in tablets, for which starch with a small granule size is ideal. In the food industry, starch is used as a binding agent in processed foods in addition to being a carbohydrate source. For this, starches with low amylose contents are often preferred for their stable gelling properties when heated in water (i.e. gelatinised). Native starches are often pre-treated either chemically or physically in order to improve or deliver the required properties required by the various branches of the food industry. In the paper industry starch is used as a coating agent. For this, starches are pre-treated with harsh chemical methods to insert charged

groups. In this context, naturally phosphorylated starch is used for paper industry. Starch phosphorylation is the only natural modification of the glucose units that introduces a charged group. For this reason, there is an interest in increasing in total phosphate bound to native starches. This could potentially decrease the pre-treatments or even make them unnecessary. Omitting such harsh chemical treatments would reduce processing costs and be better for the environment.

#### **1.4. Composition of starch and its architecture**

Starch is an inert polyglucan composed of two molecules, amylose and amylopectin. Amylose has an estimated molecular weight of  $10^5$ - $10^6$  Daltons (Perez and Bertoft, 2010) consists of essentially linear  $\alpha$ -1,4 linked glucose chains with a low proportion of  $\alpha$ -1,6 linkages (branch points). Amylopectin has an estimated molecular weight of  $10^7$ - $10^9$  Daltons (Yoo and Jane, 2002) and consists of shorter, linear  $\alpha$ -1,4 linked chains with high degree of  $\alpha$ -1,6-branches (~5%; Perez and Bertoft, 2010). Amylopectin is responsible for the crystalline structure of starch granules. The branches of  $\alpha$ -1,6 linkages lay within a specific layer, the so called amorphous layer. Amorphous layers alternate with crystalline layer that contain mainly linear  $\alpha$ -1,4 linked glucan chain segments. These linear chains form double helices then pack in an ordered pattern. This arrangement gives starch its insoluble, semi-crystalline properties as water molecules are expelled. These alternating layers make up the crystalline zones of starch granules which can be viewed as a ring-like structure (Figure 1.1). The formation of helices and their arrangement differ depending on the starch type and plant species. The helices arrange either in an A-, or B- type crystallinity. In an A-type starch, typically found in cereals, the helices are packed together densely in a monoclinic unit cell, whereas in a B-type starch, characteristic for tuberous starch, is arranged in an open hexagonal way with water-filled space between the helices (Blennow and Engelsens, 2010). In some species, a mixture of A- and B-type packing is observed. This so-called C-type starch is found in pea and cassava (reviewed in Damager et al., 2010).



**Figure 1.1 The composition and structure of starch granules**

The relationship between the starch granule (composite image of potato granules, *left*) and amylopectin structure. Crystalline and amorphous lamellae arrange to form blocklets that make up the growth rings. (Zeeman et al., 2010)

### 1.5. Carbon assimilation and storage: From source to sink tissue

Starch is a storage compound found in many plant tissues. In leaves carbon for starch biosynthesis comes from the assimilated  $\text{CO}_2$  during photosynthesis. In contrast, in heterotrophic tissues, the carbon for starch biosynthesis comes from sucrose transported from the leaves. Despite the differences in the way of carbon is supplied, there are many similarities in how starch itself is synthesized. In the following part, the two pathways will be described.

### 1.6. Carbon assimilation by photosynthesis

Plants assimilate carbon during the day via photosynthesis. Photosynthesis is partitioned into light-dependent and light-independent reactions. In the light reactions light energy is captured and converted into chemical energy, used to catalyse the light-independent reactions where atmospheric carbon dioxide is incorporated into carbon compounds that fuel cellular metabolism and biomass production. Light is captured in the chloroplast thylakoid membranes by chlorophyll and accessory pigments of the light harvesting complexes. This energy is transferred to the photosystem II, where it excites electrons derived from the splitting of water molecules. Electrons flow along the photosynthetic electron transport chain, a series of redox reactions, transports protons across the thylakoid membrane into the lumen, creating a proton gradient. This gradient is used by the proton-

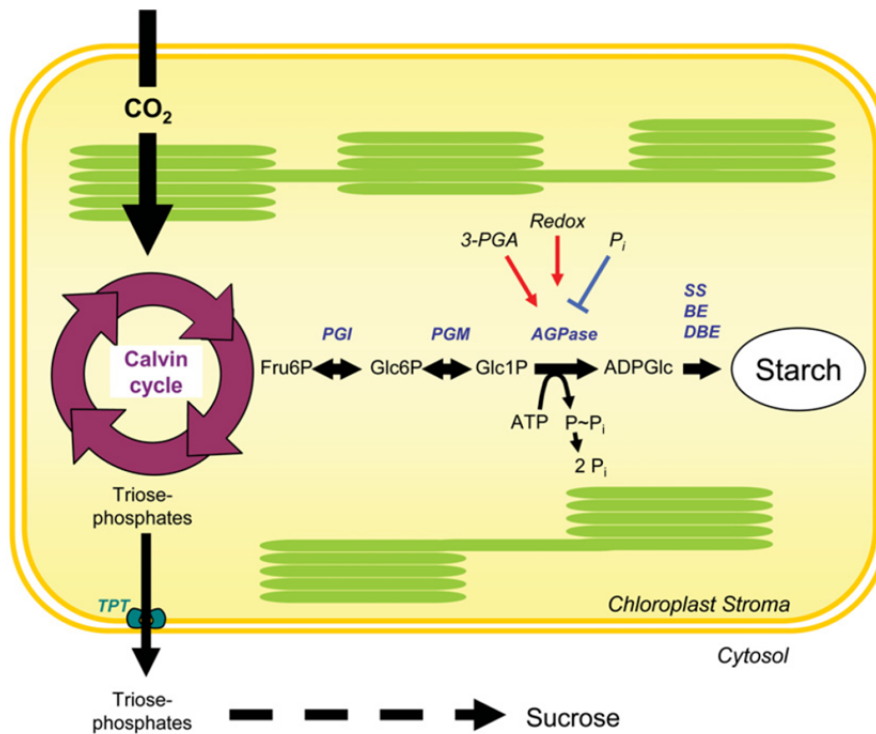
driven ATPase to synthesise ATP from ADP and inorganic phosphate. The electrons are further energised in photosystem I and then used to reduce NADP to NADPH.

The generated chemical energy and reducing power is primarily used in the assimilation of atmospheric CO<sub>2</sub> via the Calvin Cycle (the light-independent reactions of photosynthesis).

The cycle has 3 parts: carboxylation, reduction and regeneration. During carboxylation atmospheric CO<sub>2</sub> is assimilated by the enzyme Ribulose-1,5-bisphosphate-carboxylase/-oxygenase (RuBisCo) yielding 2 molecules of 3-phosphoglycerate (3-PGA). This is converted to triose-phosphates (TP), a fraction of which can be transported to the cytosol for sucrose synthesis or retained in the chloroplast for starch synthesis. Most TP is used to regenerate RuBP via several enzymatic steps. One of the intermediates generated is fructose 6-phosphate (F6P) that is used for starch synthesis. Assimilated carbon is partitioned between starch and sucrose synthesis. Sucrose is the primary product, synthesized in the cytosol and exported to heterotrophic tissues via the phloem. Starch in the chloroplast is transiently stored during the day and degraded during the subsequent night, when no photosynthesis occurs. The extent of partitioning into starch depends on the need of the plant and on the species. For *Arabidopsis* it was reported that during the day up to 50% of the assimilated carbon is subjected into starch (Zeeman and Ap Rees, 1999).

### 1.7. Starch synthesis in chloroplasts

In the starch synthesis pathway (Figure 1.2), F6P is first isomerized by phosphoglucoseisomerase (PGI) to glucose-6-phosphate (G6P). Then, G6P is converted to glucose 1-phosphate (G1P) by phosphoglucomutase (PGM). ADPglucose pyrophosphorylase (AGPase) catalyses the committed step in starch biosynthesis, using G1P and ATP to generate ADP-glucose and inorganic pyrophosphate (PPi). AGPase is highly regulated and, although it catalyses a reversible reaction, the action of inorganic pyrophosphatase hydrolyses pyrophosphate rendering ADPglucose production essentially irreversible *in vivo*. AGPase is regulated by redox regulation on the one hand (Hendriks et al., 2003). On the other hand, its activity is controlled allosterically by the levels of inorganic phosphate (Pi), an inhibitor, and triose-P, an activator (Preiss et al., 1988).



**Figure 1.2 Pathway of starch synthesis in chloroplasts**

Carbon assimilated via the Calvin cycle is partitioned with a fraction exported to the cytosol for sucrose synthesis and a fraction retained in the chloroplast for starch synthesis. Redox activation and allosteric regulation of AGPase controls the flux of carbon into starch. Abbreviations: Fru6P, fructose 6-phosphate; Glc1P, glucose 1-phosphate; Glc6P, glucose 6-phosphate; TPT, triose-phosphate/phosphate translocator. (Zeeman et al., 2007)

The ratio of these two metabolites changes according to supply of photo-assimilates and the demand for them, helping to regulate partitioning into starch. It was also shown that T6P, a compound involved in sucrose signalling, is able to promote the redox-activation of AGPase. Evidence for the linear pathway of starch synthesis comes from Arabidopsis mutants lacking each of the three enzymes (PGM, PGI, AGPase; Caspar et al., 1985; Lin et al., 1988; Yu et al., 2000). Compared to wild-type plants these mutants are unable to accumulate significant amounts of starch in their leaves. Instead they accumulate 4-5 times more soluble sugars during the day than wild-type plants (Caspar et al., 1985; Gibon et al., 2002).

Starch itself is synthesized by the coordinated activities of three enzymes. Linear chains of amylopectin and amylose are synthesized by starch synthases. Starch synthases transfer the activated glucosyl moiety of ADP-glucose to the non-reducing end of a pre-existing glucan chain, elongating it by one glucose unit. There are five classes of starch synthases in plants (Ball and Morell, 2003). Four of these starch synthases are soluble (SS1-4) whereas the fifth is termed granule bound starch synthase (GBSS). The granule bound starch synthase (GBSS), as the name

implies is found within the starch granules and is responsible for amylose synthesis. Mutant plants (e.g. maize *waxy* mutant) lacking the GBSS activity are amylose free (Shure et al., 1983).

The synthesis of amylopectin involves several steps in order to achieve its semi-crystalline structure. Soluble starch synthases (SS1-SS3) elongate existing glucan chains. Mutant analysis revealed that each isoform prefers to elongate glucan chains of different lengths. The SS1 isoform is thought to synthesis short glucan chains with a degree of polymerization (DP) of around 10 glucose units (Delvalle et al., 2005). The SS2 and SS3 isoforms elongate longer glucan chains. Pea mutants lacking SS2 have amylopectin with an altered structure, containing excessive small (DP<10) and long (DP>25) glucan chains (Craig et al., 1998), but deficient in intermediate length chains. The SS2 knock-out mutant of *Arabidopsis* also has amylopectin with decreased numbers of intermediate-length chains (DP12 to DP28; Zhang et al., 2005). For SS3 no change in amylopectin synthesis was observed in *Arabidopsis* mutant (Zhang et al., 2005), though the double mutant *Atss2ss3* displayed a strong reduction in DP12 to DP28 chains, and it was suggested that these two isoforms have some redundancy in their activities (Zhang et al., 2008).

The SS4 isoform is somewhat unique in that it appears to have a role in starch granule initiation. *Arabidopsis* mutants lacking SS4 had a decreased number and altered morphology of starch granules compared to the wild type, even though no change in amylopectin structure was observed. Interestingly, the double mutant *Atss3ss4* is essentially starch free suggesting that SS3 can partially compensate for SS4 in granule initiation (Roldan et al., 2007; Szydowski et al., 2009). Starch granule initiation is far from understood.

To introduce  $\alpha$ -1,6-branch points, branching enzymes (BE) act on the linear substrates generated by starch synthases. Branching enzymes cut  $\alpha$ -1,4 linked glucan chains and transfer the cut segment to a C6 hydroxyl of a glucose unit on the same or an adjacent chain, introducing an  $\alpha$ -1,6 linkage. In plants, two classes of branching enzyme exist, SBEI and SBEII. The BEs act on linear glucan chains with a minimum length of DP12, transferring at least 6 glucose units (Takeda et al., 1993). A third enzyme class helps to give starch its semi-crystalline structure by selectively removing some branch points. This is done by the debranching enzymes (DBE), which hydrolyse  $\alpha$ -1,6 glucose linkages. The DBEs can be classified into two sub-groups; isoamylases (ISA) and limit-dextrinases (LDA). LDA acts preferentially on substrates with small side chains, like the yeast pullulan ( $\alpha$ -1,4 linked maltotriose linked together with  $\alpha$ -1,6 bond) and limit dextrins produced during starch breakdown. ISAs do not act on pullulan, probably needing longer linear chains before the branch point. However, ISAs can further be divided into three sub-classes ISA1-3. For starch synthesis, ISA1 and ISA2 are most relevant. Mutants lacking ISA1 have been described in several species. All accumulate a glycogen-like glucan polymer as well or instead of insoluble granules. Thus, ISA1 is proposed to trim the glucans to

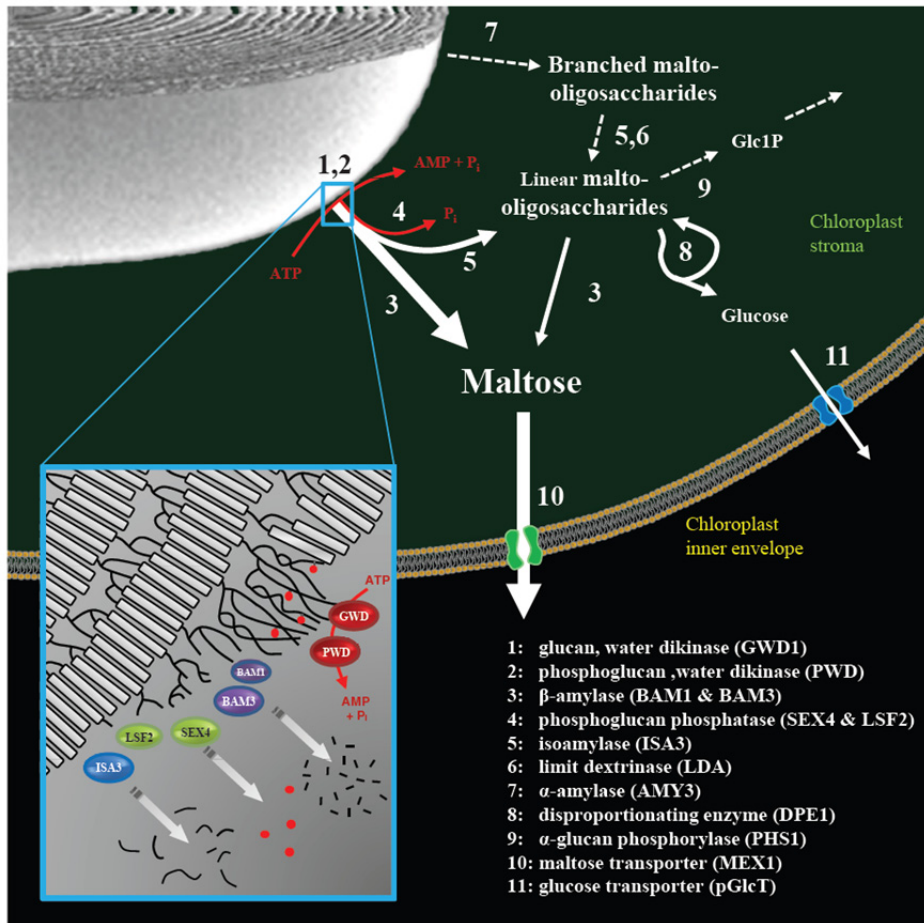


promote the formation of semi-crystalline structures. In most species examined, ISA2 is non catalytic and acts in a complex with catalytic ISA1 subunits (Hussain et al., 2003). ISA3 is, by contrast, implicated in starch degradation (see below).

### **1.8. Starch degradation in leaves**

#### **1.9. Starch phosphorylation**

To meet the carbon need in the dark, leaf starch is degraded. The initial step of starch degradation requires glucan phosphorylation. In potato, the isolation of proteins capable of binding starch led to the identification of glucan, water dikinase (GWD). Repression of this enzyme in potato led to an 85% drop in total starch bound phosphate and increased starch levels in leaves (Lorberth et al., 1998). Similarly, the *Arabidopsis gwd* mutant (*sex1*) displays elevated starch levels and slow growth, as the plants lack carbon supply during the dark period (Yu et al., 2001). GWD catalyses the transfer of the  $\beta$ -phosphate of ATP to the glucose moieties while the  $\gamma$ -phosphate of ATP is concomitantly released (transferred to water) to produce orthophosphate (Pi) and AMP (Mikkelsen et al., 2005). It was shown that GWD phosphorylates the C6 position of the glucose residues (Ritte et al., 2006). Upon phosphorylation by GWD, a second kinase, phosphoglucan water dikinase (PWD) catalyzes the phosphorylation of the C3 position of different glucose residues (Baunsgaard et al., 2005; Kotting et al., 2005). PWD, as its name implies, needs the pre-phosphorylation by GWD. Starch phosphorylation was shown to solubilize starch granule surface, presumably by disrupting and unwinding the helical structures of amylopectin (Blennow and Engelsen, 2010). This solubilisation renders starch granules accessible for hydrolytic enzymes involved in starch degradation, such as exoamylases (i.e. BAM1, BAM3) and debranching enzymes (i.e. ISA3) (Edner et al., 2007) (Figure 1.3).



**Figure 1.3 The pathway of starch degradation in chloroplasts and the role of transient glucan phosphorylation**

Maltose and malto-oligosaccharides are released from the surface of the starch granule during degradation. Malto-oligosaccharides are metabolized in the stroma. Maltose and glucose are exported to the cytosol. Estimated fluxes are indicated by relative arrow size. Dashed arrows represent the minor steps in *Arabidopsis*. Inset is a model depicting the role of phosphorylation by GWD and PWD in disrupting the packing of amylopectin double helices (gray boxes). This allows the release of maltose and malto-oligosaccharides (black lines) by  $\beta$ -amylases (BAMs) and DBE (ISA3). Phosphate (red dots) is concomitantly released by SEX4 to allow complete degradation (Zeeman et al., 2010).

It was shown many years ago that  $\beta$ -amylases are not able to act past phosphate residues on glucan chains (Takeda and Hizukuri, 1981). Thus, for efficient starch degradation phosphate residues introduced by GWD and PWD need to be released again. In *Arabidopsis* two genes have been shown to encode active phosphoglucan phosphatases. Functional characterization of these enzymes in *Arabidopsis* (*AtSEX4*, *AtLSF2*) showed that *sex4* mutants have high starch levels and slow growth compared to wild-type plants, and also accumulate phospho-oligosaccharides. Total glucan-bound phosphate (starch and phospho-oligosaccharides) was six times higher in *sex4* mutants than in wild type plants. *SEX4* preferentially removes phosphate residues from C6-position of glucose residues (Kotting et al., 2005; Hejazi et al., 2010). The second isoform, *LSF2* was shown to specifically release phosphate from the C3 position of the glucose residues (Santelia et al., 2011). Interestingly, the single mutant *lsf2* behaves like wild-type plants with respect to starch and phospho-oligosaccharide

levels. However, total starch bound phosphate levels are 25% higher than in the wild type, specifically due to an increase in C3-bound phosphate. Although no starch excess phenotype was observed for *lsf2*, the double mutant *sex4lsf2* showed an even more severe starch-excess phenotype and the accumulation of phosphor-oligosaccharides (Santelia et al., 2011). This shows that in the *sex4* mutant lines LSF2 activity contributes to starch breakdown even if it cannot substitute for the lack of SEX4.

### 1.10. Starch hydrolysis in chloroplasts

The opening of the double helical chains makes the glucans accessible for hydrolyzing enzymes like  $\alpha$ - and  $\beta$ -amylases and debranching enzymes (LDA, ISA3). In Arabidopsis leaves starch degradation is predominantly catalyzed by the plastidial exoamylases BAM1 and BAM3, which release maltose from the non-reducing ends of  $\alpha$ -1,4 glucan chains. Maltose is the major degradation product from  $\beta$ -amylolytic hydrolysis of transitory starch (Weise et al., 2004; Fulton et al., 2008), which is transported to the cytosol through the maltose exporter 1 (MEX1; Niittyla et al., 2004). However,  $\beta$ -amylases are not able to hydrolyse  $\alpha$ -1,6 glucan bonds. Thus, they act no further than few glucose units close to the branch point.

Characterization of starch degrading enzymes in cereal endosperm revealed that  $\alpha$ -amylases are important upon germination. The situation appears to be different in Arabidopsis. There are three  $\alpha$ -amylase isoforms in Arabidopsis, but only one is localized to the plastid (AMY3). In contrast to the cereal endosperm, it was shown that  $\alpha$ -amylases play a minor role in starch degradation. Mutants lacking  $\alpha$ -amylases metabolize starch normally (Yu et al., 2005) suggesting that  $\alpha$ -amylases are not crucial in starch degradation. Only in plants already deficient in starch metabolism due to the lack of other proteins was a contribution of AMY3 observed. For example, it was shown that phospho-oligosaccharides were reduced while starch content was elevated in the double mutant *sex4amy3*, compared to the *sex4* single mutant (Kotting et al., 2009). This suggests that AMY3 releases branched oligosaccharides from the starch granule, at least in the *sex4* mutant background.

Branch points on the granule surface and in branched oligosaccharides are hydrolysed by debranching enzymes ISA3 and LDA resulting in linear glucan chains which can be further hydrolysed by  $\beta$ -amylases. In the chloroplast, a disproportionating enzyme (DPE1) recycles short maltooligosaccharides to release glucose (Critchley et al., 2001; Lu et al., 2006). Glucose is also transported to the cytoplasm by the plastidial glucose transporter (pGlcT; Cho et al., 2011). Another enzyme thought to be involved in starch mobilisation is starch phosphorylase (PHS1). PHS1 catalyses the reversible reaction which releases G1P from linear glucans. It has long been speculated that

starch phosphorylases are involved in starch degradation, however mutant analysis only displayed a phenotype under stress conditions (Zeeman et al., 2004).

Maltose, once exported to the cytosol, is further metabolized to provide substrates for downstream pathways (e.g. sucrose synthesis). The cytosolic glucosyltransferase (DPE2) splits maltose, releasing one and transferring the other to an acceptor, probably a cytosolic heteroglycan (Chia et al., 2004; Fettke et al., 2006). The free glucose is phosphorylated by hexokinase and enters the hexose-P pool. A cytosolic starch phosphorylase (PHS2) acts on the heteroglycan, releasing or adding G1P. Thus, supply of substrates for various metabolic processes is maintained during the night.

### 1.11. Sucrose synthesis

Sucrose, a non-reducing disaccharide consisting of one molecule glucose and one fructose, is synthesized in the cytosol. In most plants, sucrose is transported from the source to the sink or stored in the vacuoles. Furthermore, plants often accumulate sucrose upon cold, drought or salt stress to maintain osmotic balance and to help stabilize proteins and membranes.

As described earlier, triose-phosphates (TP) synthesized during photosynthesis are exported from the chloroplast by the triose-phosphate/phosphate translocator (TPT) in exchange for orthophosphate (Pi). In the cytosol, TP provides substrates for diverse pathways (protein synthesis, organic acid or cell wall synthesis) but most is utilized to synthesize sucrose, at least in fully expanded leaves. First, TP is condensed to fructose-1,6-bisphosphate (F1,6BP) by aldolase. F1,6BP is then dephosphorylated by fructose-1,6-bisphosphatase (F1,6BPase) to F6P. In the cytosol F6P, G6P and G1P are equilibrated by the cytosolic phosphoglucisomerase (cPGI) and phosphoglucomutase (cPGM), respectively. G1P is converted to UDP-glucose (UDPGlc) by UDPGlc pyrophosphorylase, using UTP and releasing PPi. The hexose-phosphate F6P and UDPGlc are then utilized to synthesize sucrose by the sequential reactions of sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase (SPP).

The synthesis of sucrose is strongly regulated. The activity of SPS is regulated via complex allosteric feed forward and feedback mechanisms that integrate the availability of substrates for sucrose synthesis with the demand for sucrose in sink tissues. A decreased demand in sink tissues leads to an accumulation of sucrose in leaf mesophyll cells. An increase in sucrose leads to inhibition of SPS which causes an increase in F6P since this is not further utilized. This in turn influences levels of the regulatory metabolite fructose 2,6-bisphosphate (F2,6BP) - produced from F6P by fructose 2,6-bisphosphatase (F2,6BPase). F2,6BP inhibits F1,6BPase activity, limiting hexose-P production. As Pi is released in various steps of sucrose synthesis, inhibition of the pathway leads to less Pi in the cytosol, which restricts the export of triose-phosphate from the chloroplast. Increasing stromal TP and lowered Pi concentrations promotes starch synthesis through allosteric activation of

AGPase as described above. In addition, the accumulation of sucrose frequently correlates with an increase of trehalose-6-phosphate, which redox-activates AGPase (Kolbe et al., 2005). Thus, partitioning between sucrose and starch is both flexible but also tightly regulated to allow fast responses to changes in environmental conditions, and the supply of and demand for photoassimilates.

### **1.12. Sucrose transport**

Sucrose is transported by the phloem from the site of synthesis (mature leaves; source) to the site of utilization (growing leaves, heterotrophic tissues; sink). From the mesophyll cells, sucrose diffuses symplastically through plasmodesmata, to the bundle sheath cells adjacent to the phloem, after which it is loaded into the phloem by one of three mechanisms (see below). The difference in concentration of solutes between the source, where sugars are synthesized and the sink, where sugars are unloaded and utilized, results in a hydrostatic pressure gradient that leads to a bulk flow of assimilates between the source and sink tissues.

In plants three mechanisms of phloem loading exist; apoplastic loading, symplastic loading and polymer trapping (reviewed in De Schepper et al., 2013). Species that transport raffinose instead of sucrose use polymer trapping, where sucrose is symplastically transported to specialized companion cells – intermediary cells - via plasmodesmata. There sucrose is converted to a higher molecular carbohydrate, raffinose or stachyose. Because of the higher molecular size of raffinose and stachyose, it is proposed that these carbohydrates cannot diffuse back through the plasmodesmata and are thus trapped in the companion cells and then transported in the phloem. In many woody species, phloem is symplastically loaded down a concentration gradient, without up-concentration, hence a completely passive way. However, the most common phloem loading is via the apoplast where the phloem cells are symplastically isolated from the mesophyll. Sucrose is transported symplastically to the phloem parenchyma transported into the apoplast by an efflux transporter (SWEET; Chen et al., 2012).

Sucrose is then actively loaded into the companion cells via sucrose/H<sup>+</sup> co-transporters (SUT1, AtSUC2; Stadler and Sauer, 1996; Sauer, 2007). The proton motive force is built up via an H<sup>+</sup>-ATPase in the companion cell membrane.

In sink tissues sucrose unloading can be apoplastically or symplastically. For potato tuber it was shown that during tuberization the unloading changes from apoplastic to symplastic. In the tuberization initiation phase, when the stolon tip starts to grow, phloem tubers are symplastically isolated hence phloem unloading occurs via the apoplast (Viola et al., 2001). In contrast, phloem loading occurs symplastically in the developing potato tubers themselves. However, in detached

potato tubers exhibiting bud outgrowth, the phloem function shifts to apoplastic loading (Viola et al., 2007).

### 1.13. Sucrose metabolism in heterotrophic tissues

Sucrose produced in the source tissues is transported to non-photosynthetic tissues and unloaded according to the demand of these sink tissues. In sink cells sucrose is either hydrolysed by an invertase (INV) to fructose and glucose or metabolized by sucrose synthase (SUS) to fructose and UDP-glucose using UDP (Koch, 2004). Alternatively it can be transported to the vacuole. There are 3 types of invertases: cell wall (cwINV), cytosolic (cINV), and vacuolar (vINV) invertases. SUSs are localized to the cytosol.

Depending on the developmental stage or ongoing physiological processes, sucrose may be preferentially cleaved by INVs or by SUSs. In general, hexoses favour cell division and expansion and INV is often seen to mediate the initiation and expansion of many new sink structures, often with vacuolar activity preceding that in cell walls. The action of cwINV coincides with the elevated expression of hexose transporters in some systems (Koch, 2004). Later, transition to storage and maturation phases is facilitated by changes in the hexose/sucrose ratio, and by shifts from INV to SUS mediated sucrose cleavage (Koch, 2004). SUS activity in mature storage tissues may be advantageous because it is energy-efficient compared with INVs. SUS releases only one hexose in contrast to INV which releases two this means that double the ATP is used in order to phosphorylate the hexose products of INVs. It was observed that O<sub>2</sub> concentration decreases within solid tissues in a potato tuber

(Geigenberger et al., 2000), and correlating with this was a decrease in ATP/ADP ratio. This suggests that respiration and hence ATP synthesis may be lower. Hence, releasing only one free hexose by SUS might save energy within the glycolytic pathway. On the other hand, INV is thought to play a role in delivering hexose based signals and it can define the osmotic strength for sucrose unloading or in the vacuole.

In order to investigate the possibility to increase sink strength transgenic potato tubers expressing yeast INV either in the apoplast or in the cytosol were analysed. This analysis revealed that cytosolic expression led to large changes of metabolites, decrease in starch and increase of respiration. The apoplastic INV increased potato tuber size due to increase in water content. The authors of these studies suggested that the sucrose/hexose ratio depends on how and where sucrose is cleaved. This can lead to unpredicted changes in sugar signalling. Thus, an increase in extracellular hexose promoted respiration rather than increasing starch synthesis (Ferreira and Sonnewald, 2012).

#### **1.14. Starch synthesis in amyloplasts**

In heterotrophic tissues, starch synthesis occurs in the amyloplast via a similar pathway as in chloroplasts (see above). Unlike in chloroplasts, carbohydrates need to be transported from the cytosol across the plastid membrane to fuel starch synthesis. Cleavage of sucrose by SUS in the cytosol results in UDPGlc and fructose. Fructose is phosphorylated by hexokinases (HXK) or fructokinase (FK) yielding F6P, which is equilibrated with G1P and G6P by the activity of PGI and PGM. Carbohydrate is imported in form of G6P (or occasionally G1P) from the cytosol into the amyloplast (Hill and Smith, 1991; Kosegarten and Mengel, 1994; Kammerer et al., 1998). In the amyloplast stroma, subsequent steps convert G6P to ADPGlucose as described above. The cereal endosperm is an exception as the precursor for starch synthesis; ADPG is also produced by a cytosolic AGPase and transported into the plastid from the cytosol. Indications that G6P is the major compound transported to the amyloplast in other species comes from transgenic or mutant plants lacking PGM in the plastid, which leads to decreased starch content in heterotrophic tissues (Caspar et al., 1985; Harrison et al., 1998; Fernie et al., 2002). The activity of AGPase in the amyloplast relies on the availability of ATP. This is maintained by an ADP/ATP translocator (Neuhaus et al., 1997).

#### **1.15. Starch degradation in heterotrophic tissue upon germination and re-growth**

Starch mobilization in heterotrophic tissue was shown to be different from the transitory starch degradation like cereals where starch degradation was described to occur by an amylolytic enzyme activity (Fincher, 1989). Thereby the accumulated starch in the endosperm is degraded upon the gibberellic acid signal released by the germinated seed. This signal stimulates the aleurone cell layer to initiate the production and secretion of  $\alpha$ -amylase and other hydrolytic enzymes. Free glucose released in the endosperm space is taken up by the embryo, phosphorylated by hexokinases to hexose phosphates which supply the non-photosynthetic seedling with respiratory substrates and carbon skeletons for diverse cellular structures. However, knowledge about starch mobilization in heterotrophic tissues apart from cereals is very poorly understood.

#### **1.16. Adding value to cassava as a starch crop**

Cassava starch is low in protein and fat content compared to seed crops like rice, or wheat (Jobling, 2004). As aforementioned (Chapter 1.1) both its good, low-input growth and the physico-chemical properties of its starch make cassava a valuable starch crop. One drawback of cassava, though, is limited genetic variation, as it is multiplied by vegetative propagation. To introduce new desirable traits to cassava by breeding is challenging. In general, inbreeding by consecutive self-pollination is used to identify useful recessive traits. However, cassava is a monoecious plant where flowering time of female and male at the same branches is often separated in time. Hence, it is

challenging to define the right time to be able to perform crosses (Jennings and Iglesias, 2002; Ceballos, 2004). Moreover, generating seeds is slow, taking up to a year.

To increase cassava germplasm and to add value to it as a starch crop, identification of new lines was performed earlier, either by mutant screening (Carvalho et al., 2004; Ceballos et al., 2007; Ceballos et al., 2008) or by transgenic modification (Raemakers et al., 2005). Through genetics, plant lines affected in GBSSI (Raemakers et al., 2005; Ceballos et al., 2007), and putatively in ISA (Ceballos et al., 2008) and BE2 (Carvalho et al., 2004) have been described. All of these lines show differences in starch architecture and starch properties, potentially suitable for various industrial applications. With the progress made in biotechnology during the last decades it is now possible to generate transgenic lines (Bull et al., 2009) in cassava in which foreign genes are expressed or endogenous genes are repressed. This method provides the possibility to study the biochemical roles of one or more gene in more detail. However, in order to analyse gene functions in cassava starch metabolism knowledge about the basic growth behaviour and metabolic pathways are first needed. On the biochemical level very few genes involved in cassava starch metabolism were studied to date. It was shown that *SBE2* transcript expression undergoes a diurnal oscillation and is induced upon sugar supply (Baguma et al., 2003; Baguma et al., 2008). Transcriptional analysis revealed the presence of a plastidic ATP/ADP transporter in a wide range of cassava plant tissues (Yuen et al., 2009). Moreover, a gene encoding for an  $\alpha$ -amylase (designated as *MeAMY2*) was isolated from cassava storage roots (Tangphatsornruang et al., 2005). The authors reported that the sequence contains the active site and a carbohydrate binding domain. However, sequence analysis revealed closer homology to *AtAMY2*-like (At1g76130) than *AMY3* from *Arabidopsis*.

### 1.17. Scope of the work

The increased use of cassava in starch industry leads to increased demand for variation in starch traits. Although some genes involved in cassava starch metabolism were isolated, information about biological relevance is missing thus far. To generate and analyse transgenic cassava plants basic knowledge about the carbohydrate metabolism is needed.

I first analysed cassava plants grown in a greenhouse, monitoring growth performance, the capacity for carbon assimilation and carbon allocation. Second, I sought to identify key enzymes involved in starch metabolism, with the main focus on starch mobilization. This is important in understanding the biological background on the one hand and to unravel target enzymes for future biotechnological applications on the other. Third, I attempted to introduce new starch traits to the cassava germplasm through biotechnology. Transgenic lines were designed based partly on current knowledge and partly according to my new findings.



---

## 2. Material and Method

All studies were performed with the African Cassava variety *Manihot esculenta* Crantz (cv. 60444) from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

### 2.1. Greenhouse grown cassava

Cassava plants were propagated by taking stem cuttings from the lower, hardwood part of the stem of mother plants. The cuttings contained at least 2 axillary buds or a stem with 15 cm length. The stem cuttings were planted in soil (70% Klasmann Substrat 2 [pH 5.5 (CaCl<sub>2</sub>), fertilization 2.0 g/L], 30% Perlite) in a squared pot size (7x7x6 cm, V=195 cm<sup>3</sup>). Within 2 months, the rooted cuttings were transferred to bigger round pots (diameter 13 cm, V = 880 cm<sup>3</sup>) containing 40% Klasmann Substrate 2, 10 % Perlite, 50% lawn soil from Ricoter (40 % sand, 10% Perlite, 25% garden compost, 25% "Weisstorf", 5kg m<sup>-3</sup> chipples made of horn) and 6 g Osmocot extract from Scotts (11% N, 11% P, 18% K, 2% MgO). Mother plants grown for cuttings were cultivated and grown in 5-L pots containing 40% Klasmann Substrate 2, 10% Perlite, 50% lawn soil from Ricoter and 16 g Osmocot extract from Scotts. The plants were grown under greenhouse conditions under a 14 h light period at 20 kilolux minimal light intensity, 24°C, 60% humidity, and 10 h night period, 17°C and 50% humidity.

### 2.2. Growth analysis

#### 2.2.1. Age determination

The developmental stage of cassava plants were determined by the height, number of axillary buds and leaves. The height was determined from the origin of sprouting to the apex. The number of nodes and leaves were counted from the apex leaf down to the origin of sprouting. The apex leaf (being visible from top view) was considered as youngest leaf 1 (L1) (Figure 3.1 A).

### 2.3. Photosynthetic capacity

Photosynthetic capacity of greenhouse grown cassava plants was determined using a portable Li-6400XT from LI-COR. The Li-6400XT device was connected to an infrared gas analyser (IRGA). Calibration of the device was performed as described in the manual. For each series of measurement a new CO<sub>2</sub> cylinder (Licor Environmental GmbH, Bad Homburg, Germany) was used and the soda lime and the desiccant were exchanged if necessary. All measurements were performed with the following parameter settings: a stomata ratio of 0.5, an air flow of 250 μmol m<sup>-2</sup> s<sup>-1</sup> and a CO<sub>2</sub> concentration of 400 ppm at ambient air humidity in the chamber. The light intensity varied

depending on the experimental set up (see paragraphs 2.3.1 and 2.3.2.). For the measurements, a chamber with integrated LED source is clamped over a single leaf and the gas exchange of a defined area (6 cm<sup>2</sup>) of the leaf was measured. As the area of cassava leaves is greater than 6 cm<sup>2</sup>, the measurements were performed on each of the three middle lobes of a specific leaf.

### **2.3.1. Photosynthesis at different light intensities**

To determine the photosynthetic rate at different light intensities, a series of light quanta was applied to a mature leaf (L4). The light series comprised 0, 25, 50, 100, 200, 400, 600, 800, 1200, 1500  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ . For each position of the chamber, three measurement points per light intensity were logged with an interval of 10 s and a dead time of 90 s after the intensity changes. The three middle lobes per leaf were analysed for three individual plants.

### **2.3.2. Photosynthesis of leaves at different developmental stages**

To measure the photosynthetic rate dependent on leaf age, the measurements were performed at a constant light intensity of 100  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ . On each of the three middle lobe of the leaf, three independent measurement points were logged when the value was stable. The data was collected from leaves of different developmental stage (L4, L7, L10, L13, L16, L19) of three individual plants.

## **2.4. Iodine staining, microscopy**

Starch visualization by iodine staining was performed as described in Hostettler et al. 2011. Briefly, leaf material was destained in hot 80% ethanol and submerged in iodine solution (I<sub>2</sub>-KI) solution (0.34% (w/v) I<sub>2</sub> and 0.68% (w/v) KI) for staining. Stem and storage root tissue was stained directly with iodine.

### **2.4.1. Microscopy**

The plant tissue samples (leaf, stem, storage root) were cut into lateral sections of 20  $\mu\text{m}$  using a Vibratom (Leica VT1200 S) and stained with iodine. The samples were analysed under a Zeiss Axio Imager Z2 microscope.

## **2.5. Carbohydrate extraction**

Insoluble and soluble carbohydrate extraction was performed as described in Hostettler et al. 2011. Plant material of leaf, stem and storage root was harvested and immediately frozen in liquid nitrogen. For leaf tissue, 8 discs with an area of 3.41 mm<sup>2</sup> each were punched out of the lobes. Except for the youngest leaf, which was too small, the disks did not contain the middle vein. Each tissue sample

consisted of 100-200 mg plant material. Stem and storage root samples were pulverized with the Geno/Grinder® 2010 from SPEX SamplePrep prior to further extraction. All tissue samples were grinded in an ice-cold 5-mL all-glass homogenizer with 3 mL ice-cold 0.7M perchloric acid. 2.7 mL of the homogenate were transferred into a 13-mL culture tube and spun down (3.000 *g*, 10 min, 4°C). After centrifugation, 2.2 mL of the supernatant was transferred into a new tube and neutralized to pH 6-7 by adding the appropriate volume of neutralization buffer (2 M KOH , 0.4 M MES). After precipitation of the potassium perchlorate (14.000 *g*, 10 min, 4 °C), aliquots were taken from the supernatant and stored at -20°C. The insoluble fraction was washed with 4 mL ddH<sub>2</sub>O and spun down (3.000 *g*, 10 min, 4 °C). The supernatant was discarded and the pellet was washed 3 times with 80% ethanol. Between the washing steps the suspension was spun down as before. The pale or colourless pellet was dried and re-suspended in water to a final volume of 1 mL. The insoluble fraction was stored at -20 °C.

### 2.6. Carbohydrate measurements

#### 2.6.1. Insoluble carbohydrate determination

For the carbohydrate measurements, the starch in the samples was first hydrolysed to glucose. This involved a gelatinization step at 95°C for 10 min after which the samples were mixed with digestion buffer (220 mM Na-acetate pH 4.8, 10 units  $\alpha$ -amylase [EC 3.2.1.98, Roche Diagnostics], 12.6 units amyloglucosidase [EC 3.2.1.3, Roche Diagnostics]) or control digestion buffer (220 mM Na-acetate pH 4.8) in a 1:1 (v:v) ratio and incubated at 37°C for 4 h. The digest was spun down at 16.000 *g* for 10 min at 20°C. For glucose quantification 100  $\mu$ L sample (for  $\Delta OD_{340}$  0.2-0.5) was mixed in a 200  $\mu$ L assay containing 25 mM Hepes-KOH pH 7.5, 1 mM MgCl<sub>2</sub>, 1 mM ATP, 1 mM NAD<sup>+</sup>, 1.4 units hexokinase (EC2.7.1.1, Roche Diagnostics). The initial OD at 340 nm ( $OD_{340}$ ) was determined. The addition of 1 unit glucose-6-phosphate dehydrogenase (EC1.1.1.49, Roche Diagnostics) to the assay triggers the conversion of glucose-6-phosphate to 6-gluconolactone thereby reducing NAD<sup>+</sup> to NADH which was spectrophotometrically detected with a microplate reader (TECAN Infinite® M1000). The amount of NADH detected corresponds to the equivalent amount of glucose.

#### 2.6.2. Soluble carbohydrate determination

##### 2.6.2.1. Enzymatically

The contents of soluble sugars such as glucose, fructose and sucrose were analysed spectrophotometrically using an enzymatic assay. The enzymatic assay was comprised of three steps.

The first steps of the assay involved the measurement of glucose levels and were already described before (Chapter 2.6.1.). When the kinetic reaction for glucose reached the endpoint, fructose levels were determined by adding 0.7 units phosphoglucosomerase (PGI 2mg/mL, EC5.3.1.9, Roche Diagnostics). PGI converts fructose 6-phosphate (F6P) to glucose 6-phosphate (G6P) that then enters the same reaction pathway as described before and NADH is released and measured. When fructose kinetic reaction reached the saturation 10 units invertase (INV, EC3.2.1.26, baker yeast Fluka) was added to hydrolyse sucrose into glucose and fructose. Both, glucose and fructose then enter again the reaction pathway via G6PDH either directly or through PGI and can be monitored indirectly via the formation of NADH.

### 2.6.2.2. HPLC-PAD

Soluble sugars (glucose, fructose, sucrose and maltose) in the supernatant were measured using high pH anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) as described in Fulton et al. (2008) with minor modifications. Samples of the neutralized soluble fraction (100  $\mu$ L) were applied to sequential 1.5-mL columns of Dowex 50 W and Dowex 1 (Sigma-Aldrich, Buchs, Switzerland). The neutral compounds were eluted with 4 mL of water, lyophilized, and re-dissolved in 100  $\mu$ L of water. The sugars were separated on a Dionex PA-20 column according to the following conditions: eluent A, 100 mM NaOH; eluent B, 150 mM NaOH and 500 mM sodium acetate. The gradient was as follows: 0 to 7 min, 100% A; 7 to 26.5 min, a concave gradient to 20% A, 80% B (elution of sugars); 26.5 to 32 min, 20% A, 80% B (column wash step); 32 to 40 min, 100% A (column re-equilibration). Peaks were identified by co-elution with known sugar standards. Peak areas were determined using Chromeleon software (Dionex, Olten, Switzerland).

## 2.7. Protein extraction

### 2.7.1. Soluble protein extraction for Immunoblot analysis and native PAGE

Three hundred mg of frozen, pulverized tissue was homogenized with 1 mL ice-cold extraction buffer (100 mM Mops pH 7.2, 1 mM EDTA, 10% [v/v] ethanediol, 1% [w/v] PVPP, 1mM DTT, 1x proteinase inhibitor [Complete, Mini, EDTA-free, Roche Applied Science]). The proteins were homogenized using ice-cold all-glass homogenizer. The homogenate was sedimented by centrifugation (10min, 16.000g, 4°C) and the supernatant immediately used for native protein activity assays or stored at -80°C for further experiments.

### 2.7.2. Protein amount determination by Bradford

For total protein content determination, the BioRad Protein Assay (BioRad, Hercules, California) was performed following the manufacturer's instruction. Bovine serum albumin (BSA, Sigma-Aldrich) served as a standard.

### 2.8. SDS PAGE

Soluble proteins were separated by SDS-PAGE. The stacking gel was composed of 3.75% (w/v) acrylamide/ bisacrylamide (37.5/1), 125 mM Tris-HCl pH 6.8, 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate, 0.1% (v/v) TEMED and the separating gel of 7.5% (w/v) acrylamide/bisacrylamide (37.5/1), 375 mM Tris-HCl pH 8.8, 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate, 0.05% (v/v) TEMED.

Proteins were mixed in a 1:9 (v/v) ratio with SDS sample buffer (0.1 M Tris-HCl pH 6.8, 40% [v/v] glycerol, 3% [w/v] SDS, 0.015% [w/v] bromophenol blue, 1.1% [w/v] DTT). Electrophoresis was performed in running buffer (25 mM Tris-HCl pH 8.3, 192 mM glycine, 0.1% [w/v] SDS) at 22°C at constant current of 15 mA per gel. PrecisionPlusProtein™ Standards (Biorad) were used as molecular weight markers. To visualize proteins on SDS gel the proteins were first fixed in 12% trichloroacetic acid (TCA) solution for 30 min and then the staining solution (5% [v/v] MeOH, 12.5% Ammonium sulfate, 2.5% ortho-phosphoric acid, (1-2 mL) 5% Coomassie blue G250) was added.

### 2.9. Western Blot

Proteins separated by SDS PAGE were transferred onto a PVDF-membrane by electroblotting in 119 mM Tris, 40mM glycine, pH8.3, 10% (v/v) methanol (blotting buffer) at constant 100V for 1 h at 4°C. Following transfer, the membrane was washed in TBS (20mM Tris-pH7.5, 500mM NaCl) for 10 min and incubated for 1h at 25°C in blocking solution (2% or 3% (w/v) non-fat dry milk in TTBS (TBS with 0.1% Tween-20) to block unspecific binding sites. After the blocking step the membranes were rinsed in TTBS for 1 min and subsequently the membrane was washed 3 times 10 min. The blots were incubated overnight with the primary antibody against GWD (Eurogentech, Cologne, Germany) or against the FLAG Tag (M2, Sigma Aldrich) in a dilution of 1/3000 or 1/1000, respectively. The antibodies were raised in rabbit (GWD) and mouse (M2). The corresponding secondary antibodies tagged with horse radish peroxidase (HRP) were mixed with 3% non-fat dry milk in TTBS and incubated at 20°C for 2h. The membrane was washed with TTBS once for 1 min and subsequently three times for 10 min. Chemiluminescence was detected using the Chemiglow West substrate following the manufacturers protocol (Proteinsimple, Santa Clara, California).

## 2.10. Determination of amylolytic activity

### 2.10.1. Native PAGE

For native gels, soluble proteins were mixed 1:9 (v/v) with 10x native sample buffer (50% [v/v] glycerol, 0.05% [w/v] bromophenol blue) and separated using 1 mm thick gels consisting of a stacking gel (3.75% [w/v] acrylamide/ bisacrylamide (37.5/1), 125 mM Tris-HCl pH 6.8, 0.05% [w/v] ammonium persulfate, 0.1% [v/v] TEMED) and a separating gel (6% [w/v] acrylamide/bisacrylamide (37.5/1), 375 mM Tris-HCl pH 8.8, 0.05% [w/v] ammonium persulfate, 0.05% [v/v] TEMED). Electrophoresis was performed in running buffer (25 mM Tris pH 8.3, 192 mM Glycine) at 4°C, 100 V for 20 min followed with 20 mA constant current for 1 h. To detect amylolytic protein activity, the following substrates were added to the gel: 0.1% (w/v) amylopectin from potato starch (Sigma-Aldrich), 0.1% (w/v)  $\beta$ -limit dextrin (Megazyme International, Bray, Ireland) or 1% (w/v) red-pullulan (Megazyme International).

## 2.11. Proteome analysis, sample digestion, mass spectrometry and spectra analysis

### 2.11.1. Protein preparation

Total proteins were extracted according to a modified protocol from (Saravanan and Rose, 2004). Briefly, root material was homogenized on ice using a glass homogenizer in 1% (w/v) PVPP, 0.7 M sucrose, 0.1 M KCl, 0.5 M Tris-HCl, pH 7.5, 100 mM EDTA, 2% (v/v)  $\beta$ -mercaptoethanol, 2x protease inhibitor cocktail (Roche Applied Science, Penzberg, Germany). 1:1 ratio (v/v) phenol, pH 8.0 (Sigma-Aldrich) was added and spun down at 30 min at 4,000 *g*, 4°C. Proteins in the phenol phase were precipitated overnight at -20°C in 5 volumes of 0.1 M ammonium-acetate-100% methanol and after centrifugation (5 min at 4,000 *g*, 4°C) washed in 100% methanol and subsequently in 80% acetone. The air-dried precipitated proteins were resuspended in 4% SDS, 40 mM Tris pH 6.8, 2x protease inhibitor cocktail (Roche Applied Science). Protein concentration was determined using a BCA Protein Assay Kit (Thermo Scientific, Waltham, Massachusetts) before adding 40 mM DTT. Proteins were boiled 5 min at 95°C and 100  $\mu$ g proteins were subjected to SDS-PAGE on 12% gels. The gels were Coomassie-stained according to standard procedures and subsequently sliced into 14 fractions. Each gel slice was diced into small pieces. In-gel protein digestion was performed according to a modified protocol from Shevchenko et al (1996). After digestion, dried peptides were resuspended in 3% (v/v) acetonitrile 0.2% (v/v) trifluoroacetic acid and

cleaned up using Sep-Pak Cartridges (Waters, Milford, Massachusetts, USA). Clean samples were dried and resuspended in 12  $\mu$ L 3% (v/v) acetonitril, 0.2% (v/v) formic acid for mass spectrometry.

### 2.11.2. Mass spectrometry

Mass spectrometry analysis and database searches were done accordingly to Bischof et al., 2011 and Bischof et al., 2013. Peptides were analyzed on a LTQ Orbitrap mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) coupled to an Eksigent-Nano-HPLC system (Eksigent Technologies, Dublin (CA), USA). Peptide mixtures were loaded onto laboratory-made capillary columns (75  $\mu$ m inner diameter [BGB Analytik, Böckten, Switzerland], 8 cm length, packed with Magic C18 AQ beads, 3  $\mu$ m, 100 Å [Michrom BioResources, Auburn, CA, USA]). Peptides were eluted from the column by an increased acetonitrile concentration in the mobile phase from 5% (v/v) acetonitrile, 0.2% (v/v) formic acid to 40% (v/v) acetonitrile, 0.2% (v/v) formic acid over 74 min, followed by a 10 min wash step at 5% (v/v) acetonitrile, 0.2% (v/v) formic acid. Full-scan MS spectra (300–2000 m/z) were acquired with a resolution of 60000 at 400 m/z after accumulation to a target value of 500000. Collision induced dissociation (CID) MS/MS spectra were recorded in data dependent manner in the ion trap from the six most intense signals above a threshold of 500, using a normalized collision energy of 35% and an activation time of 30 ms. Charge state screening was enabled and singly charge states were rejected. Precursor masses already selected for MS/MS were excluded for further selection for 120 s and the exclusion window was set to 20 ppm. The size of the exclusion list was set to a maximum of 500 entries.

### 2.11.3. Protein identification and label-free peptide quantification.

MS/MS spectra were searched with Mascot (Matrix Science, London, UK) version 2.3 against the cassava protein database *Mesculenta\_147\_peptide.fa* ([ftp://ftp.jgi-psf.org/pub/JGI\\_data/phytozome/v7.0/Mesculenta/](ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v7.0/Mesculenta/)) with a concatenated decoy database supplemented with contaminants. Information about Arabidopsis AGI-homologue and protein description were obtained from the file *Mesculenta\_147\_annotation\_info.txt*. (download from [ftp://ftp.jgi-psf.org/pub/JGI\\_data/phytozome/v7.0/Mesculenta/](ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v7.0/Mesculenta/)) using a small awk script. The search parameters were: requirement for tryptic ends, one missed cleavage allowed, mass tolerance of  $\pm$  5 ppm. Beside carbamidomethylation of cysteines as fixed modification, oxidation of methionine was included as variable modification. Peptide identification was accepted with a minimal Mascot ion score of 23 and a Mascot expectation value  $\leq$  0.05. To increase protein identification confidence, a minimum of two unique peptides for each identified protein was required. The spectrum false discovery rate was calculated by dividing the number of decoy database spectrum assignments by



the number of spectrum assignments in the final dataset. The false positive rate was below 1% for all measured biological replicates. For the Progenesis analysis, a merged peaklist was generated.

Progenesis LC-MS analysis was done according to (Greer et al., 2012). Peptides were detected and quantified with Progenesis LC-MS software (version 2.5; Non Linear Dynamics) using default settings (no deconvolution/deisotoping, 200 most intense MS/MS peaks). Peak areas were calculated by Progenesis from imported Thermo RAW mass spectrometry files. The 14 gel lanes, each containing six biological samples were analysed separately and resulting quantification information was merged to obtain final protein abundances. For peak alignment, one sample was set as reference and the retention times of all other samples within the same gel lane were aligned. Around 20 manual landmarks were set before automatic alignment to create a maximal overlay of the two-dimensional feature maps. Features with only one charge or more than 3 charges were excluded from further analyses and all remaining features were used to calculate a normalization factor for each sample that corrects for experimental variation. For quantification, all unique peptides (with Mascot score  $\geq 25$  and  $p < 0.05$ , see above) of an identified protein were included and the total cumulative abundance was calculated by summing the abundances of all peptides allocated to the respective protein. No minimal thresholds were set for the method of peak picking or selection of data to use for quantification. Finally, quantification information of all 14 analyzed gel lanes were merged and statistical analysis of variance (ANOVA) was used to calculate p-values based on the sum of the normalized abundances across all 84 runs.

### 2.12. Construct design

#### 2.12.1. Overexpression of potato *StGWD* in cv. 60444

Two pCAMBIA2300 plasmid constructs harbouring either the wild-type (pCAMBIA2300::*StGWD*) or redox-insensitive (pCAMBIA2300::*StGWD*<sub>C1084S</sub>) coding sequence (CDS) of potato GWD were obtained from Mikkel Glaring (University of Copenhagen, Copenhagen, Denmark). In both cases, the constructs were under the control of the 35S promoter and contained the native transit peptide of *StGWD* (JG388473; NCBI database) at the N-terminus. At the C-terminus, a FLAG-tag (Einhauer and Jungbauer, 2001) was added. The redox-insensitive sequence contains a nucleotide modification (5'-GC-3251-CT-3') leading to an amino acid substitution of cysteine 1084 to a serine (C1084S) at the peptide level. Genotyping and verification of mutation sequence was performed with primers as indicated in Table 2.1.

**Table 2.1 Primer list for genotyping and sequencing the wild-type and redox-insensitive *StGWD* constructs** (in bold the sequence for restriction sites).

| Primer          | Sequence (5'-3')                 |
|-----------------|----------------------------------|
| StGWD_BamHI_rv  | gc <b>ggatcc</b> TCACTTATCATCAT  |
| StGWD_BamHI_fw2 | c <b>ggatcc</b> ATGAGTAATTCCTTAG |
| StGWD5F         | TCCAATGGTGGAGACAACCA             |

### 2.12.2. RNAi: Hairpin design

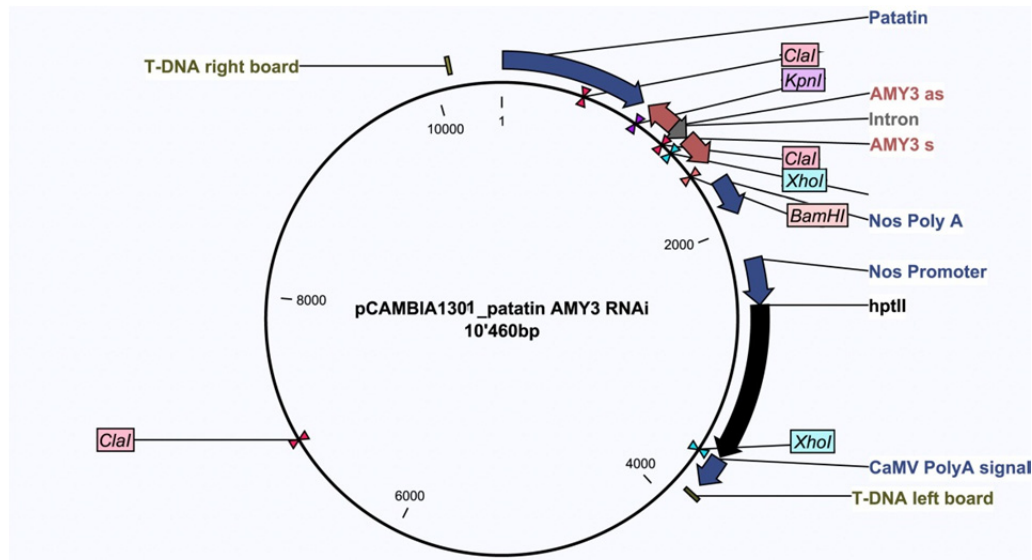
For RNA interference (RNAi), a hairpin construct was designed against three genes: *MeSEX4*, *MeLSF2* and *MeAMY3*. For each gene, a 190- 205 bp long template was amplified from the coding sequence which was prior tested to be unique to the specific transcript by performing a nucleotide BLAST search on the cassava genome database ([www.phytozome.net](http://www.phytozome.net)). Accession numbers and sequence positions of the hairpin templates are given in Table 2.2. The target sequence of *MeSEX4* was first cloned in the pCR8 vector using the TOPO® TA Cloning® Kit from Life Technologies (Carlsbad, California). The *MeAMY3* sequence was first cloned in the pJET1.2 vector using the CloneJET PCR Cloning kit from Thermo Scientific. All primers are given in Table 2.3, set A). In a second step, the *MeSEX4* and *MeAMY3* sequences were re-amplified (primers in set B, Table 2.3) in the forward and reverse orientation and cloned into a modified plasmid of pBluescriptSKII (from H. Vanderschuren, ETH Zurich, Switzerland) containing a synthetic plant intron sequence forming a loop (57-165 bp of the M27939 sequence; Goodall and Filipowicz, 1989). The hairpin construct of *MeLSF2* (target sequences in both orientations linked with the loop) was *de novo* synthesized and ligated in the pBluescriptSKII by Eurofins MWG Operon (Ebersberg, Germany). All RNAi constructs were each subcloned into a modified pCAMBIA1301 vector containing the *Solanum tuberosum* class I patatin promoter (GQ352473.1 aligning 11-970bp, Naumkina et al., 2007) at the 3' end (Figure 2.1).

**Table 2.2 Sequence position of the RNAi hairpin construct**

| Construct | Accession (Phytozom.org) | CDS (bp) | Sequence position for hairpin construct (bp) |
|-----------|--------------------------|----------|----------------------------------------------|
| SEX4 RNAi | cassava4.1_009735m.g     | 1140     | 197-402                                      |
| LSF2 RNAi | cassava4.1_013314m       | 858      | 41-231                                       |
| AMY3 RNAi | cassava4.1_001362        | 2691     | 1-210                                        |

**Table 2.3 Primer list used for hairpin cloning and genotyping** (in bold the sequence for restriction sites).

| Primer name                       | Sequence<br>(5'-3')                                         |
|-----------------------------------|-------------------------------------------------------------|
| <b>Set A</b>                      |                                                             |
| MeS4fw2                           | ATGAGAACATGAGAAAATTT                                        |
| MeS4rv8                           | ATTCTGCTTGCAACAGGACC                                        |
| AMY3 forward                      | ATGTCGACCGTTGCCATTGAG                                       |
| AMY3 reverse                      | AAAAGTTTCAAGAAGAGCGGT                                       |
| <b>Set B</b>                      |                                                             |
| AMY3 anti-sense forward<br>(Clal) | AT <b>ATCGATAATCTTACCTCACGAGTGGTACATGTCGACCGTTGCCATTGAG</b> |
| AMY3 anti-sense reverse<br>(KpnI) | AT <b>GGTACCAAAGTTTCAAGAAGAGCGGT</b>                        |
| AMY3-sense forward (XhoI)         | AT <b>CTCGAGATGTCGACCGTTGCCATTGAG</b>                       |
| AMY3-sense reverse (BamHI)        | AT <b>GGATCCAAAAGTTTCAAGAAGAGCGGT</b>                       |
| MeS4as_Hp_rv_Kpn                  | <b>GGGTACCGGTCCTGTTGCAAGCAGAAT</b>                          |
| MeS4_Hp_rvBamHI                   | <b>CGCGGATCCGGTCCTGTTGCAAGCAGAAT</b>                        |
| MeS4_HpF_Cla                      | <b>ATATCGATAATCTTACCTCACGAGTGGTACAGGAGGACAAGGGAAAGTCT</b>   |
| MeS4as_Hp_rv_Kpn                  | <b>ATCTCGAGAGGAGGACAAGGGAAAGTCT</b>                         |
| <b>Set C</b>                      |                                                             |
| 35S For (GWD)                     | GCACAATCCCACTATCCT                                          |
| stR1-r1 (GWD)                     | CTTGGGCAAGGTCATCAGGTA                                       |
| pp2A fw                           | TGCAAGGCTCACACTTTCATC                                       |
| pp2A rv                           | CTGAGCGTAAAGCAGGGAAG                                        |
| patatinFW                         | TGCGTATTAGTTTTAGCGACGAAG                                    |
| patatinRV                         | AAA CAG ATT CTC TCC CTC GCA C                               |
| terminatorFW                      | TGA ATC CTG TTG CCG GTC TTG                                 |
| terminatorRV                      | AGC GCA ACG CAA TTA ATG TGA G                               |



**Figure 2.1 Representing vector map of modified pCambia1301-patatin used for RNAi constructs**

The patatin promoter is upstream of the insert consisting of the antisense-sequence of the target transcript, followed by an artificial hairpin (110 bp), the sense-sequence of the target transcript, and the Nos Poly terminator.

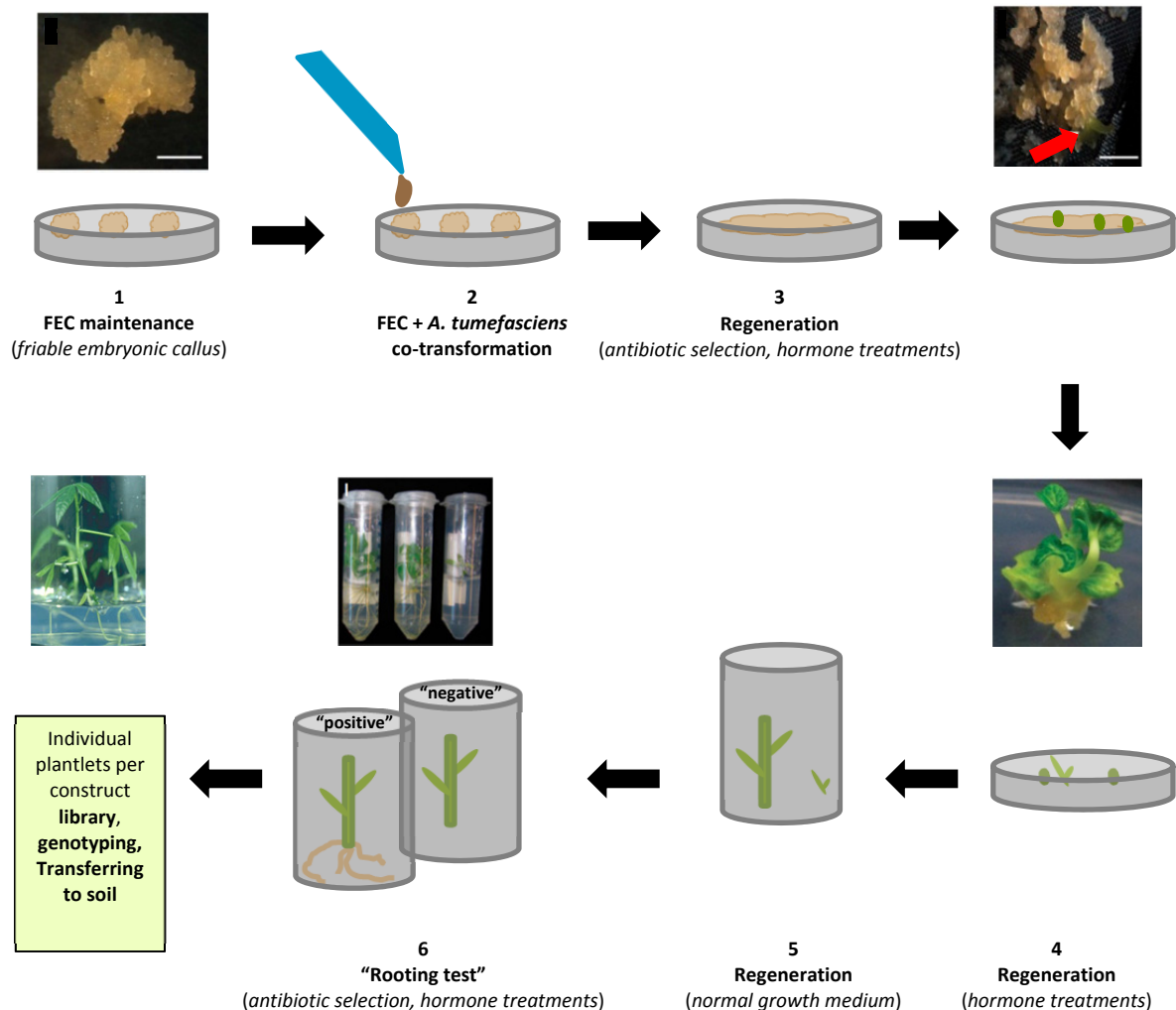
### 2.12.3. *Agrobacterium tumefaciens* transformation

Electrocompetent *Agrobacterium tumefaciens* strain LBA4404 (Rif<sup>50</sup> Strep<sup>100</sup>) were transformed with an RNAi construct or the full length CDS of StGWD-Flag tag, respectively. One  $\mu$ L the plasmid of interest was added to a 50  $\mu$ L aliquot of competent cells of *A. tumefaciens*, hold on ice. The mix was transferred to pre-cooled Gene Pulser cuvettes (BioRad) and an electro shock of 1.5kV, 200  $\Omega$  was applied. After transformation, the cells were put back on ice and 750  $\mu$ L of YEB liquid medium (5 g/L Beef extract, 1 g/L yeast extract, 5 g/L peptone, 5 g/L sucrose, 0.5 g/L MgCl<sub>2</sub>) was added. After 90 min, the cells were grown on YEB medium containing the respective antibiotics at 28°C for 2 days.

### 2.12.4. Tissue culture and FEC transformation

Cassava plantlets of the African genotype cv. 60444 were grown in a SANYO plant growth facility (Type MLR, Panasonic Biomedical Sales Europe B.V.) under a day-night regime (16h light, 8h dark, 27°C, 50  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). Transgenic cassava lines were obtained with *Agrobacterium*-mediated friable embryonic callus (FEC) transformation described in (Bull et al., 2009; Niklaus et al., 2011). The important steps of transformation are illustrated in a scheme in Figure 2.2. After transformation with *A. tumefaciens* harbouring the plasmid of interest, the transformed FECs were pre-selected on regenerating medium containing hygromycin antibiotics (Figure 2.2, step 2 and 3). Until step 4 the growth media are supplied with antibiotics for selection though in step 4 and 5 the media contain no antibiotics (Figure 2.2). In these steps (4, 5) new plantlets are regenerated. To confirm that the re-generated cassava plantlets contain the construct of interest a rooting test was performed on

growth media containing selection antibiotics (Figure 2.2, step 6). DNA was extracted and the insertion of the construct verified by PCR amplification using primers listed in Table 2.3 (set C).



**Figure 2.2 Main steps involved cassava transformation and regeneration.** This schematic illustration depicts the late phase of maintaining and transforming cassava friable embryonic calli (FEC). The transformation steps were performed according Bull et al., 2009. 1) FECs are multiplied and maintained on GD medium 2) Inoculation of FEC with *A. tumefaciens* 3) FECs are regenerated by transferring the transformed FECs on selective media. After 1-2 weeks leafy structures are generated. 4) To regenerate plantlets the leafy structures are transferred on growth media without antibiotics. 5) Plantlets are transferred on growth media to regenerate plantlets including roots. 6) For positive selection part of the stem of regenerated plantlets are subjected to "rooting test". The growth medium is supplied with antibiotics. Plantlets containing the plasmid produce roots, negatives not. The positive lines are grown on growth medium and kept for further analysis and as library. The pictures derive from Bull et al. (2009).

### 2.13. Construct functional analysis

#### 2.13.1. Starch isolation from transgenic cassava storage root and $^{31}\text{P}$ NMR analysis

To measure the content of starch-bound phosphate, starch granules were purified from cassava storage roots according to Hostettler et al., 2011. The frozen material was first pulverized using a

Geno/Grinder® 2010 from SPEX SamplePrep. After resuspension in ice-cold starch extraction buffer (50 mM Tris-HCl, pH 8.0, 0.2 mM EDTA, 0.5% (v/v)), the mix was homogenized for 3 min using a Waring blender. The homogenate was filtered through a 100 µm nylon mesh and the filtrate was spun down (15 min, 3.000 g, 20°C). The pellet was resuspended in starch extraction buffer and filtered through a 75 and 60 nylon mesh. The filtrate was overlaid on a 10 ml cushion of 95% (v/v) Percoll and 5% (v/v) 500 mM Tris-HCl, pH 8.0 and after centrifugation (15 min at 2.500g), the pellet was resuspended in SDS buffer (0.5% SDS [w/v]). The samples were spun down at 20.000g for 1 min and washed again in 1ml SDS buffer. The centrifugation and re-suspension steps with SDS buffer were repeated several times to get a clean, white pellet of starch granules. After this, SDS was washed away by five centrifugation/resuspension steps with water and a final wash with 80% EtOH. The starch pellet was dried under vacuum for 48 h.

Sample preparation for <sup>31</sup>P-NMR analysis was done according to Santelia et al. (2011). Briefly, the starch samples were resuspended in a salt solution (3 mM NaCl, 1 mM CaCl<sub>2</sub>, pH6) and digested with α-amylase from pig pancreas (Roche Applied Science) and amyloglucosidase from *Aspergillus niger* (Roche Applied Sciences). <sup>31</sup>P-NMR analysis was performed on an Avance III 600-MHz spectrometer equipped with a QCI CryoProbe (Bruker) at 303K.

### 2.13.2. Genomic DNA extraction

Leaf material was harvested and frozen in liquid nitrogen. After pulverisation with a mixer mill (MM301, Retsch) in a 1.5-mL Eppendorf tube containing glass beads, the plant material was resuspended in 900 µL DNA extraction buffer (7M Urea, 0.3 M NaCl, 50 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH 8). Following centrifugation (13.000g, 20 °C, 15 min), the supernatant was mixed in a 1:1 (v/v) ratio with phenol: chloroform: isoamylalcohol (25:24:1; Carl Roth, Karlsruhe, Germany) and spun down for 15 min, 13.000g, at 20°C. Six hundred µL from the aqueous phase was transferred to a 1.5-mL Eppendorf tube containing 1:10 (v/v) 3 M Sodium Acetate, pH 5.2 and 1 µL RNaseA (20 mg/mL) was added and mixed well. To precipitate the DNA, isopropanol in a 1:1 (v/v) was added to the mixture and incubated for 1 h at -20°C. The precipitated DNA was spun down at 13.000g for 15 min at 20°C. The pellet was washed twice with 1 mL 70% ethanol and once with 100% ethanol. Between the washing steps the sample was spun down 13.000g, 15 min at 20°C. After drying the pellet under the air-flow it was re-suspended in 30 µL ddH<sub>2</sub>O.

### 2.13.3. RNA extraction

Up to 200 mg of pulverized storage root samples (using a GenoGrinder, described in 2.6) were mixed with 600 µL of RNA extraction buffer (150 mM Tris-boric acid, pH 7.5, 2% SDS, 50 mM EDTA, pH 8.0)

## *2. Material and Method*

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during 2-5 min using a vortex. After adding 150  $\mu\text{L}$  of 100% EtOH and mixing for 1 min on a vortex, 66  $\mu\text{L}$  5M potassium acetate were added and mixed for 1 min. Following the addition of 750  $\mu\text{L}$  chloroform to denature proteins, the samples were mixed for 1 min and spun down at 13.000  $g$  for 3 min at 20°C. Of the RNA containing aqueous phase, 500  $\mu\text{L}$  was transferred to a new 2-mL Eppendorf tube. To the aqueous phase phenol: chloroform was added in a 1:1 (v/v) ratio. The mixture was spun down at 13.000  $g$ , 1 min, 20°C. From the upper aqueous phase 400  $\mu\text{L}$  was transferred to a new 2-mL Eppendorf tube. One mL of 100% RNase-free EtOH was added and mixed well. The RNA was precipitated at -80°C for 30 min and pelleted by 13.000  $g$ , 30 min, 4°C. The supernatant was removed and 170  $\mu\text{L}$  80% RNase-free EtOH was added. The RNA was subsequently pelleted at 13.000  $g$ , 3 min, 20°C. The supernatant was removed and the pellet re-suspended in DEPC water. To precipitate RNA, 25  $\mu\text{L}$  8M DEPC LiCl was added and incubated at -20 °C overnight. The RNA was pelleted at 13.000  $g$ , 30 min, 4 °C and the supernatant was removed. To wash the pellet 170  $\mu\text{L}$  80% RNase-free EtOH was added and spun down (13.000  $g$ , 3 min, 20 °C). The supernatant was removed and the pellet air dried under the flow hood for 5 min. Then the pellet was re-suspended in 25-50  $\mu\text{L}$  DEPC water.

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### 3. Cassava growth carbon assimilation and allocation analysis

#### 3.1. Introduction

In plants two kinds of starches can be distinguished which fulfil different storage requirements. Transitory starch in photosynthetic tissues like Arabidopsis leaves undergoes a diurnal cycle of synthesis and degradation. In non-photosynthetic tissues (i.e. potato tubers, cassava storage roots, maize kernels) carbohydrate is translocated from the photosynthetic tissue, imported into the amyloplast and converted to starch. Starch in amyloplasts is built-up and stored over a long-term period (Geigenberger, 2003; Sonnewald and Kossmann, 2013). Cassava produces both transient and storage starch, but neither process has been studied in depth at the molecular genetic level in this species.

I first studied carbohydrate metabolism in leaves and storage root of cassava with the aim of drawing a basic framework of starch and carbohydrate metabolism. I measured physiological parameters and correlate them with the carbohydrate metabolism. My experiments were performed with greenhouse-grown cassava plants, grown under defined conditions to reduce environmental influences on the metabolism (such as variability in water availability, temperature, light intensities or soil-nutrient availability). Cassava physiological characteristics and yield performance in response to natural growth conditions as well as stress conditions were investigated in several studies performed on field grown cassava plants (El-Sharkawy et al., 1984; El-Sharkawy, 1990; Angelov et al., 1993). Comparisons of biomass with parameters such as photosynthetic rate and stomatal conductance revealed a positive correlation between the root biomass and photosynthesis (El-Sharkawy, 1990). Only few studies are reported dealing with greenhouse grown cassava plants (Edwards et al., 1990; Calatayud, 2000; Cruz, 2003).

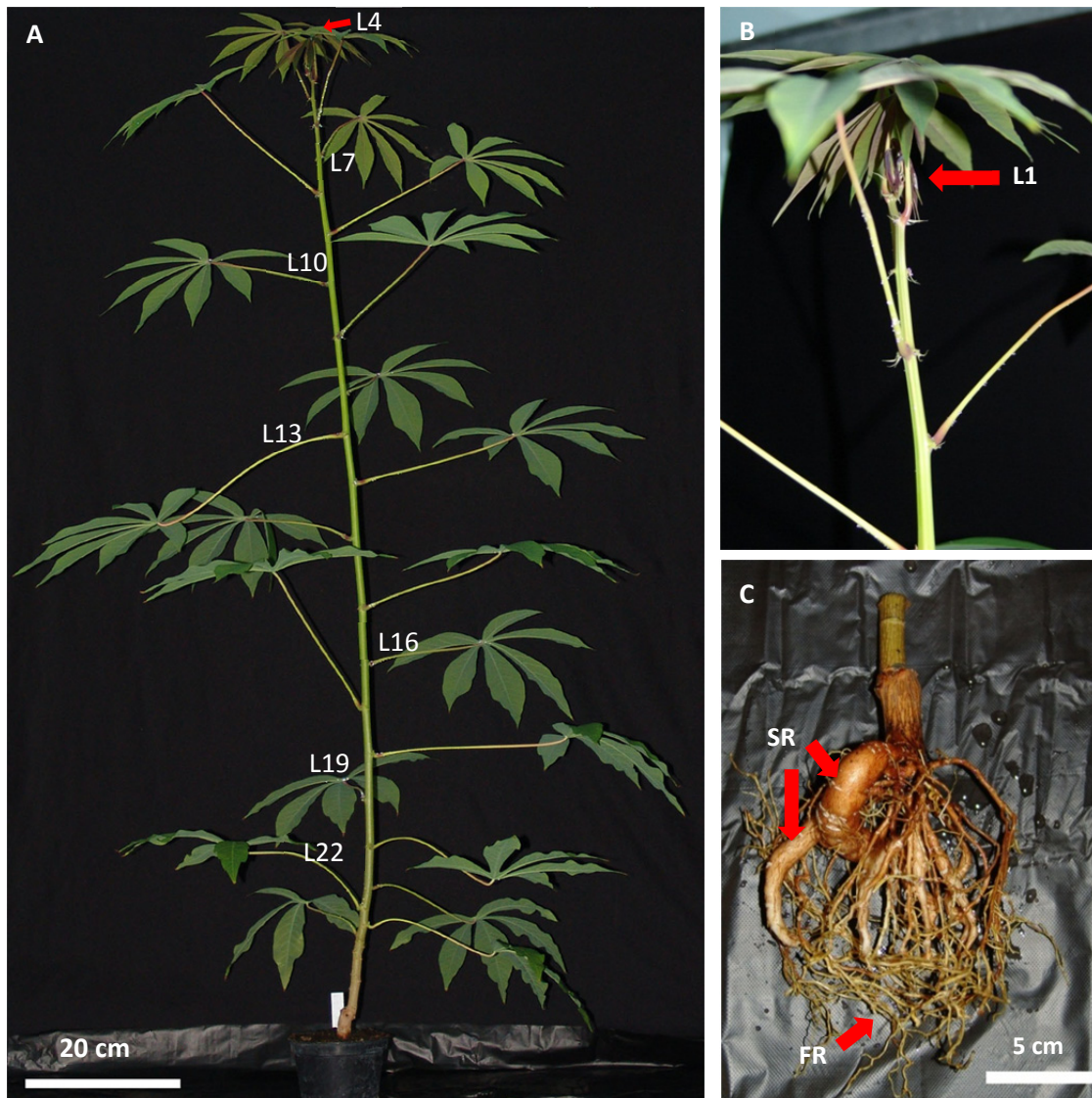
To investigate the general performance of cassava in our greenhouse conditions, experiments were performed to unravel the general performance, in respect of growth and carbon assimilation. Therefore, the characteristics of several batches of greenhouse grown cassava plants was assessed and compared with photosynthetic capacity and the distribution of carbohydrates in different tissues at various developmental stages.

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## **3.2. Growth analysis of greenhouse-grown cassava plants**

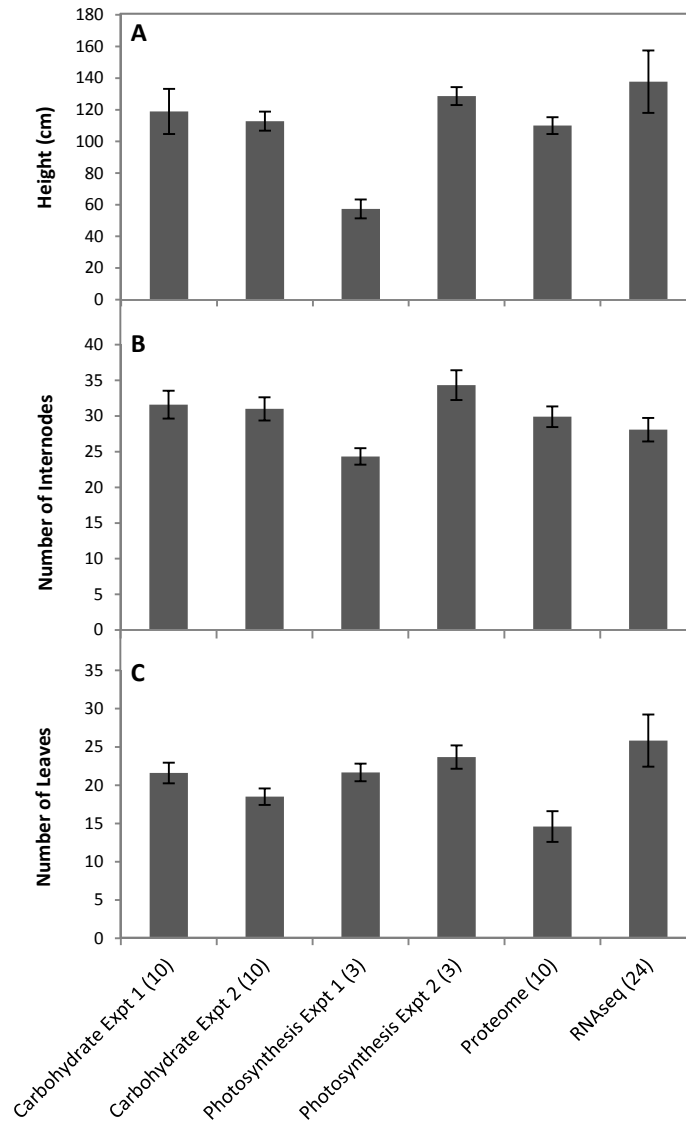
### **3.2.1. Growth analysis of plant batches grown at different time points**

*Manihot esculenta* Crantz plant batches were generated by stem cuttings from different mother plants. The plants were grown in a greenhouse for 4-5 months under defined light, climate, and soil-nutrient conditions (Chapter 2.1). For this thesis, six batches of plants were harvested at different times of the year for six individual experiments. Although the conditions were controlled, minimal variation of external, environmental influences like day length and light intensity could not be excluded. For the harvested batches, the homogeneity of the plants was determined. Individual plant height was measured from the apex - the emerging point of the youngest visible leaf - to the sprouting initiation site at the bottom. The number of leaves was assigned by the top-down counting, starting with the youngest leaf (number one) down to the initiation of sprouting (Figure 3.1 A). The number of leaves was determined by counting them from top to bottom. The numbering of internodes was done the same way where the first internode section was that between the first and second leaf. Although at the point of harvesting the plants were at a similar developmental stage, differences in height, number of leaves and of internodes were observed. Therefore, the above definition of tissue age facilitated the comparisons of plants and tissues at the same developmental stages in the different experiments (i.e. young, developing, mature, and aged leaves).



**Figure 3.1** *Manihot esculenta* Crantz (cv. 60444) grown under greenhouse conditions for 4-5 months  
 A) The tissue was numbered according to the age and counted from the young, undeveloped to the old, mature tissue (L = leaf). B) Enlargement of the apex of cassava plant showing the youngest leaves, arrow pointing at the youngest leaf. C) Cassava fibrous roots (FR) and storage roots (SR)

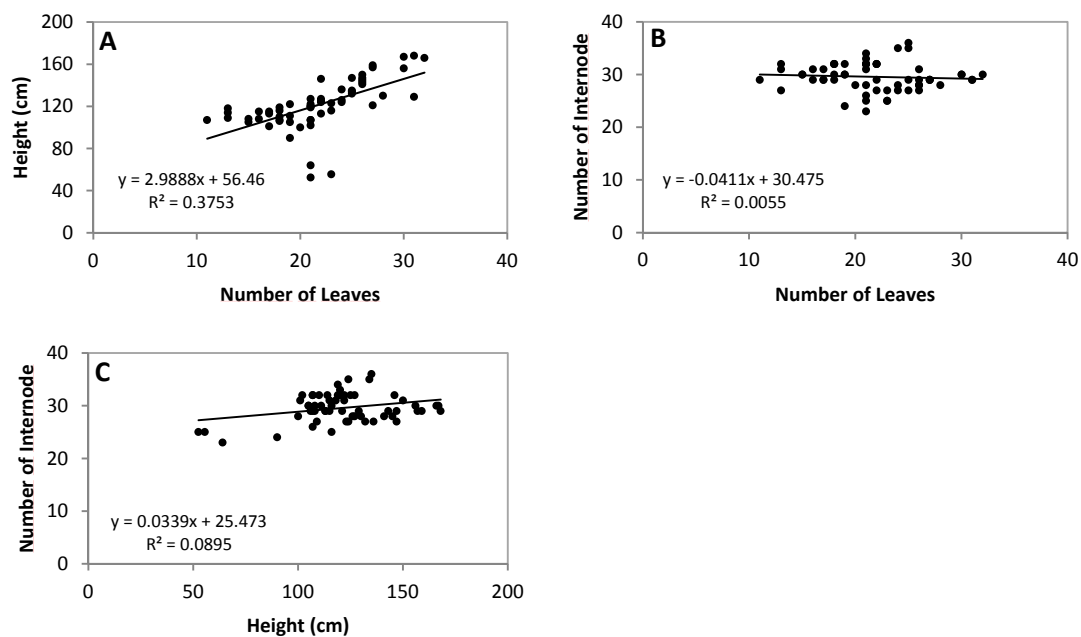
Comparing the growth of the six plant batches, the mean plant height of the different batches was 110 cm ranging between  $57 \pm 5.97$  cm (Photosynthesis Expt 1) to  $137.79 \pm 19.72$  cm (RNAseq) (Figure 3-2 A). Variation in the number of internodes was between  $24.3 \pm 0.67$  (Photosynthesis Expt 1) and  $34.3 \pm 1.2$  (Photosynthesis Expt 2) (Figure 3-2 B). And the number of leaves ranged from  $14.6 \pm 2.01$  (Proteome) to  $25.83 \pm 3.41$  (RNAseq) (Figure 3-2 C).



**Figure 3.2 Plant batch growth analyses**

For the six plant batches used in the different experiments for this thesis the three measures height (cm), number of internodes and number of leaves were determined. Mean  $\pm$  SD (N = 3, 10, 24 as indicated)

Although the average of leaf number varied between batches, minimal variation was observed within each batch. An eventual connection between the parameters they were plotted against each other to reveal any dependency. Thus, a pairwise-comparison was performed on the collected data and linear regression was investigated (Figure 3-3). The comparison revealed that, within the harvested plants, none of these parameters were strongly correlated.



**Figure 3.3** Pairwise comparisons over all six plant batches used for the described experiments

The comparison was performed between A) height (cm) vs. number of leaves, B) Number of internodes vs. number of leaves, C) number of internodes vs. height (cm).  $R^2$  = linear correlation coefficient (N = 60).

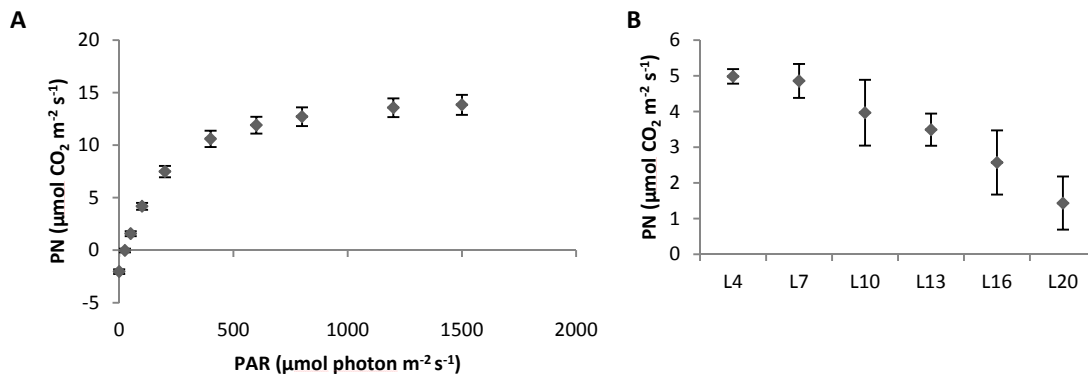
### 3.3. Photosynthetic capacity measurements

I measured the photosynthetic capacity of greenhouse grown cassava plants using a LI-COR 6400 XT device connected to an integrated infrared-gas analyser (IRGA). This device has a chamber with integrated LED source, which is clamped over a single leaf and the gas exchange of a defined area ( $6 \text{ cm}^2$ ) of the leaf is measured. Cassava leaves are hand-shaped with multiple lobes and a surface area larger than  $6 \text{ cm}^2$ . Thus, photosynthetic measurements were performed on the 3 middle lobes in triplicate (i.e. nine measurements) and the mean value taken as a representative value for photosynthetic performance. I investigated the influence of both light intensity and leaf age on the photosynthetic capacity so as to be able to relate this to carbohydrate content. For the analysis,  $\text{CO}_2$  concentration and air flow were kept constant ( $400 \text{ ppm}$ ;  $250 \text{ mol s}^{-1}$ ).

#### 3.3.1. Photosynthetic capacity of cassava leaves in dependence of light and age

The dependency of photosynthesis on light was investigated by applying a series of light intensities (photosynthetically active radiation [PAR] from 0 to  $1500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) to a fully-expanded leaf (L4) and measuring gas exchange (Figure 3.4 A). As expected, photosynthetic rate increased with increasing light intensity. In the dark, respiration led to negative photosynthetic rate (i.e. an increased  $\Delta\text{CO}_2$ ). At a PAR of  $25 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  photosynthetic rate was close to zero, and the

acquired data allowed me to calculate a light compensation point of PAR 24  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (where the rate of photosynthesis equals that of respiration/photorespiration). Between PAR 50 to 200, the photosynthetic rate increased in a near-linear way ( $R^2 = 0.9853$ ), reaching 7.48  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ . The positive influence of light intensity on the photosynthetic rate was limited beyond PAR 400 and the  $\text{CO}_2$ -assimilation curve flattened reaching a maximum of 13.84  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$  as photosynthesis became light-saturated (Figure 3.4 A).



**Figure 3.4 Photosynthetic rate in greenhouse-grown cassava plants**

A) at increasing light intensities (0-1500  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) from L4 B) from leaves at different developmental stages (L4-L20) at constant light intensity (100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Mean  $\pm$  SD (N=3)

The influence of leaf age on photosynthesis was measured at a light intensity of 100  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ , which was similar to that measured within the leaf canopy in the greenhouse on a typical day. The youngest fully developed leaves measured (L4 and L7) showed highest photosynthetic rates (around 5  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ; Figure 3.4 B). From leaf 10 to the oldest leaves, photosynthetic capacity decreased progressively to 1.43  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ . Thus, leaf age indeed has a major negative influence on the photosynthetic rate even with the same, non-saturating PAR.

### 3.4. Carbohydrate accumulation in cassava leaves and stem

#### 3.4.1. Carbohydrates in cassava leaves of different developmental stage and time points

Based on my measurements of photosynthetic rate, I calculated the theoretical accumulation of carbohydrate for a defined light period. These calculations revealed that in mature leaves up to 298.76 mg hexoses g<sup>-1</sup> FW at high light intensities (PAR 1500) could be assimilated in a 14 h light period (Table 3.1). While at low light intensities the theoretical assimilation of hexoses decreases 89% (PAR 50; Table 3.1). The potential of hexose assimilation depends on leaf age where the theoretical carbohydrate accumulation was lower in older leaves with L20 having only 29% the assimilation rate of L4 (Table 3.2).

**Table 3.1 Theoretical carbon assimilation according the photosynthetic rate measurements** Calculation was based on both the photosynthetic rate measurements in dependency of light intensity and the measurement that 1 g leaf tissue has an area of 0.01414 m<sup>2</sup>.

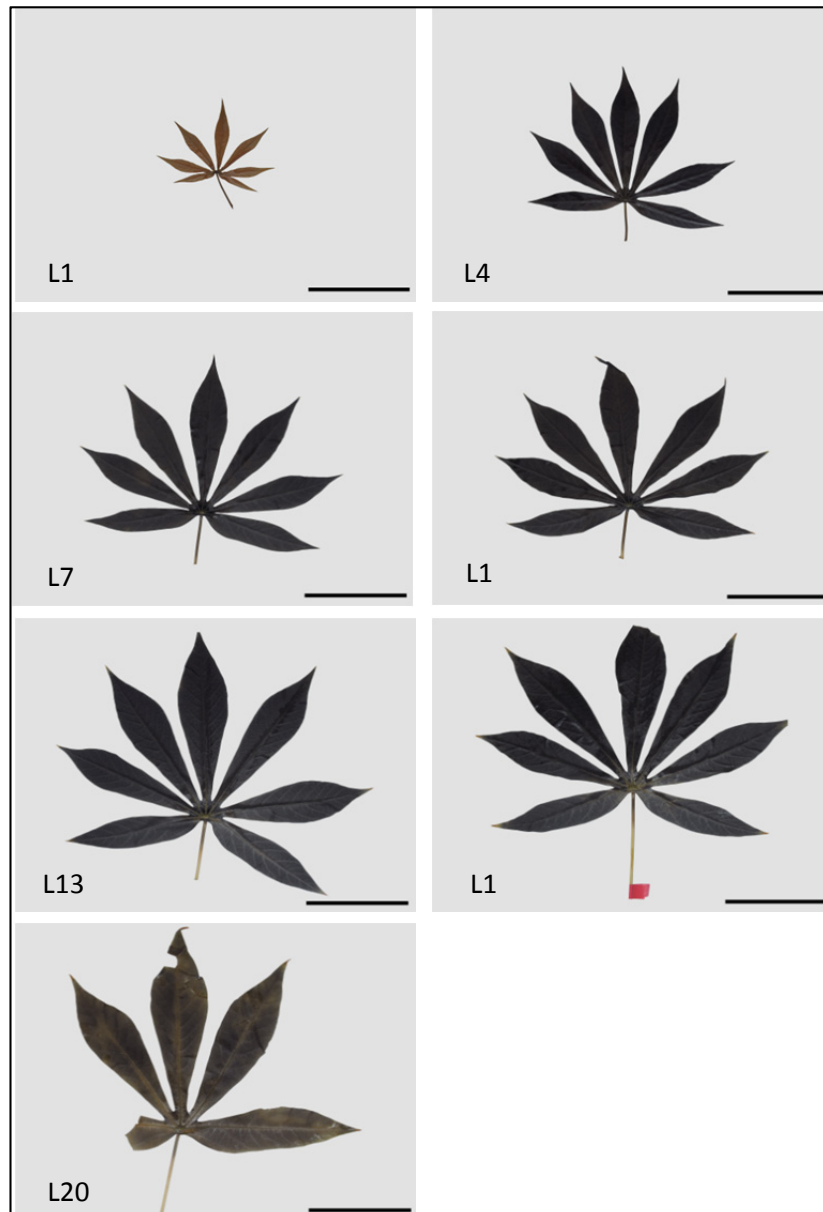
| Light intensity<br>( $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) | Carbon assimilation<br>(mg hexose g <sup>-1</sup> FW) |  |
|--------------------------------------------------------------------|-------------------------------------------------------|--|
|                                                                    | 14h light period                                      |  |
| 0                                                                  | -44.02                                                |  |
| 25                                                                 | -0.62                                                 |  |
| 50                                                                 | 33.89                                                 |  |
| 100                                                                | 90.14                                                 |  |
| 200                                                                | 161.56                                                |  |
| 400                                                                | 228.76                                                |  |
| 600                                                                | 257.02                                                |  |
| 800                                                                | 274.37                                                |  |
| 1200                                                               | 292.76                                                |  |
| 1500                                                               | 298.76                                                |  |

**Table 3.2 Theoretical carbon assimilation according the photosynthetic rate measurements** Calculation was based on both the photosynthetic rate measurements in dependency of light intensity and the measurement that 1 g leaf tissue has an area of 0.01414 m<sup>2</sup>.

| Leaf | Carbon assimilation<br>(mg hexose g <sup>-1</sup> FW) |                  |
|------|-------------------------------------------------------|------------------|
|      | 9h light period                                       | 14h light period |
| L4   | 68.54                                                 | 106.61           |
| L7   | 66.77                                                 | 103.86           |
| L10  | 54.53                                                 | 84.83            |
| L13  | 47.98                                                 | 74.64            |
| L16  | 35.36                                                 | 55.01            |
| L20  | 19.72                                                 | 30.68            |

PAR: 100  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$





**Figure 3.5 Visualisation of starch in cassava leaves by iodine staining**

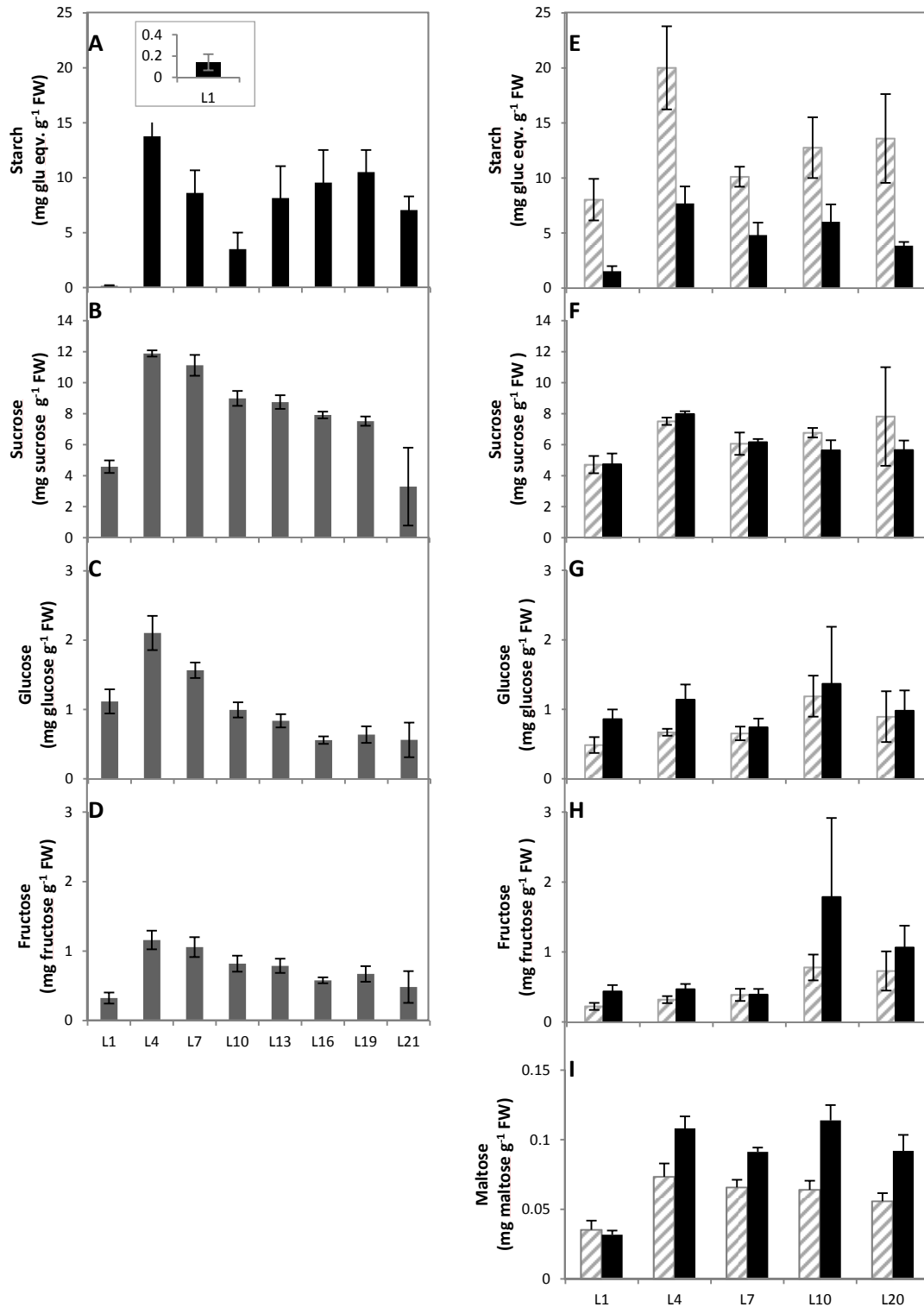
Whole leaves stained with iodine, harvested 4h into the light period. L1= leaf one, L4=leaf 4, L7= leaf 7, L10= leaf 10, L13= leaf 13, L16= leaf 16, L20= leaf 20. Scale bar 10 cm.

The theoretical hexose accumulation during the light period leads to the question about real level of carbohydrates in cassava leaves at different developmental stages. The finding of reduced photosynthetic rate in older leaves suggests that they might have a lower degree of starch and soluble sugar accumulation during the day. To investigate this, I harvested every 3<sup>rd</sup> leaf – from the first, undeveloped leaf (L1) down to the oldest leaf (L20) (Figure 3.1). Initially, whole cassava leaves harvested 4h into the light were stained with iodine to visualize starch. This showed no obvious differences in starch levels between the differently aged leaves, except that the youngest leaf hardly stained (Figure 3.5 A). This suggests that starch is either not fully degraded during the night or starch

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synthesis is very rapid during the first 4h light period. To complement this semi-quantitative iodine staining, leaf material was harvested in two experiments to quantify insoluble and soluble carbohydrates. In the first experiment, leaves of different ages (every 3<sup>rd</sup> leaf) were sampled both at midday (9h into the light) and in the second experiment the comparable leaves were sampled both at the end of the day (EoD) and end of the night (EoN). Insoluble (starch) and soluble (sucrose, glucose, fructose and maltose) carbohydrates were extracted and measured as described (Chapter 2.6.). For these measurements leaves of plants from 'carbohydrate Expt1' and 'carbohydrate Expt2' were analysed (Figure 3.2).

The measurement of starch at midday showed that carbohydrate allocation into this storage pool differed depending on the leaf age and position. In L4 the starch level was highest ( $13.76 \pm 1.98$  mg glu eqv.  $g^{-1}$  FW) whereas only in the youngest, undeveloped leaf (L1) it was lowest ( $0.142 \pm 0.08$  mg glu eqv.  $g^{-1}$  FW). For the other leaves investigated (L7-L21), starch levels varied between  $3.50 \pm 1.51$  mg (L10) and  $10.52 \pm 2.01$  (L19) mg glu eqv.  $g^{-1}$  FW (Figure 3.6 A). The analysis of soluble sugars revealed a pattern similar to that described by Angelov et al. (1993) where sucrose levels are low in the very youngest ( $\sim 6$  mg suc  $g^{-1}$  FW) and oldest ( $\sim 11$  mg suc  $g^{-1}$  FW) leaves but higher in the most of the fully-expanded leaves in between ( $\sim 14$ - $15$  mg suc  $g^{-1}$  FW). In comparison to the sucrose levels the amount of glucose and fructose are low with maximal values in L4 ( $2.1 \pm 0.25$  mg gluc  $g^{-1}$  FW;  $1.16 \pm 0.13$  mg fruc  $g^{-1}$  FW) (Figure 3.6 B-D). Generally, in my analysis the carbohydrate levels were slightly lower than what was measured in field studies, but comparable in terms of the division between the different carbohydrate pools. The carbon partitioned into starch after 9h in cassava leaves, is comparable to the amounts typically observed in Arabidopsis at the end of the day (in a 12h light period) (Gibon et al., 2004; Fulton et al., 2008).



**Figure 3.6 Analysis of insoluble (starch) and soluble (sucrose, glucose, fructose and maltose) carbohydrate allocation in leaves at different developmental stages**

A-D) harvested at the 9h into the light period and E-I) harvested at end of the day (grey, stripe bar) and end of the night (black bar) A, E) starch, B, F) sucrose, C, G) glucose, D, H) fructose I) maltose. L1=young, newly emerged leaf at the top, L21=oldest leaf at the bottom. Mean  $\pm$  SE, N = 5, 4, 3.

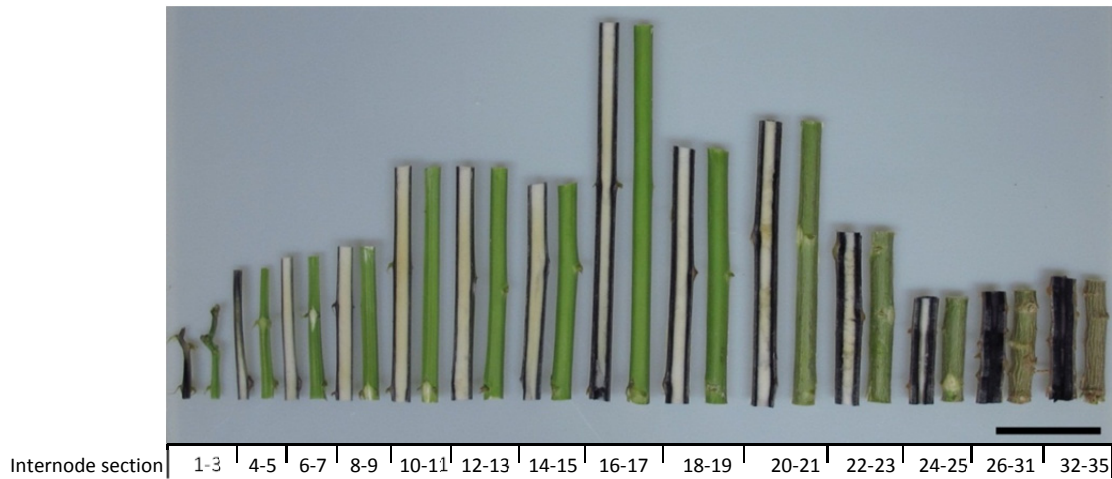
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To investigate the extent of leaf starch accumulation during the day and degradation in the subsequent night, leaf tissue was harvested at the EoD and EoN. These measurements give insight into the extent of diurnal, transitory starch turnover in a 14h light regime. For these measurements leaves from plants 'carbohydrate Exp2' were analysed (Figure 3.2). Higher starch levels were observed at the EoD, with the highest value of  $20.01 \pm 3.78$  mg glu eqv.  $g^{-1}$  FW in L4 and the lowest in L1 ( $8.03 \pm 1.89$  mg glu  $g^{-1}$  FW). As in the previous experiment, starch levels in the older leaves (L7, L10 and L20) were comparable to each other and lower than in L4. At the end of the night between 80.1 % (L1) and 52.3 % (L7) of the transient starch level was degraded. Interestingly, the levels for soluble sugars (sucrose, glucose and fructose) were unchanged at EoD and EoN (Figure 3.6 F-H). These data are consistent with the idea that leaf starch is degraded to maintain sugar levels during the dark.

Lower amounts of starch at the EoN than at the end of the previous day suggests that in cassava leaves, diurnal starch metabolism is occurring in a similar way as has been described in the model plant *Arabidopsis* (Gibon et al., 2004; Fulton et al., 2008). In *Arabidopsis*, transient leaf starch is degraded during the night by a cascade of enzymes (see Chapter 1.6). In the main transient starch degradation pathway,  $\beta$ -amylases are involved, producing maltose as an intermediate metabolite (Niittyla et al., 2004; Weise et al., 2004). The comparison of maltose levels at the EoD and EoN showed increased maltose levels at the EoN in all leaves except L1, where maltose levels remained unchanged. (Figure 3.6 I) This finding suggests that in cassava leaves, maltose is an intermediate metabolite in transitory starch metabolism, as in *Arabidopsis*.

### **3.4.2. Carbohydrates in cassava stem at different developmental stages and time points**

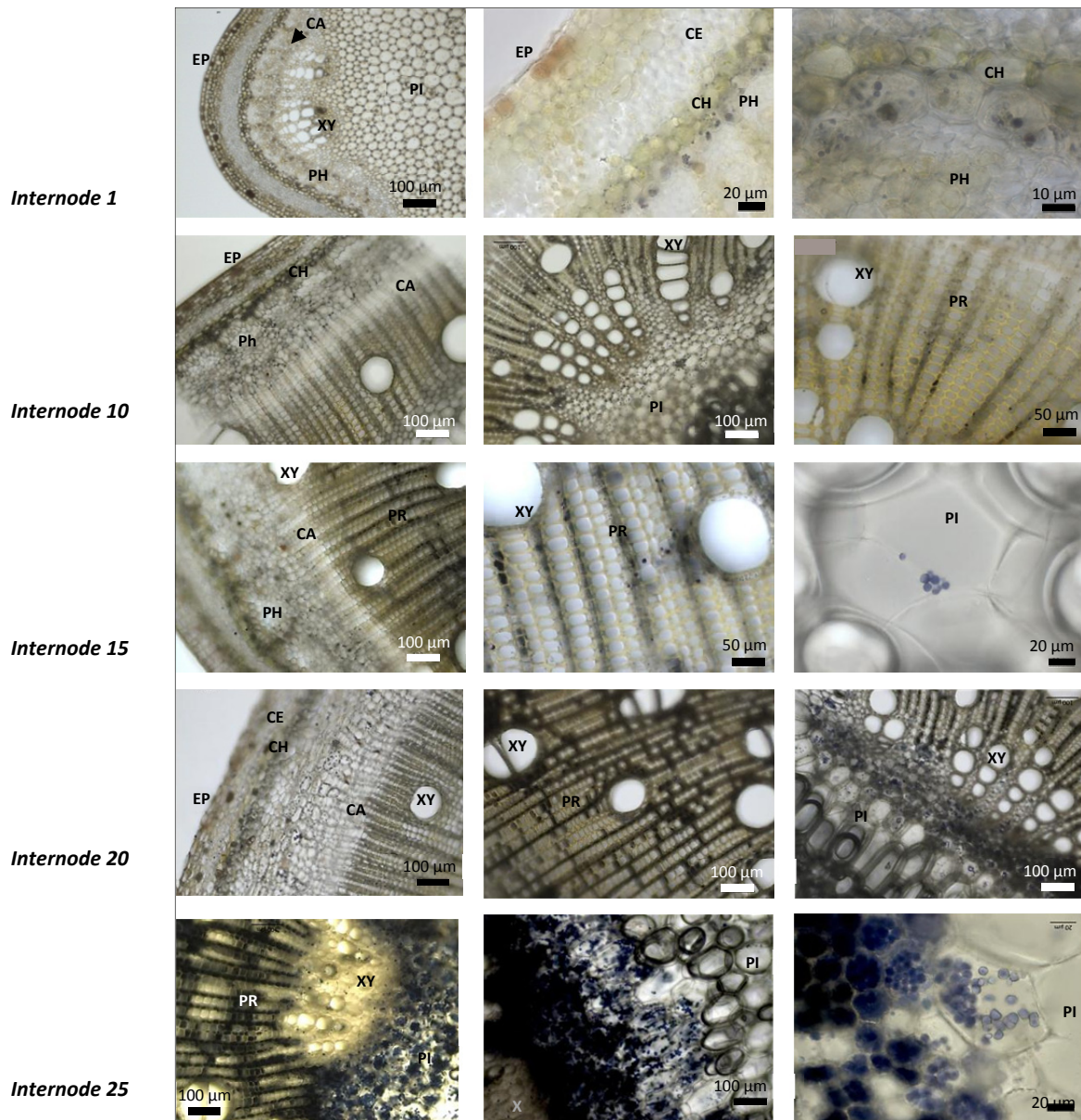
In daily life cassava is propagated by stem cuttings from mother plants. The older part of the stem is cut into 15-30 cm long sections and planted vertically to soil. This is because this lower part is full of nutrients needed to fuel re-growth of new leaves and stem (El-Sharkawy, 2004).



**Figure 3.7 Starch visualization of cassava stem with iodine staining**

Iodine staining of bisected part and outer stem part are shown. Below the stem sections the respective numbering is indicated. Scale bar = 5cm

I stained the laterally bisected stem with Lugol to reveal a positive correlation between tissue age and starch accumulation. This is in accordance with the stem being a storage organ. Starch accumulates mainly in the pith ray at the outermost layers of the stem. In the younger stem tissue, the middle pith remains unstained, whereas in older tissue, the staining is observed in all layers (Figure 3.7). Analysis of differently aged transverse stem sections under the light microscope (Int1, Int10, Int15, Int20, and Int25) confirmed the preliminary finding of starch accumulation in different cell layers. In the youngest part of the stem (Int1), starch granules are formed in the chlorenchyma (Figure 3.8). In internode 10, starch granules appear in the cells around the phloem, and start to accumulate at the margins of the pith. From Int10 to Int15, radial growth from the cambium is observed. The cell layer containing the pith ray and xylem are enlarged and starch granules start to accumulate in pith ray close to the cambium, and eventually also in the pith cells (Figure 3.8). Within the pith ray and the central pith layer, starch granules increase in number and volume.



**Figure 3.8 Starch visualization of transversal cassava stem section**

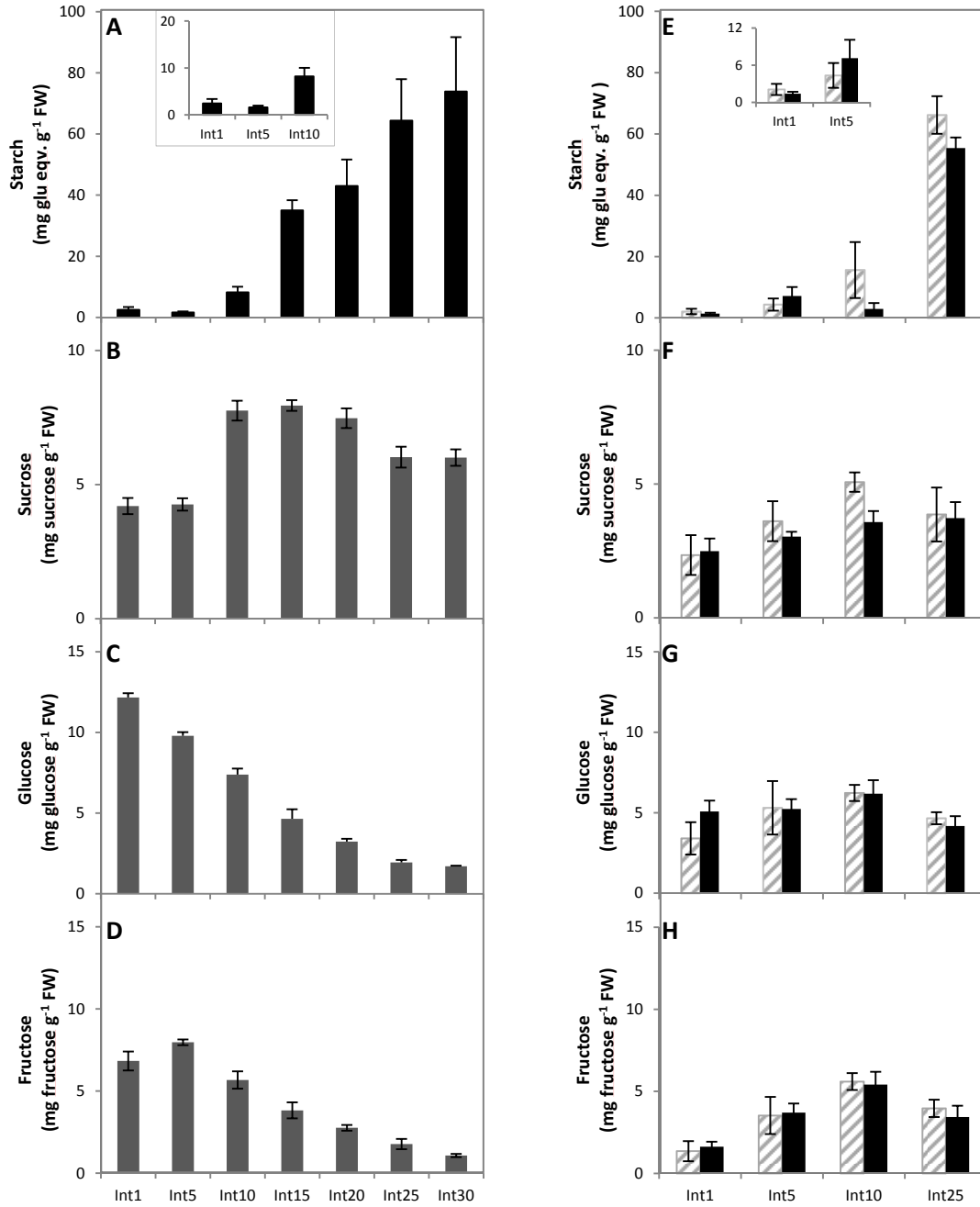
Light microscopy pictures at different developmental stages. EP= epidermis; PH= Phloem; XY = Xylem; PI = Pith; CA = Cambium; CE = Collenchyma; CH = Chlorenchyma; PR = Pith ray. Scale bar as indicated.

The accumulation of starch in the older part of the stem was also shown quantitatively; every 5<sup>th</sup> internode section (counting from the top to the bottom) was harvested from 5 replicate cassava plants. For these measurements stem sections of plant batch 'carbohydrate Expt1' (Figure 3.2) were analysed. I observed that the starch content increased with the age of the tissue (Figure 3.9 A). Starch levels below 10 mg glu eqv. g<sup>-1</sup> FW were detected in Int1, Int5 and Int10. From Int15 down to Int30, close to the sprouting initiation site, starch levels doubled from 35.05 ± 3.3 mg to 73.93 ± 17.79 mg glu eqv. g<sup>-1</sup> FW (Figure 3.9 A).

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Sucrose levels showed quite similar levels throughout the analysed samples, ranging from  $4.2 \pm 0.3$  mg to  $7.95 \pm 0.2$  mg suc  $g^{-1}$  FW. Int1 and Int5 had the lowest levels of sucrose. Compared to the younger stem part sucrose levels were higher in the middle of the stem (Int10 to Int20) and the oldest stem sections (Int25 and Int30) (Figure 3.9 B). The levels of glucose and fructose behaved in a reciprocal manner compared to starch accumulation. These soluble sugars were considerably lower in the older stem tissue. For example, glucose levels were 87% lower in Int30 compared with Int1. Fructose levels, like those of glucose were highest in the youngest part of the stem (Figure 3.9 C-D).

Analysis of insoluble and soluble carbohydrate accumulation at the end of the day and at the end of the night, measured from stem sections of plant batch 'carbohydrate Expt2' (Figure 3.2), revealed little or no diurnal fluctuation in stem carbohydrate levels. Diurnal starch turnover was not observed in investigated parts of the stem (Figure 3.9 E-H). The values for starch at the end of the day were comparable to the values measured in samples harvested after the following dark period. Except for Int25 where a difference in starch levels at the indicated time points was determined though below significance ( $t$ -test  $> 0.05$ ) (Figure 3.9 E). No major differences were observed between the end of the day and the end of the night for sucrose, glucose and fructose (Figure 3.9 F-G). Only in Int10 were sucrose levels 30% lower at the end of the night than at the end of the day. Glucose and fructose levels showed a similar pattern of accumulation to each other; levels increased from Int1 to Int10 and were lower again in Int25 (Figure 3.9 G-H). In comparison to the measurements on plant batch 'carbohydrate Expt1', lower soluble sugar levels were determined. For the two hexoses, glucose and fructose the levels differed between 2 and 80% in younger internode sections compared to the previous measurements. In contrast for Int25 the analysed hexoses were 60% higher. The changes in sucrose levels were between 16-35% lower compared to the 'carbohydrate Expt1'.



**Figure 3.9 Analysis of insoluble (starch) and soluble (sucrose, glucose, and fructose) carbohydrate allocation in stem at different developmental stages. A-D) harvested at the 9h into the light period and E-H) harvested at the end of the day (grey, stripe bar) and end of the night (black bar) A, E) starch, B, F) sucrose, C, G) glucose, D, H) fructose. Int1= youngest internode section, on the top Int25 = oldest internode section at the bottom. Mean  $\pm$  SE, N=5, 4, 3.**



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### **3.5. Conclusions drawn from the analysis of carbohydrate assimilation in cassava plants**

#### **3.5.1. Analysis of plant batches used for subsequent experiments**

In this chapter I showed that small differences in height, number of internodes and leaves were observed between the batches of plants used for my individual experiments (Figure 3.2). However, considering that the plants were grown from cuttings of different mother plants and grown under semi-controlled greenhouse conditions at different times of year, the batches used for experiments were broadly homogenous. The number of internodes is a good parameter to define the plants developmental stage and showed the least variation. The variation of leaf number between the batches can be explained by the fact that in cassava, the oldest leaves undergo senescence and are eventually shed (Alves, 2002). Nevertheless, for most of the plants, the number of leaves, plant height and the number of internodes was the same. Even in the batches where one parameter varied (e.g. plant height in the batch for the photosynthesis experiment) the other two parameters were still similar to other batches. Therefore, it is reasonable to conclude that the developmental stage of the batches was similar and that the results from the different experiments can be compared with each other.

#### **3.5.2. Capacity of cassava plants to perform photosynthesis and carbon assimilation**

The photosynthetic rate measurements accurately quantified the influence of light intensity on the carbon assimilation rate in my material (Figure 3.4 A). The light intensity inevitably varied in my plants, depending on the season, although this was augmented to some extent by supplementary lighting in the glasshouses. The maximal photosynthetic rates reported here are lower than was found in other studies (Edwards et al., 1990; Angelov et al., 1993; Calatayud et al., 2000). This might be because my measurements were gained on greenhouse-grown plants and/or because the variety used (cv. 60444) is rather a model than an agricultural cultivar. A clear negative correlation between leaf age and the capacity to assimilate carbon could also be shown (Figure 3.4 B). This was also observed in field grown plants (Angelov et al., 1993). The collected photosynthetic rate data are important as they allowed me to calculate the potential carbon assimilation, which can be compared with the absolute measurements of non-structural carbohydrates in the leaves. For example, comparing the theoretical assimilation of carbon with the starch accumulation during a day suggests that young, fully expanded leaves partitioned only 5-11% of assimilated carbon into starch. In the oldest leaf (L20) as much of 22% goes into starch. However, these values are somewhat speculative as light intensity is likely to have varied throughout the day and throughout the leaf canopy.

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All leaves degraded their starch to some extent during the night, although some degraded more than others, and most contained residual starch at the end of the dark period (Figure 3.6 E). This is in contrast to findings in the model plant *Arabidopsis*, which partitions a higher proportion of its assimilates into starch and degrades almost all of it at night. Nevertheless, as in *Arabidopsis*, I observed increased maltose levels at the end of the night (Figure 3.6 I) suggesting that cassava leaves metabolise their starch via a comparable pathway.

Cassava differs from *Arabidopsis* in another important way, accumulating about 10 times more sucrose (Gibon et al., 2004), together with high levels of glucose and fructose. In fact, the levels of total soluble carbohydrates were comparable to the amount of starch in the leaves. It is likely that much of this sugar is stored in the vacuoles of the palisade and sponge mesophyll cells. Interestingly, the levels of these sugars were almost unchanged at the end of the night, suggesting that starch is degraded to support night-time metabolism and maintain sugar levels (Figure 3.9 E-H).

Total non-structural carbohydrate levels were lowest in the oldest leaves (i.e. those close to senescence and with low photosynthetic rates) and the very youngest leaves (i.e. those that are still actively growing; Figure 3.6). The highest levels were observed in the fully expanded leaves at the top of the canopy (L4 and L7; Figure 3.6), which can be partly explained by their location (exposed to more light) and their high photosynthetic rate. Moving down the stem, the older leaves are more prone to being shaded by the upper canopy (Figure 3.1) and display lower photosynthetic rates, possibly as the process of senescence begins and the photosynthetic machinery is degraded (Figure 3.4 B). These findings are in agreement with the data shown by Angelov et al. (1993) where a similar trend of carbohydrate levels in different aged leaves was found, although the absolute values they observed were higher than those reported here. Again, these differences in absolute amounts could be explained by the growth conditions and the model cultivar used in our studies.

Carbohydrate levels measured for the developmental stages and diurnal fluctuations revealed differences in absolute values and pattern in respect of tissue age. Especially soluble sugars were lower in the diurnal analysis compared to the levels seen for the developmental stages. Depending on the analysed tissue and the age variation was up to 80% i.e. in stem sections Int1 at the end of the day compared to middle of the day (Figure 3.9). A reason for these substantial differences might be explained by differences in light conditions in the greenhouse and the difference in plant material (carbohydrate Expt1 and Expt2, Figure 3.2). Another reason for the observed differences might be explained by a diurnal fluctuation of carbohydrates throughout the day night cycle. Here I present three time points thus, it might be that soluble sugars throughout the light period are accumulated at midday and decrease towards the end of the day. To test this hypothesis a 24-h harvesting

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experiment could be performed. However, the experimental-setup I present here was designed in such a way that the results can be considered to be valuable.

Fully-expanded leaves serve as source tissue delivering carbohydrates to sink tissues. Export is probably highest during the day in cassava, but might continue during the night. However, if the calculated partitioning into starch is correct, it suggests that only a small fraction of the day-time assimilates is stored for night-time metabolism. Clearly, the developing root and shoot apices represent strong sinks, as does the starch-accumulating storage root. However, my data also show that starch accumulates in the stem – particularly the older parts (Figure 3.9 A) – to very high levels (up to 74 mg glu eqv. g<sup>-1</sup> FW). This suggests that the stem is also a strong sink tissue. The stem starch does not appear to follow the diurnal fluctuations observed for transitory starch in leaves, and can be considered as a storage starch pool that accumulates over time in the pith (Figure 3.8). This starch probably serves an important role during the propagation of cassava from stem cuttings, fuelling regrowth of new roots and shoots.

Further evaluation of the pattern and amounts of carbon partitioned between storage in the leaf and export to the various sinks would be valuable. This could be evaluated by feeding cassava leaves with <sup>14</sup>C-labelled CO<sub>2</sub>. After supplying a pulse of <sup>14</sup>CO<sub>2</sub>, the amount of <sup>14</sup>C label in the insoluble or soluble carbohydrate fractions in different plant parts could be determined during a chase period. Performing such experiments for differently aged leaves could reveal also where carbon, fixed in different parts of the canopy are primarily exported to. In *Arabidopsis* and other species, it was shown that the phloem network between leaves is established in with a defined way, related to the phyllotaxis of the plant. Thus, sucrose exported from a specific source leaf ends up in sink leaves defined by the vascular connections (Busse and Evert, 1999).

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## 4. Carbohydrate metabolism in Cassava storage roots after induction of sink-to-source transition

### 4.1. Introduction

As described in the General Introduction, transitory leaf starch metabolism has already been studied in detail and well-described in the literature (Smith and Stitt, 2007; Streb and Zeeman, 2012). In contrast, storage starch metabolism in heterotrophic tissues - especially starch remobilization - is less well understood. Early biochemical studies dealing with starch remobilization in cereal grains revealed major differences between the degradation pathways of transitory and storage starch. For example, whereas the breakdown of transitory starch in *Arabidopsis* leaves is highly dependent on the hydrolytic activity of  $\beta$ -amylases, storage starch remobilization in the cereal endosperm is mainly catalysed by  $\alpha$ -amylases (Fincher, 1989). Although other starch metabolizing enzymes are found to be present in the endosperm of germinating seeds (i.e. LDA,  $\alpha$ -glucosidase,  $\beta$ -amylase) a large increase in  $\alpha$ -amylase production is observed upon germination. This led to the general acceptance that cereal starch mobilization is initiated by  $\alpha$ -amylolytic hydrolysis. On the other hand, in potato tubers evidence was presented showing that  $\beta$ -amylases may be the main hydrolytic enzymes, responsible in remobilizing starch (Nielsen et al., 1997; Viola et al., 2007). It is well known that potato tubers stored at cold temperatures for several days increase break down some of their starch and accumulate soluble sugars, a process called cold-sweetening. Studies to investigate the molecular mechanism and discover the enzymes involved revealed a correlation between increased  $\beta$ -amylase activity and cold-sweetening. For white clover, it was also shown that starch mobilization upon defoliation leads to increase in soluble sugars and decrease in starch content in the storage roots. This was shown to correlate with an increase in  $\alpha$ -amylase activity (Gallagher et al., 1997). Other studies showed that  $\alpha$ -amylase activity can be isolated from starch granules of poplar wood ray cells and potato tubers (Witt and Sauter, 1994; Witt and Sauter, 1996). Although no detail about the functional involvement of these hydrolytic enzymes in heterotrophic starch metabolism are provided, the results give indications that starch remobilization differs from what is described in leaf tissue.

The aim of this study was to identify key proteins involved in the remobilization of starch in cassava storage roots using a proteomics approach. Therefore a simple method was developed to shift the metabolism in cassava roots from starch synthesis to degradation - or in other words to induce a sink to source transition. In potato tubers, studies involving sink-source transition are performed on tubers detached from the mother plants. By doing so, bud dormancy is released and new sprouts are made (Viola et al., 2007). In this process, starch is remobilized and sucrose is made and transported

to the bud, indicating that new sink is created. Unlike potato tubers, which derive from modified stems, cassava roots do not sprout once detached from the mother plant. In contrast, detached cassava roots undergo a rapid post-harvest physiological deterioration (PPD) within 24-48h (Sanchez et al., 2006). The first step is black discoloration of the vascular parenchyma and later of the storage parenchyma. This is followed by a secondary PPD, attributed to microbial infection. Thus, to study starch remobilization in cassava storage roots, another experimental setup was needed to provoke the transition from sink-source in cassava roots. Studies aiming to delay PPD showed that pruning before the harvest minimises deterioration. Additionally, it was shown that starch content of storage roots decreased near linearly up to 26 days, after which starch starts to accumulate again (van Oirschot et al., 2000). Thus, pruning seems to be a valuable method to study starch remobilization in cassava storage roots, and was used here.

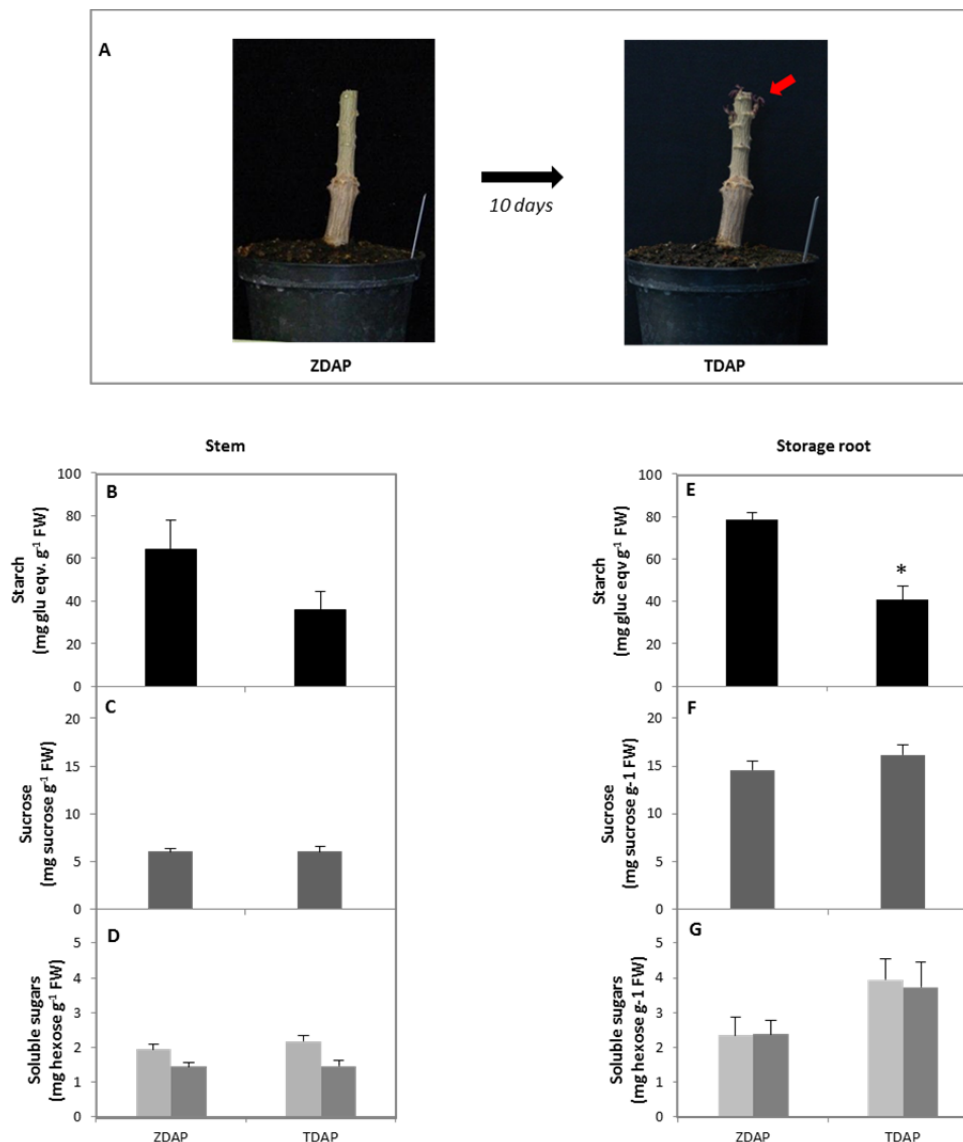
In the past decades, mass spectrometry-based proteomics has become an important method to monitor metabolic network components at a system-wide level (Domon and Aebersold, 2010; Schulze and Usadel, 2010). Nevertheless, peptide and therefore protein identification depends upon available genome sequence information. Sequencing of the cassava genome began 2003 and resulting EST sequences were used for pioneer transcriptomics and proteomics studies (Lopez et al., 2004; Sheffield et al., 2006; Reilly et al., 2007; Li et al., 2010; Mitprasat et al., 2011; Owiti et al., 2011). Proteomics studies based on 1 or 2 dimensional gel electrophoresis analysed several cassava tissues including fibrous and storage root (Sheffield et al., 2006), secondary somatic embryos (Baba et al., 2008), leaves at different growth stages (Mitprasat et al., 2011) and storage roots subjected to post-harvest deterioration (Owiti et al., 2011). These studies successfully identified up to 1110 proteins in storage roots by combining available EST sequences with bioinformatics approaches to optimize protein identification in non-sequenced organisms (Owiti et al., 2011). Recently, the first annotated draft of the cassava genome, predicted to contain 30666 protein-coding loci, was released ([ftp://ftp.jgi-psf.org/pub/JGI\\_data/phytozome/v5.0/Mesculenta](ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta); reviewed in Prochnik et al., 2012) thus paving the way for the application of large-scale shotgun proteomics.

Various methods have been developed to provide absolute or relative quantitative information for identified proteins (Domon and Aebersold, 2010; Schulze and Usadel, 2010). First, protein abundances can be determined prior to analysis by peptide isobaric labelling, during acquisition by multiple reaction monitoring or in a post-acquisition manner by label-free strategies (Vaudel et al., 2010; Owiti et al., 2011). Second, it has been shown that the number of identified peptides is to some extent proportional to the protein abundance, enabling protein quantification by label-free spectra counting (Lu et al., 2007). Third, softwares such as SuperHirn or Progenesis LC-MS

#### 4. Metabolic shift in cassava storage root from sink-to-source

have been developed to determine peptide and protein abundances according to the intensity of eluting ions (Azimzadeh et al., 2012; Fischer et al., 2012; Greer et al., 2012). Implementation of statistical tests in Progenesis LC-MS has proven to be excellently suited for obtaining sensitive and robust quantification of proteomics data (Azimzadeh et al., 2012; Fischer et al., 2012; Greer et al., 2012), and this approach was used here to identify proteins involved in the remobilization of starch in cassava storage roots.

#### 4.2. Remobilization of carbohydrates from storage organs



**Figure 4.1 Pruning of cassava plants and the influence on the carbohydrates**

A) Cassava plants were pruned and storage roots harvested immediately (ZDP) or ten days after pruning (TDP). Within this time-period a new shoot appeared (red arrow); Greenhouse, 14h light. A-D) Carbohydrate allocation in stem at the cutting side ZDP and TDP. E-G) Carbohydrate allocation in cassava storage roots ZDP and TDP, A,E) starch, C,F) sucrose and D,G) soluble sugar level glucose (dark grey), fructose (light grey). Values are mean  $\pm$ SE (N=5), \*t-test < 0.01



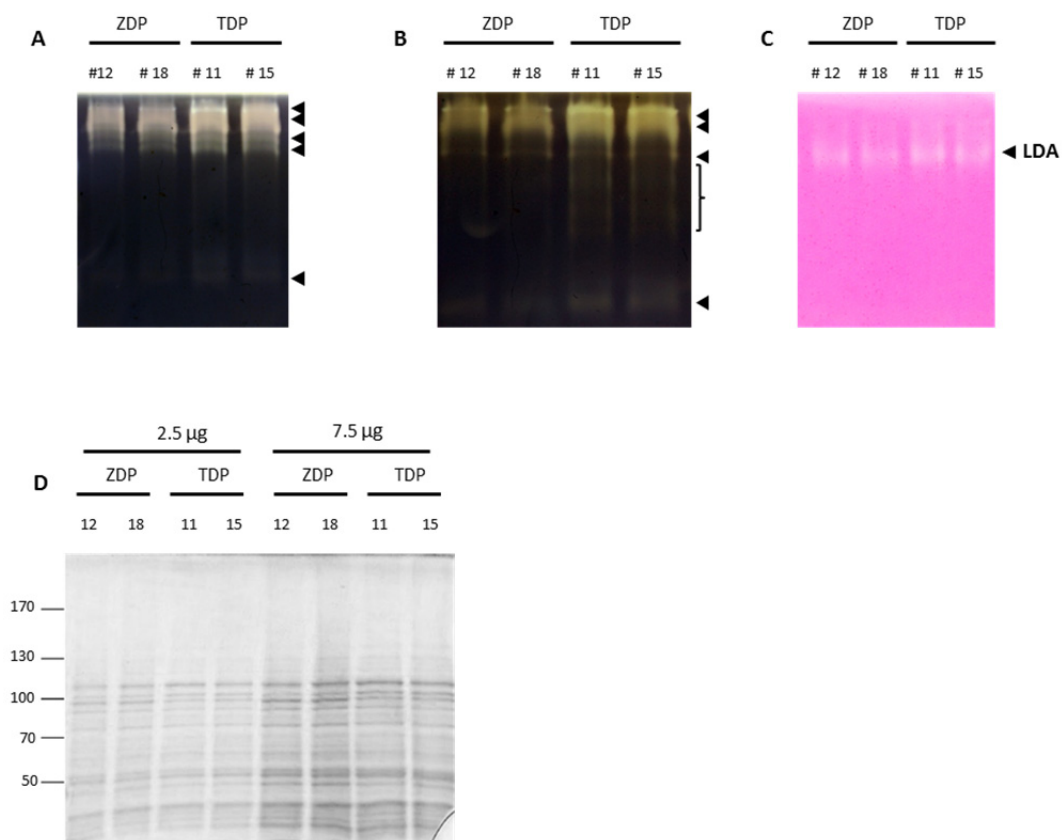
To investigate starch catabolism in cassava storage roots, I analysed changes in carbohydrate levels and amylolytic activity upon cutting off the photosynthetic above-ground tissues. The storage roots of greenhouse-grown cassava plants (plant batch 'Proteome', Figure 3.2) were harvested either uncut as a control (Control; zero days of pruning, ZDP) or 10 days after cutting off the above-ground photosynthetic part (ten days of pruning, TDP) (Figure 4.1 A). The emergence of new shoots from internodes could be observed within this time period, but the leaves were tiny and not yet photosynthetic source tissues (Figure 4.1 A).

To investigate the remobilization of carbohydrates from both stem and root storage tissues, levels of starch, sucrose, glucose, and fructose were determined at both time points. Starch levels in stem tissue (immediately below the cutting side) and storage roots were decreased by 44.6% and 48.1%, respectively (Figure 4.1 B, E). The level of sugars remained unchanged for the two time points in both tissues (Figure 4.1 C, D, F, G).

activities cannot be determined unambiguously. Ceballos et al. (2008) performs similar analyses and suggested that ISA and SBE activities migrate at the top of the gel (Figure 4.2 A). However, isoforms of both  $\alpha$ - and  $\beta$ -amylases also run in this location in *Arabidopsis* leaf extracts. A faint activity band appears further down in the gel for the TDP protein samples, which may represent limit dextrinase (LDA) activity. The substrate  $\beta$ -limit dextrin (amylopectin digested with an excess of commercial  $\beta$ -amylases; Figure 4.2 B) cannot be digested by  $\beta$ -amylase. As seen on amylopectin, the enzymatic activities on  $\beta$ -limit dextrin were increased TDP in comparison to the control samples. Activity of the top two bands appeared the same as on amylopectin and were increased in TDP samples, as was the lowest band. Two minor bands visible on amylopectin were not visible on  $\beta$ -limit dextrin, suggesting that they may be  $\beta$ -amylases. The chromogenic substrate red-pullulan (partially depolymerised pullulan containing Procion MX-5B dye) is a specific substrate to reveal LDA activity (Figure 4.2 C). For LDA activity, 3 times more total protein was loaded per lane (7.5  $\mu$ g) compared to the native PAGE containing amylopectin and  $\beta$ -limit dextrin. The activity of LDA on red-pullulan also appeared to be increased in the TDP samples compared to ZDP control samples (Figure 4.2 C). With the exception of LDA activity, these analyses do not reveal clearly which hydrolytic enzymes are involved in the process of starch degradation upon the sink-source-transition.

#### 4. Metabolic shift in cassava storage root from sink-to-source

I was interested if the decrease in starch levels is caused by a changed hydrolytic activity of starch degrading enzymes. Therefore, protein extracts from two replicate cut and control storage roots were analysed by native PAGE containing 0.1% amylopectin, 0.1%  $\beta$ -limit dextrin or 1% red pullulan (Figure 4.2 A-C). Using amylopectin as a substrate reveals starch hydrolysing/modifying enzyme activity. Activity bands appeared at the top of the native PAGE with a markedly increased activity in storage root extract after cutting compared to the control (Figure 4.2 A). The identity of these The decrease in starch levels and the increase in amylolytic activity show that storage starch is mobilized in stems and storage roots for the production of new leaf tissue. Therefore, this treatment appears as a suitable system in which to further analyse the switch from a sink to a source tissue.

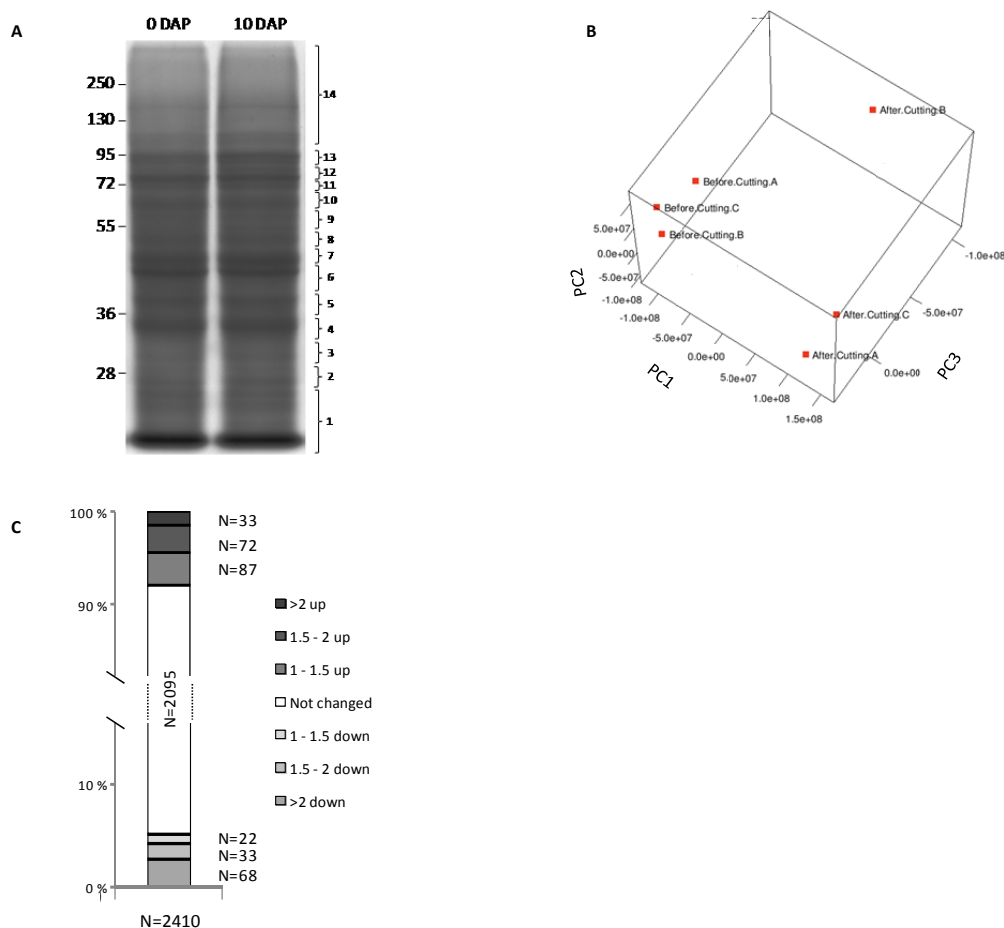


**Figure 4.2 Amylolytic enzyme activity PAGE**

Total storage root protein extract from ZDP and TDP were subjected on a 6% native PAGE containing A) 0.1% potato amylopectin, B) 0.1%  $\beta$ -limit dextrin and C) 1% red pullulan. Protein loading was 2.5  $\mu$ g for A and B, and 7.5  $\mu$ g for C. D) 6% SDS-PAGE stained with co-omassie for protein loading control, either 2.5  $\mu$ g or 7.5  $\mu$ g per lane.

### 4.3. Cassava storage root proteome comparison: Unravelling enzymes involved in carbohydrate metabolism in storage roots before and after pruning

A large-scale proteome study of storage roots was performed and conducted by Dr. Sylvain Bischof, primarily to identify important proteins involved in storage starch metabolism. Therefore, proteins from storage roots harvested before (ZDP) and after pruning (TDP) were extracted and equivalent concentrations of total proteins were separated by 1-dimensional SDS-PAGE (Figure 4.3 A). Each lane was cut into 14 fractions to decrease the sample complexity, thereby increasing the probability to identify individual proteins. Proteins in each fraction were in-gel digested with trypsin and analyzed by mass spectrometry (MS) using an Orbitrap mass spectrometer. Three biological replicates were analysed for each time point. Measured spectra were identified using the cassava genome database ([www.phytozome.net](http://www.phytozome.net)) and quantitative information for each identified peptide and protein was obtained using the software Progenesis LC-MS.



**Figure 4.3 Evaluation of proteomics data set**

A) Total protein separated on a SDS-PAGE for proteomics and the slices subjected for digestion B) Correlation analysis between the biological replicate represented in a PCA plot. Before Cutting = ZDP; After Cutting = TDP C) Total number of proteins indicating the significantly up- and down-regulated as a percentage in respect of fold changes. Data obtained by Dr. Sylvain Bischof, ETH Zürich.

In total, 20177 peptides were identified across all six datasets analysed. Peptide identification was accepted with a minimal Mascot ion score of 23 and a maximum expectation value of  $\leq 0.05$ . To increase protein identification confidence, a minimum of two unique peptides for each protein was required. Proteins identified with only a single peptide hit were excluded. This led to a total of 2410 proteins identified with a high confidence (Supplemental Table 4.1). Of these, 2409 proteins were found in both time points while only one protein (cassava4.1\_005818m; homologue to Arabidopsis CYTOCHROME P450 86 A1) was found only at ZDP (Supplemental Table 4.1).

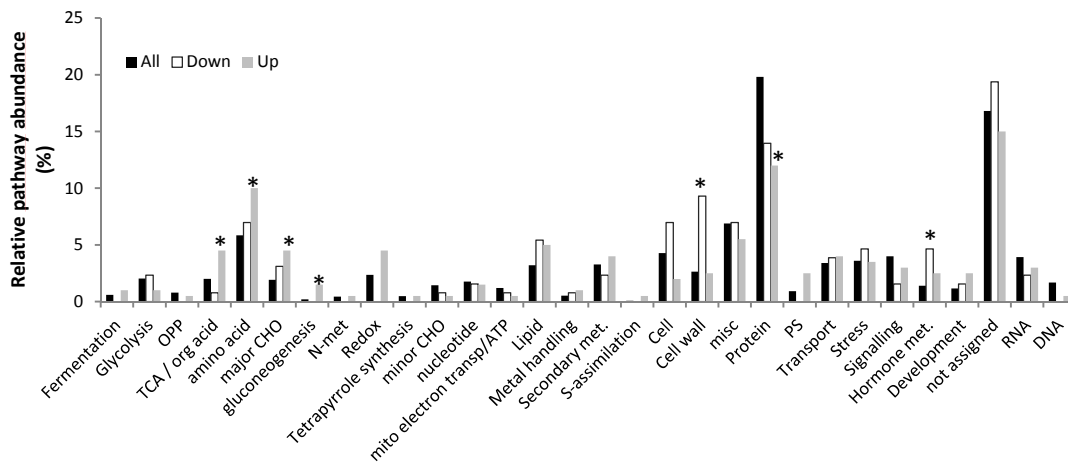
To investigate homogeneity among the biological replicates, a principle component analysis (PCA) was performed. PCA analysis revealed a clear separation of the biological replications of “Before Cutting” (ZDP) and “After Cutting” (TDP) (Figure 4.3 B). In PC1, which contributes 53% of the variance, clearly separates the six replicates according the treatment “Before cutting” and “After cutting”. While the replicates for “Before Cutting” cluster together for PC2 (20.9%) and PC3 (11.6%) “After Cutting” B separates from the two other replicates for PC3 (Figure 4.3 B). However, the clear separation between the treated and untreated samples indicates clear protein regulation.

To identify proteins significantly differently expressed after pruning (compared to the untreated control), an analysis of variance (ANOVA) test was performed (Supplemental Table 4.2). To reveal proteins that were de-regulated (up or down) after pruning, the mean average of protein abundance TDP was compared to mean average of protein abundance of the untreated control samples. This comparison revealed a total of 315 proteins with ANOVA p-values  $\leq 0.05$  (Figure 4.3 D). Investigations of the fold change of the deregulated proteins revealed that 127 proteins were up-regulated, 105 and 33 of which were at least 1.5-fold or 2-fold higher, respectively. One hundred and eighty proteins were down-regulated, 101 and 68 of which were at least 1.5-fold or 2-fold lower, respectively. (Figure 4.3 D; Supplemental Table 4.2).

Next, I used the publicly-available functional annotations of *Arabidopsis thaliana* proteins to identify the cassava proteins (see Material and Methods for details). Several Arabidopsis proteins had more than one close cassava protein homolog suggesting that DNA duplications have resulted in multiple gene copies in the cassava genome. Sixteen out of 2410 cassava proteins could not be assigned to any Arabidopsis homolog, suggesting that they are specific to cassava (Supplemental Table 4.1).

To gain insight into the metabolic processes affected by pruning, I categorized the 2394 genes according to their metabolic functions using the MapMan software (Supplemental Table 4.1; mapman.mpimp-golm.mpg.de; Usadel et al., 2009). As some proteins are involved in more than one metabolic pathway, several cassava homologs were found to be allocated to two or more functional

categories. In order to identify processes significantly altered by pruning, the proportion of deregulated proteins in each category was compared with the proportion of total proteins in the same category. After pruning (TDP), proteins belonging to the functional categories ‘cell wall’ and ‘hormone metabolism’ were significantly under-represented compared to the control (ZDP; Figure 4.4 A). In contrast, proteins assigned to the categories ‘major CHO metabolism’, ‘TCA/Organic acid transformation’, ‘amino acid metabolism’, and ‘protein’ were significantly over-represented after pruning (Figure 4.4 A).



**Figure 4.4 Evaluation of the MapMan categorization**

The relative pathway abundance was calculated by the number of features assigned to a MapMan category in relation to the total number of features found over all categories (Black bar). White bar reflect the relative abundance of significantly up-regulated features TDP to the total number of features significantly up-regulated TDP. Grey bar reflect the relative abundance of significantly down-regulated features TDP to the total number of features significantly down-regulated TDP. The probability of features found to be over- or underrepresented within one category was determined with a Hypergeometric distribution test. \* p-value  $\leq 0.01$

### 4.3.1. Investigation of metabolic pathway changes after pruning

#### 4.3.1.1. Changes in the abundance of proteins involved in primary carbon metabolism

The transition of cassava root from sink-to-source is connected to significant changes in starch and sucrose metabolism. This is not surprising as storage roots, as a sink tissue, relies on carbohydrates (sucrose) transported from the photosynthetic source tissues. The sucrose is unloaded from the phloem and can either be cleaved by invertase (into fructose and glucose) or by sucrose synthase (SUS; which converts UDP and sucrose into fructose and UDP-glucose). In subsequent steps the generated hexoses are metabolised further giving substrates for diverse metabolic pathways (e.g.

respiration), or they are transported to the vacuole for storage, or transported into the amyloplast and converted into starch. In heterotrophic tissues that have switched into a source tissue, some of these reactions are reversed. For example, starch is degraded and the products are used to synthesise sucrose, which then is exported to new sink tissue via phloem transport. Sucrose is synthesised in the cytoplasm from F6P and UDP-glucose by consecutive activities of sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase (SPP). To elucidate differences in storage root primary carbohydrate metabolism in untreated (sink) and pruned (source) roots, I investigated the protein levels for enzymes involved in starch and sucrose metabolism in more detail.

#### *Starch metabolism*

In *Arabidopsis thaliana*, 53 proteins are known to be directly or indirectly involved in starch metabolism (Streb and Zeeman, 2012). The obtained cassava proteome data were mined for cassava homologs of Arabidopsis proteins involved in starch metabolism. A total of 31 cassava proteins were found (Table 4.1). For the other 22 proteins assigned to the starch metabolism in Arabidopsis no peptides corresponding to a cassava homolog were identified. Of the 31 proteins identified, six pairs were each annotated to the same Arabidopsis protein. A closer investigation of the cassava genome revealed that these cassava homologs are duplicate. Hence a total of 25 starch related proteins were found in this proteome analysis. The duplicates were indicated with a prefix “a” or “b”. The case of LDA was an exception; a closer analysis showed that the two cassava sequences (cassava4.1\_004771; cassava4.1\_024672) lie next to each other on the same scaffold. An alignment of these two peptide sequences to the AtLDA as a query revealed sequence homology of either the N- or C-terminus without common sequence overlaps. Therefore, the two samples were taken together. From these 25 proteins 8 were identified to be involved in starch synthesis and 15 in degradation, (Table 4.1; Streb et al., 2012).

Of the 30 proteins involved in starch metabolism, five were significantly ( $p\text{-value} = \leq 0.05$ ) deregulated in the TDP sample compared to the ZDP control (Table 4.1). Of these, three proteins were up- and two proteins were down-regulated.

Among the proteins induced by pruning was a protein containing a starch-binding domain and a coiled-coil domain (COC, cassava4.1\_012932) which was increased in abundance by 1.7 fold. The Arabidopsis homolog was shown to be a chloroplast-localized protein which binds to starch, preferentially to amylose (Lohmeier-Vogel et al., 2008). Furthermore, starch synthase 1 (SS1; cassava4.1\_004619) was significantly more abundant TDP (1.2-fold) compared to the ZDP control. However, the most striking change was a 6.3-fold increase in the plastidial endoamylase  $\alpha$ -amylase 3 (AMY3; cassava4.1\_001362). AMY3 is involved in the release of branched malto-oligosaccharides

from the starch granule. Analysis of down-regulated proteins in TDP samples revealed APL3 (a large-subunit of AGPase cassava4.1\_005409) and PWD (phosphoglucan water dikinase, cassava4.1\_000497) to be 2-fold and 1.3-fold less abundant, respectively. APL3 is specific for root tissue in Arabidopsis, while APL1 is more abundant in leaves (Crevillen et al., 2005). PWD (cassava4.1\_000497) plays a role in starch phosphorylation, acting on starch granules after pre-phosphorylation by GWD (Baunsgaard et al., 2005; Kotting et al., 2005).

The other proteins assigned to the starch synthesis pathway include the plastidial PGM1 isoform, three isoforms of starch synthases (SS2, cassava4.1\_002278; SS4, cassava4.1\_003800; and GBSSI, cassava4.1\_003884), two branching enzymes isoforms (SBE2-a cassava4.1\_003773; SBE2-b, cassava4.1\_001686; and SBE3, cassava4.1\_001595), and the debranching enzymes ISA1 and ISA2 (cassava4.1\_001932; cassava4.1\_001414). Amongst the proteins assigned to starch degradation were the debranching enzymes ISA3 (cassava4.1\_008945) and LDA (cassava4.1\_004771, cassava4.1\_024672), the phosphoglucan phosphatase SEX4 (cassava4.1\_009735) and its homolog LSF1 (cassava4.1\_025886). Both plastidial and cytosolic disproportionating enzymes, DPE1 (cassava4.1\_008552) and DPE2 (cassava4.1\_001086), which recycle short maltooligosaccharides to release glucose were identified. Homologs of the plastidial and cytosolic  $\alpha$ -glucan phosphorylase (PHS1, cassava4.1\_004717, cassava4.1\_002614 and PHS2, cassava4.1\_001626, cassava4.1\_004717) were identified. PHS1 and PHS2 catalyze the reversible reaction of glucose-1-phosphate release from linear glucans (Steup and Schachtele, 1981; Shimomura et al., 1982). In addition, a plastidial hexose-phosphate translocater (GPT1, cassava4.1\_009268) and a glucose transporter (GlcT, cassava4.1\_004822) could be identified. GPT1 was described in maize and Arabidopsis heterotrophic tissues and transports glucose-6-phosphate across the amyloplast membrane (Kammerer et al., 1998; Andriotis et al., 2010), providing substrates for either starch synthesis or the plastidial oxidative pentose phosphate pathway. GlcT was shown to be important to export glucose from the Arabidopsis chloroplasts during the dark period (Cho et al., 2011).

#### 4. Metabolic shift in cassava storage root from sink-to-source

**Table 4.1 Starch related proteins** Up- or down-regulation of starch –related proteins TDP. Significant ANOVA p-value  $\leq 0.05$  are indicated in bold. Fold change is represented in respect of TDP (N=3).

| Enzyme | Cassava accession                                                       | Arabidopsis Accession | Fold change  | Anova (p)          |
|--------|-------------------------------------------------------------------------|-----------------------|--------------|--------------------|
| AMY3   | cassava4.1_001362m PACid:17970800                                       | AT1G69830             | 6.271784075  | <b>0.023873795</b> |
| SS1    | cassava4.1_004619m PACid:17988337                                       | AT5G24300             | 1.244623178  | <b>0.027163229</b> |
| COC    | cassava4.1_012932m PACid:17987585                                       | AT5G39790             | 1.769509262  | <b>0.045000512</b> |
| GBSSI  | cassava4.1_003884m PACid:17959989                                       | AT1G32900             | 1.766510779  | 0.059838338        |
| SS4    | cassava4.1_003800m PACid:17972839                                       | AT4G18240             | 1.388017137  | 0.06039722         |
| ADG1   | cassava4.1_005518m PACid:17993865                                       | AT5G48300             | 1.399319996  | 0.102984919        |
| SEX4   | cassava4.1_009735m PACid:17978895                                       | AT3G52180             | 2.142486305  | 0.135771612        |
| GlcT   | cassava4.1_004822m PACid:17987618                                       | AT5G16150             | 1.647159078  | 0.143239995        |
| PHS2_b | cassava4.1_002466m PACid:17978799                                       | AT3G46970             | 1.304470706  | 0.14731939         |
| PHS1_a | cassava4.1_002614m PACid:17978880                                       | AT3G29320             | 1.403512667  | 0.159453092        |
| LDA    | cassava4.1_004771m PACid:17981254/<br>cassava4.1_024672m PACid:17981243 | AT5G04360             | 1.431089875  | 0.170502706        |
| pGPT   | cassava4.1_009268m PACid:17980520                                       | AT5G54800             | 1.598831466  | 0.196091071        |
| SS2    | cassava4.1_002278m PACid:17978759                                       | AT3G01180             | 1.373885415  | 0.235049795        |
| ISA2   | cassava4.1_001414m PACid:17986210                                       | AT1G03310             | 1.361153605  | 0.253063848        |
| DPE2   | cassava4.1_001086m PACid:17969334                                       | AT2G40840             | 1.250485455  | 0.264003241        |
| SBE3   | cassava4.1_001595m PACid:17966954                                       | AT2G36390             | 1.319348939  | 0.273468576        |
| PHS1_b | cassava4.1_004717m PACid:17991231                                       | AT3G29320             | 1.420309134  | 0.275694189        |
| DPE1   | cassava4.1_008552m PACid:17989108                                       | AT5G64860             | 1.064269048  | 0.576997277        |
| LSF1   | cassava4.1_025886m PACid:17963084                                       | AT3G01510             | 1.206461149  | 0.694236214        |
| SBE2_a | cassava4.1_003773m PACid:17961099                                       | AT5G03650             | 1.111449633  | 0.702243606        |
| PGI_a  | cassava4.1_006414m PACid:17980631                                       | AT4G24620             | 1.052986579  | 0.721798105        |
| PGM2_a | cassava4.1_004332m PACid:17969721                                       | AT1G70730             | 1.124431419  | 0.762414575        |
| ISA1   | cassava4.1_001932m PACid:17965320                                       | AT2G39930             | 1.157479924  | 0.772568075        |
| PGM1   | cassava4.1_003452m PACid:17960838                                       | AT5G51820             | 1.033803977  | 0.831527628        |
| PHS2_a | cassava4.1_001626m PACid:17966328                                       | AT3G46970             | 1.021786578  | 0.912783717        |
| APL3   | cassava4.1_005409m PACid:17968441                                       | AT4G39210             | -2.088975338 | <b>0.011351981</b> |
| PWD    | cassava4.1_000497m PACid:17989907                                       | AT5G26570             | -1.321020787 | <b>0.026783934</b> |
| PGI_b  | cassava4.1_031246m PACid:17981861                                       | AT4G24620             | -1.049204492 | 0.575652002        |
| PGM2_b | cassava4.1_004336m PACid:17982342                                       | AT1G70730             | -1.03259972  | 0.739905026        |
| SBE2_b | cassava4.1_001686m PACid:17972441                                       | AT5G03650             | -1.02787995  | 0.83351531         |
| ISA3   | cassava4.1_008945m PACid:17990994                                       | AT4G09020             | -1.031881174 | 0.847019054        |



*Sucrose metabolism*

Amongst the significantly deregulated proteins were several enzymes involved in sucrose metabolism. A set of 100 proteins involved in sucrose metabolism and transport in *Arabidopsis* was used to identify homologs in cassava. I could assign 49 cassava proteins to *Arabidopsis* homologs (Table 4.2). After correcting for duplicated (or triplicated genes), a total of 34 *Arabidopsis* homologs were found in the cassava proteome (Table 4.2). Amongst the 49 proteins, four were significantly up-regulated and two significantly down-regulated ( $p\text{-value} \leq 0.05$ ) in TDP samples compared the ZDP control samples (Table 4.2). After pruning fructokinase (FK; cassava4.1\_011584) and cytosolic fructose-1,6-bisphosphatase (F1,6BPase; cassava4.1\_011197) (which produce fructose 6-phosphate from free fructose or from fructose 1,6-bisphosphate respectively) were down-regulated compared to the control. Mutant analysis in *Arabidopsis* showed that F1,6BPase is involved in fructose sensing and signalling, independent to the catalytic activity (Cho and Yoo, 2011). After pruning, there was significant up-regulation of plastidial fructose-1,6-bisphosphate aldolase (FBA3; cassava4.1\_009233), cytosolic invertase2 (cINV2; cassava4.1\_005201), cytosolic/nuclear hexokinase1 (HXK1; cassava4.1\_006138) and plastidial hexokinase3 (HXK3; cassava4.1\_007221). FBA3 converts fructose 1,6-bisphosphate into dihydroxyacetonephosphate (DHAP) and glyceraldehyde-3-phosphate (GAP; triose-phosphate). The metabolites DHAP and GAP are substrates for diverse metabolic pathways in different subcellular compartments (OPPP, glycolysis and TCA cycle, sucrose synthesis). The cINV2 hydrolyses sucrose into glucose and fructose. HXK1 and HXK3 phosphorylate various hexoses (e.g. fructose and glucose) (Claeyssen and Rivoal, 2007). As for F1,6BPase, HXK1 is involved in sugar-signalling in *Arabidopsis*, sensing glucose independently of its catalytic activity (Moore et al., 2003).

#### 4. Metabolic shift in cassava storage root from sink-to-source

**Table 4.2 Sucrose related proteins** Proteins involved in sucrose metabolism up- or down regulated TDP. . Significant ANOVA p-value  $\leq 0.05$  are indicated in bold. Fold change is represented in respect of TDP (N=3).

| Enzyme                   | Cassava accession                 | Arabidopsis Accession | Fold change  | Anova (p)          |
|--------------------------|-----------------------------------|-----------------------|--------------|--------------------|
| <b>FBA3</b>              | cassava4.1_009233m PACid:17991143 | AT2G01140             | 1.31375782   | <b>0.016611607</b> |
| <b>cINV2</b>             | cassava4.1_005201m PACid:17960338 | AT4G09510             | 1.279162588  | <b>0.035931392</b> |
| <b>HXK3</b>              | cassava4.1_007221m PACid:17990129 | AT1G47840             | 1.512944971  | <b>0.038045423</b> |
| <b>HXK1</b>              | cassava4.1_006138m PACid:17985703 | AT4G29130             | 1.559017046  | <b>0.039299266</b> |
| <b>cPGI</b>              | cassava4.1_004581m PACid:17978999 | AT5G42740             | 1.095756629  | 0.071848769        |
| <b>FK</b>                | cassava4.1_025703m PACid:17973403 | AT1G17160             | 1.843801599  | 0.083268321        |
| <b>FBA3</b>              | cassava4.1_009217m PACid:17976496 | AT2G01140             | 1.711619311  | 0.088076604        |
| <b>UGPase1</b>           | cassava4.1_003947m PACid:17969276 | AT3G03250             | 1.973510332  | 0.099628734        |
| <b>SUS3</b>              | cassava4.1_001840m PACid:17981623 | AT4G02280             | 1.601670806  | 0.108474525        |
| <b>PFK5</b>              | cassava4.1_005217m PACid:17974485 | AT2G22480             | 1.670368678  | 0.117662228        |
| <b>cTPI</b>              | cassava4.1_014503m PACid:17981974 | AT3G55440             | 1.722312487  | 0.124504536        |
| <b>cTPI</b>              | cassava4.1_014432m PACid:17967798 | AT3G55440             | 1.550469005  | 0.153717454        |
| <b>F1,6BPase</b>         | cassava4.1_008978m PACid:17990898 | AT5G64380             | 1.397492877  | 0.198494275        |
| <b>pTPI</b>              | cassava4.1_012016m PACid:17981009 | AT2G21170             | 1.375325681  | 0.206730912        |
| <b>FBA6</b>              | cassava4.1_010509m PACid:17974511 | AT2G36460             | 1.152940825  | 0.222164189        |
| <b>FBA6</b>              | cassava4.1_010502m PACid:17961042 | AT2G36460             | 1.275518191  | 0.243091463        |
| <b>cTPI</b>              | cassava4.1_014454m PACid:17988950 | AT3G55440             | 1.180865848  | 0.305726618        |
| <b>pTPI</b>              | cassava4.1_012057m PACid:17986071 | AT2G21170             | 1.237065937  | 0.344269378        |
| <b>SUS6</b>              | cassava4.1_027790m PACid:17979357 | AT1G73370             | 1.132328345  | 0.356569843        |
| <b>FK</b>                | cassava4.1_009589m PACid:17989580 | AT1G66430             | 1.059469231  | 0.51925422         |
| <b>SPSA1</b>             | cassava4.1_000732m PACid:17989703 | AT5G20280             | 1.146477123  | 0.538192248        |
| <b>SUS6</b>              | cassava4.1_001874m PACid:17972883 | AT1G73370             | 1.103336919  | 0.639252054        |
| <b>FK</b>                | cassava4.1_018514m PACid:17973431 | AT1G17160             | 1.003602549  | 0.680104111        |
| <b>pPGI</b>              | cassava4.1_006414m PACid:17980631 | AT4G24620             | 1.052986579  | 0.721798105        |
| <b>PFP</b>               | cassava4.1_003757m PACid:17962621 | AT1G20950             | 1.070408139  | 0.737585428        |
| <b>PGM2</b>              | cassava4.1_004332m PACid:17969721 | AT1G70730             | 1.124431419  | 0.762414575        |
| <b>TMT2</b>              | cassava4.1_002419m PACid:17972054 | AT4G35300             | 1.166354201  | 0.784652062        |
| <b>SPP2</b>              | cassava4.1_008254m PACid:17994099 | AT2G35840             | 1.085711675  | 0.811210375        |
| <b>PGM1</b>              | cassava4.1_003452m PACid:17960838 | AT5G51820             | 1.033803977  | 0.831527628        |
| <b>UGPase2</b>           | cassava4.1_006965m PACid:17961642 | AT5G17310             | 1.029815509  | 0.836268835        |
| <b>cPGI</b>              | cassava4.1_008526m PACid:17960520 | AT5G42740             | 1.030880805  | 0.874555718        |
| <b>FBA2</b>              | cassava4.1_009140m PACid:17966251 | AT4G38970             | 1.026531768  | 0.944833158        |
| <b>SUS6</b>              | cassava4.1_001283m PACid:17991130 | AT1G73370             | 1.139926359  | 0.957015817        |
| <b>FK</b>                | cassava4.1_011584m PACid:17982732 | AT3G59480             | -1.475956124 | <b>0.013297578</b> |
| <b>cF1,6BPase, FINS1</b> | cassava4.1_011197m PACid:17981706 | AT1G43670             | -1.217314961 | <b>0.039438558</b> |
| <b>FBAL</b>              | cassava4.1_032962m PACid:17968079 | AT1G18270             | -1.171850871 | 0.051744173        |
| <b>SUS4</b>              | cassava4.1_001864m PACid:17992794 | AT3G43190             | -1.258455378 | 0.065106092        |
| <b>FBA2</b>              | cassava4.1_009143m PACid:17961139 | AT4G38970             | -1.391879393 | 0.137753418        |
| <b>UGPase2</b>           | cassava4.1_006979m PACid:17979348 | AT5G17310             | -1.139212866 | 0.17657924         |
| <b>SUS4</b>              | cassava4.1_001871m PACid:17965267 | AT3G43190             | -1.15987115  | 0.190463169        |
| <b>PFP</b>               | cassava4.1_004602m PACid:17991512 | AT1G12000             | -1.274753778 | 0.199752268        |
| <b>PFK3</b>              | cassava4.1_006625m PACid:17989898 | AT4G26270             | -1.371593061 | 0.200778451        |
| <b>PFP</b>               | cassava4.1_003814m PACid:17990206 | AT1G20950             | -1.352083741 | 0.201283797        |
| <b>FK</b>                | cassava4.1_011578m PACid:17975802 | AT3G59480             | -1.514375705 | 0.20581161         |
| <b>PFK3</b>              | cassava4.1_004789m PACid:17985737 | AT4G26270             | -1.119311497 | 0.295388041        |
| <b>FRK</b>               | cassava4.1_011838m PACid:17972376 | AT5G19150             | -1.102633246 | 0.325308231        |
| <b>pPGI</b>              | cassava4.1_031246m PACid:17981861 | AT4G24620             | -1.049204492 | 0.575652002        |
| <b>HXK2</b>              | cassava4.1_006251m PACid:17973163 | AT2G19860             | -1.043431483 | 0.595756134        |
| <b>PGM2</b>              | cassava4.1_004336m PACid:17982342 | AT1G70730             | -1.03259972  | 0.739905026        |

### *Carbohydrate Signalling*

Sugar signalling is well known and certain sugars and some enzymes are involved in translating the metabolic state of the cell into a transcriptional response. As mentioned, glucose can be sensed by HXK1 (Jang et al., 1997; Moore et al., 2003) and fructose can be sensed by F1,6BPase (Cho and Yee, 2011), both of which were up-regulated after pruning. Also up-regulated after pruning was KING1 (cassava4.1\_008188), the  $\gamma$ -regulatory subunit (Bouly et al., 1999) of the heterotrimeric SnRK1 (Snf1-protein kinase), which was 11-fold more abundant. Proteins homologous to SnRK1 in mammals (AMPK) and yeast (Snf1) have been described to be up-regulated under starving conditions (reviewed in Hardie et al., 1998). In plants, the role of SnRK1 proteins is still controversial. However, there are reports that SnRK1 inhibits the activity of enzymes involved in energy consuming pathways such as isoprenoid biosynthesis (3HMGCoA reductase), nitrogen assimilation (nitrate reductase) and sucrose biosynthesis (SPS) (Sugden et al., 1999). Sugden et al. (1999) showed that SPS is inactive in the phosphorylated state (Huber et al., 1989). I also identified KIN10, the catalytic  $\alpha$ -subunit of the SnRK1 (Baena-Gonzalez et al., 2007), though no change in abundance was observed.

#### **4.3.1.2. Changes in different metabolic pathways accompany the metabolic shift from sink-to-source**

Energy derived from catabolic processes (in form of ATP and reducing equivalents i.e. NAD[P]H) are required for cell maintenance and to drive energy-consuming anabolic processes. To provide energetic compounds carbohydrates and other energy-rich metabolites are respired by various pathways. Depending on the metabolite source (e.g. lipid, sugar, protein, amino acids) different pathways are involved. For example, glycolysis consumes hexose-phosphates generating pyruvate, ATP and NADH, while lipids are metabolized by  $\beta$ -oxidation to acetyl Co-A. Both pyruvate and acetyl CoA provide substrate for the tricarboxylic acid (TCA) cycle. As shown in Figure 4.4 A, proteins of the functional categories, 'protein', 'amino acid', 'gluconeogenesis' and 'TCA/ organic acid' were statistically overrepresented in the dataset TDP (Table 4.3).

### *Energy producing pathways*

Energy to fuel metabolism can be obtained by degradation of lipids. Interestingly, an enrichment of proteins functionally categorized in lipid metabolism was observed after pruning (Figure 4.4 A). Seven proteins were down-regulated in TDP compared ZDP samples, five of which are known to act in lipid synthesis. On the other hand of the ten proteins up-regulated, six are known to play a role in lipid degradation (Table 4.3). These observations indicate an induction of lipid degradation after pruning.

I observed an increased abundance of several peroxisomal proteins involved in  $\beta$ -oxidation and the glyoxylate cycle: the acyl-CoA oxidase ACX3 (cassava4.1\_002966), the multifunctional protein MFP2 (cassava4.1\_002479) and the peroxisomal ketoacetyl-CoA thiolase PKT3 (cassava4.1\_007022). Ten additional proteins involved in fatty acid breakdown were also identified (Supplemental Table 4.1; Penfield et al., 2006). Tricarboxylic acid cycle proteins were also significantly enriched and up-regulated TDP (Figure 4.4 A). Metabolites derived from sugars, fatty acids, and proteins all feed into the TCA and are metabolized to produce reducing equivalents to fuel ATP production. In total, 49 proteins of the TCA cycle were identified (Supplemental Table 4.1), of which nine were up-regulated TDP compared to the ZDP control samples. In contrast, only one TCA protein was down-regulated TDP (Table 4.3).

Gluconeogenesis allows the de-novo synthesis of glucose from non-carbohydrate carbon substrates (i.e. pyruvate, lactate, glycerol, gluconeogenic amino acids). The two gluconeogenic enzymes phosphoenolpyruvate carboxykinase PCK1 (cassava4.1\_004362; cassava4.1\_030131) and peroxisomal malate dehydrogenase pMDH1 (cassava4.1\_010585) were both found to be up-regulated in TDP samples (Table 4.3; Supplemental Table 4.1). In a sequential reaction, MDH catalyses the oxidation of malate to oxaloacetate which is further converted by PCK1 to phosphoenolpyruvate. Phosphoenolpyruvate can then be converted to hexose phosphates through gluconeogenesis, or to pyruvate through the last steps of glycolysis. In addition, the glyoxylate cycle enzyme citrate synthase CSY2 (cassava4.1\_005825; Pracharoenwattana et al., 2005) was detected but its abundance did not change.

Twenty proteins involved in the oxidative pentose phosphate pathway (OPPP) were identified, eighteen of which were unchanged (Supplemental Table 4.1). However, the glucose-6-phosphate dehydrogenase isoform G6PD4 (cassava4.1\_003566), which generates NADPH at the start of the pathway was up-regulated TDP (Table 4.3).

#### 4. Metabolic shift in cassava storage root from sink-to-source

**Table 4.3 Significant regulated proteins of the MapMan categorization with the largest changes.** MapMan categories that showed highest changes and the proteins that changes most (p-value  $\leq 0.05$ ).

| Mapman category                                  | Cassava accession | Arabidopsis Accession | Max fold change | Anova (p)          |
|--------------------------------------------------|-------------------|-----------------------|-----------------|--------------------|
| gluconeogenese/ glyoxylate cycle                 | cassava4.1_004362 | AT4G37870             | 2.842536127     | <b>0.015794126</b> |
| gluconeogenese/ glyoxylate cycle                 | cassava4.1_030131 | AT4G37870             | 2.560662975     | <b>0.029497669</b> |
| gluconeogenesis                                  | cassava4.1_010585 | AT2G22780             | 1.768182403     | <b>0.028130428</b> |
| OPP                                              | cassava4.1_003566 | AT1G09420             | 1.233848973     | <b>0.000655903</b> |
| TCA / org                                        | cassava4.1_007889 | AT5G58330             | 2.283125624     | <b>0.009206196</b> |
| TCA / org                                        | cassava4.1_010105 | AT5G43330             | 1.203658368     | <b>0.002399012</b> |
| TCA / org                                        | cassava4.1_009952 | AT4G35650             | 1.431034228     | <b>0.04786744</b>  |
| TCA / org                                        | cassava4.1_000903 | AT2G05710             | 1.241465409     | <b>0.017624705</b> |
| TCA / org                                        | cassava4.1_006853 | AT2G44350             | 1.613845419     | <b>0.033774052</b> |
| TCA / org                                        | cassava4.1_007540 | AT1G59900             | 1.398320386     | <b>0.011352076</b> |
| TCA / org                                        | cassava4.1_004864 | AT3G13930             | 1.298227513     | <b>0.042369153</b> |
| TCA / org                                        | cassava4.1_003490 | AT3G52200             | 1.263526843     | <b>0.034907379</b> |
| TCA / org                                        | cassava4.1_008387 | AT2G20420             | 1.300115518     | <b>0.043278571</b> |
| TCA / org                                        | cassava4.1_004579 | AT3G16950             | -1.119370463    | <b>0.014414463</b> |
| glycolysis                                       | cassava4.1_006596 | AT3G12780             | 1.745902602     | <b>0.009172008</b> |
| glycolysis                                       | cassava4.1_006605 | AT3G12780             | 1.61206826      | <b>0.024595073</b> |
| glycolysis                                       | cassava4.1_006818 | AT2G29560             | -1.559280338    | <b>0.013076458</b> |
| glycolysis                                       | cassava4.1_007678 | AT2G36530             | -1.184387774    | <b>0.017383169</b> |
| glycolysis                                       | cassava4.1_005990 | AT5G56350             | -1.598008389    | <b>0.000651792</b> |
| lipid metabolism                                 | cassava4.1_028937 | AT5G35360             | 1.636998465     | <b>0.001626041</b> |
| lipid metabolism                                 | cassava4.1_002966 | AT1G06290             | 2.249492405     | <b>0.003248717</b> |
| lipid metabolism                                 | cassava4.1_014036 | AT4G16210             | 1.643172336     | <b>0.007485635</b> |
| lipid metabolism                                 | cassava4.1_005575 | AT4G29010             | 1.577977396     | <b>0.008383851</b> |
| lipid metabolism                                 | cassava4.1_001538 | AT4G35790             | 1.58413125      | <b>0.018376305</b> |
| lipid metabolism                                 | cassava4.1_002951 | AT5G13640             | 1.178699477     | <b>0.023079062</b> |
| lipid metabolism                                 | cassava4.1_007181 | AT2G33150             | 2.286929361     | <b>0.024956686</b> |
| lipid metabolism                                 | cassava4.1_002479 | AT3G06860             | 1.959534388     | <b>0.026417829</b> |
| lipid metabolism                                 | cassava4.1_006559 | AT2G18730             | 1.306196433     | <b>0.038275462</b> |
| lipid metabolism                                 | cassava4.1_019325 | AT5G42890             | 1.561863456     | <b>0.04040195</b>  |
| lipid metabolism                                 | cassava4.1_000033 | AT1G36160             | -2.372967306    | <b>0.000310268</b> |
| lipid metabolism                                 | cassava4.1_004720 | AT2G26260             | -1.953040311    | <b>0.004870589</b> |
| lipid metabolism                                 | cassava4.1_004314 | AT3G22960             | -2.069258196    | <b>0.01550972</b>  |
| lipid metabolism                                 | cassava4.1_000041 | AT1G36160             | -4.840405433    | <b>0.022125948</b> |
| lipid metabolism                                 | cassava4.1_004405 | AT5G52920             | -1.460937398    | <b>0.029268563</b> |
| lipid metabolism                                 | cassava4.1_004230 | AT3G22960             | -1.581515844    | <b>0.03100602</b>  |
| lipid metabolism                                 | cassava4.1_006550 | AT4G36480             | -1.299408497    | <b>0.044136398</b> |
| lipid metabolism                                 | cassava4.1_023396 | AT2G07050             | -2.283239733    | <b>0.051325251</b> |
| gluconeogenese/ glyoxylate cycle                 | cassava4.1_004362 | AT4G37870             | 2.842536127     | <b>0.015794126</b> |
| gluconeogenese/ glyoxylate cycle                 | cassava4.1_030131 | AT4G37870             | 2.560662975     | <b>0.029497669</b> |
| mitochondrial electron transport / ATP synthesis | cassava4.1_009175 | AT2G20360             | 1.374081152     | <b>0.029255982</b> |
| mitochondrial electron transport / ATP synthesis | cassava4.1_012350 | AT5G40810             | 1.333333982     | <b>0.051180451</b> |
| mitochondrial electron transport / ATP synthesis | cassava4.1_014824 | AT3G54110             | 2.108727377     | <b>0.075192815</b> |

#### 4. Metabolic shift in cassava storage root from sink-to-source

##### Protein and amino acid metabolism

I observed changes in proteins associated with protein and amino acid biosynthesis. There was an increase in proteins assigned to protein ubiquitination and modification TDP compared to the ZDP controls (Table 4.4). The protein ubiquitination pathway served to conjugate ubiquitin to Lys residues within substrate proteins, thereby targeting them for degradation by proteasomes (Smalle and Vierstra, 2004). Amongst the functional category for amino acid metabolism 18 proteins were up-regulated TDP. Four of the proteins are assigned to the central amino acids metabolism like aspartate aminotransferase that catalyses the reversible transfer of the amino group from aspartate to  $\alpha$ -ketoglutarate yielding glutamate and oxaloacete. Furthermore, it was described that they are responsible to recycle carbon skeleton during ammonia assimilation (Ryan and Fottrell, 1974).

**Table 4.4 Significant regulated proteins of the MapMan categorization amino acid and protein metabolism.** MapMan categories that showed highest changes and the proteins that changes most (p-value  $\leq$  0.05).

| Mapman category       | Cassava accession  | Arabidopsis Accession | Max fold change | Anova (p)          |
|-----------------------|--------------------|-----------------------|-----------------|--------------------|
| amino acid metabolism | cassava4.1_008396  | AT1G12050             | 1.602166851     | <b>0.004113184</b> |
| amino acid metabolism | cassava4.1_019208  | AT2G43750             | 1.541881836     | <b>0.005888425</b> |
| amino acid metabolism | cassava4.1_006617  | AT1G70580             | 1.926825688     | <b>0.007351459</b> |
| amino acid metabolism | cassava4.1_012571  | AT5G54080             | 3.560328754     | <b>0.008195887</b> |
| amino acid metabolism | cassava4.1_022406  | AT4G34030             | 1.115867703     | <b>0.010464068</b> |
| amino acid metabolism | cassava4.1_010180  | AT2G17265             | 1.355471482     | <b>0.011336394</b> |
| amino acid metabolism | cassava4.1_011138  | AT3G22740             | 9.158163652     | <b>0.011499745</b> |
| amino acid metabolism | cassava4.1_004831  | AT5G62530             | 1.369684373     | <b>0.012721537</b> |
| amino acid metabolism | cassava4.1_007524  | AT1G80600             | 1.692282687     | <b>0.01966984</b>  |
| amino acid metabolism | cassava4.1_006432  | AT5G11880             | 1.417256807     | <b>0.022360409</b> |
| amino acid metabolism | cassava4.1_008771  | AT1G09795             | 1.348388951     | <b>0.02236232</b>  |
| amino acid metabolism | cassava4.1_008563  | AT2G24580             | 1.497246038     | <b>0.026408252</b> |
| amino acid metabolism | cassava4.1_006859  | AT5G46180             | 1.838310247     | <b>0.0271597</b>   |
| amino acid metabolism | cassava4.1_005703  | AT3G22200             | 1.768579566     | <b>0.032529975</b> |
| amino acid metabolism | cassava4.1_007094  | AT4G31990             | 1.344153267     | <b>0.038616693</b> |
| amino acid metabolism | cassava4.1_008844m | AT5G19550             | 1.260339511     | <b>0.04100868</b>  |
| amino acid metabolism | cassava4.1_006286  | AT4G24830             | 1.460609283     | <b>0.041827029</b> |
| amino acid metabolism | cassava4.1_007019  | AT3G57050             | 1.5727444       | <b>0.047772756</b> |
| amino acid metabolism | cassava4.1_009247  | AT4G01850             | -3.966421553    | <b>0.007795959</b> |
| amino acid metabolism | cassava4.1_009789  | AT3G61440             | -2.966724969    | <b>0.010016052</b> |
| amino acid metabolism | cassava4.1_009245  | AT4G01850             | -4.153689723    | <b>0.011618162</b> |
| amino acid metabolism | cassava4.1_012023  | AT5G65780             | -1.914311188    | <b>0.015473361</b> |
| amino acid metabolism | cassava4.1_011785  | AT4G14880             | -1.470820095    | <b>0.016861007</b> |
| amino acid metabolism | cassava4.1_008023  | AT1G17745             | -1.721439695    | <b>0.023508802</b> |
| amino acid metabolism | cassava4.1_009260  | AT4G01850             | -7.173982227    | <b>0.028675998</b> |
| amino acid metabolism | cassava4.1_010021  | AT3G61440             | -3.602058257    | <b>0.042572879</b> |
| amino acid metabolism | cassava4.1_009356  | AT2G36880             | -3.634983363    | <b>0.042766793</b> |

#### 4. Metabolic shift in cassava storage root from sink-to-source

**Table 4.4 Significant regulated proteins of the MapMan categorization amino acid and protein metabolism.**

|         |                   |           |              |                    |
|---------|-------------------|-----------|--------------|--------------------|
| protein | cassava4.1_008257 | AT2G38000 | 2.281747913  | <b>0.022194642</b> |
| protein | cassava4.1_007163 | AT4G38220 | 1.607520884  | <b>0.025997167</b> |
| protein | cassava4.1_007251 | AT4G38220 | 1.539019794  | <b>0.035387818</b> |
| protein | cassava4.1_002552 | AT1G50380 | 1.485492241  | <b>0.039766223</b> |
| protein | cassava4.1_009672 | AT3G54360 | 1.46021094   | <b>0.00386717</b>  |
| protein | cassava4.1_006459 | AT1G06110 | 1.752486433  | <b>0.001786115</b> |
| protein | cassava4.1_013730 | AT3G27430 | 1.842292617  | <b>0.02456257</b>  |
| protein | cassava4.1_008212 | AT1G53750 | 1.524920926  | <b>0.037236273</b> |
| protein | cassava4.1_008421 | AT5G58290 | 1.485892869  | <b>0.023993294</b> |
| protein | cassava4.1_015645 | AT3G60820 | 1.17945286   | <b>0.022298921</b> |
| protein | cassava4.1_008374 | AT5G09900 | 1.165663759  | <b>0.022289753</b> |
| protein | cassava4.1_022803 | AT3G13235 | 1.252930263  | <b>0.005124596</b> |
| protein | cassava4.1_007929 | AT1G51710 | 1.247724727  | <b>0.000184834</b> |
| protein | cassava4.1_015319 | AT2G18110 | 1.568825174  | <b>0.01445178</b>  |
| protein | cassava4.1_007130 | AT1G04170 | 1.411027139  | <b>0.012777074</b> |
| protein | cassava4.1_009232 | AT1G53880 | 1.323855844  | <b>0.049159598</b> |
| protein | cassava4.1_011934 | AT2G40010 | 1.172392775  | <b>0.013602982</b> |
| protein | cassava4.1_024858 | AT5G07090 | 1.360660378  | <b>0.0453126</b>   |
| protein | cassava4.1_003839 | AT3G03060 | 1.337480979  | <b>0.018067829</b> |
| protein | cassava4.1_009061 | AT1G45000 | 1.202475005  | <b>0.031655988</b> |
| protein | cassava4.1_006415 | AT1G63500 | 1.310031239  | <b>0.015797169</b> |
| protein | cassava4.1_004238 | AT3G25800 | 1.170059726  | <b>0.044526673</b> |
| protein | cassava4.1_006148 | AT4G35230 | 1.945108137  | <b>0.002996077</b> |
| protein | cassava4.1_022125 | AT4G20360 | 2.152845168  | <b>0.04550581</b>  |
| protein | cassava4.1_000656 | AT1G09620 | -5.828654907 | <b>0.041876401</b> |
| protein | cassava4.1_032535 | AT4G10320 | -1.554065116 | <b>0.038778811</b> |
| protein | cassava4.1_007409 | AT1G14570 | -1.67872442  | <b>0.013700506</b> |
| protein | cassava4.1_009231 | AT2G38860 | -1.360936044 | <b>0.001005953</b> |
| protein | cassava4.1_001804 | AT4G30020 | -2.780422867 | <b>0.033328303</b> |
| protein | cassava4.1_000634 | AT5G06460 | -1.512632939 | <b>0.012333321</b> |
| protein | cassava4.1_018147 | AT5G42190 | -1.633379408 | <b>0.028928969</b> |
| protein | cassava4.1_000599 | AT5G06600 | -1.88056626  | <b>0.027227639</b> |
| protein | cassava4.1_005302 | AT3G18190 | -1.595956909 | <b>0.011719071</b> |
| protein | cassava4.1_005057 | AT3G03960 | -1.244498262 | <b>0.013973317</b> |
| protein | cassava4.1_001585 | AT3G07100 | -2.204258055 | <b>0.045976556</b> |
| protein | cassava4.1_000150 | AT1G71220 | -1.661860023 | <b>0.014141749</b> |
| protein | cassava4.1_032325 | AT5G67360 | -5.267634152 | <b>0.045000855</b> |
| protein | cassava4.1_004221 | AT4G34980 | -2.534486168 | <b>0.009117001</b> |
| protein | cassava4.1_006951 | AT2G03640 | -2.356099306 | <b>0.043776796</b> |
| protein | cassava4.1_008506 | AT3G50000 | -2.277335938 | <b>0.001188611</b> |
| protein | cassava4.1_004241 | AT4G26300 | -1.243089333 | <b>0.000953209</b> |
| protein | cassava4.1_000688 | AT4G20850 | -1.6375074   | <b>0.016587509</b> |

#### 4. Metabolic shift in cassava storage root from sink-to-source

##### Cell wall biosynthesis

The plant cell wall consists of cellulose, hemicellulose and pectins. These different polymers make up the cell wall layers, giving it the rigidity. Plant cell walls undergo constant modifications in order to allow cell expansion and division in growing tissues. Biosynthesis of the cell wall includes polymerization and several modification steps. Of the proteins described to be involved in cell wall synthesis and hemicellulose modifications, 12 were down-regulated (e.g. RHM1, UDP-GlcNAc TDP compared to the ZDP controls, while five others were up-regulated (like expansin-like 1 pectin methylesterase inhibitor [PME inhibitor] and xylanase 1; Table 4.5). PME was shown to be involved in cell wall modifications required for pollen tube growth (Jiang et al., 2005). The finding that a putative PME inhibitor is up-regulated, while most other cell wall biosynthetic proteins were down-regulated, indicates that cell wall modifications and growth may be reduced.

**Table 4.5 Significant regulated proteins of the MapMan categorization cell wall.** MapMan categories that showed highest changes and the proteins that changes most (p-value  $\leq$  0.05).

| Mapman category | Cassava accession | Arabidopsis Accession | Max fold change | Anova (p)          |
|-----------------|-------------------|-----------------------|-----------------|--------------------|
| cell wall       | cassava4.1_004339 | AT3G14310             | 1.481605442     | <b>0.003386575</b> |
| cell wall       | cassava4.1_003278 | AT1G62440             | 1.599930163     | <b>0.005963094</b> |
| cell wall       | cassava4.1_001252 | AT1G58370             | 1.475983414     | <b>0.007256631</b> |
| cell wall       | cassava4.1_004821 | AT4G02320             | 1.689617201     | <b>0.028879098</b> |
| cell wall       | cassava4.1_014262 | AT3G45970             | 1.465024207     | <b>0.032980867</b> |
| cell wall       | cassava4.1_013014 | AT5G13870             | -2.053823582    | <b>0.002361225</b> |
| cell wall       | cassava4.1_003705 | AT5G49720             | -4.673208839    | <b>0.005091526</b> |
| cell wall       | cassava4.1_006282 | AT1G31070             | -1.901909375    | <b>0.009644226</b> |
| cell wall       | cassava4.1_005517 | AT1G75680             | -2.472428331    | <b>0.015315386</b> |
| cell wall       | cassava4.1_026770 | AT5G07720             | -7.792853011    | <b>0.015907422</b> |
| cell wall       | cassava4.1_012617 | AT1G63000             | -2.510786326    | <b>0.017298634</b> |
| cell wall       | cassava4.1_003070 | AT1G78570             | -1.939766287    | <b>0.024894052</b> |
| cell wall       | cassava4.1_012622 | AT1G63000             | -3.058469716    | <b>0.027758688</b> |
| cell wall       | cassava4.1_006215 | AT3G61490             | -6.021070584    | <b>0.030450968</b> |
| cell wall       | cassava4.1_023284 | AT3G51160             | -1.612490451    | <b>0.032606569</b> |
| cell wall       | cassava4.1_013011 | AT3G23730             | -7.078208205    | <b>0.034907825</b> |
| cell wall       | cassava4.1_007645 | AT3G62830             | -2.474521947    | <b>0.046882028</b> |



*Changes in strigolactone biosynthesis- release of bud outgrowth*

According to our analysis the MapMan category ‘hormones’ was significantly down-regulated after pruning. Interestingly, investigations of this functional category revealed that two proteins involved in strigolactone biosynthesis, MAX1 and MAX4 (cassava4.1\_005510 and cassava4.1\_005134), to be down-regulated 2.3 and 9.4 times, respectively. For Arabidopsis it was shown that strigolactone is involved in maintaining axillary bud dormancy. It was shown that strigolactone-deficient mutant plants, *max* (more axillary growth), exhibit increased branching (Stirnberg et al., 2002; Sorefan et al., 2003; Booker et al., 2005). Recently, a strigolactone transporter ABCG40 (also known as PDR12 that transports strigolactone in petunia; cassava4.1\_027677; cassava4.1\_000229) was described (Kretzschmar et al., 2012) ABCG40 was 1.7 times more abundant after cutting (Table 4.6; Supplemental Table 4.1).

**Table 4.6 Significant regulated proteins of the MapMan categorization hormone metabolism.** MapMan categories that showed highest changes and the proteins that changes most (p-value ≤ 0.05).

| Mapman category    | Cassava accession | Arabidopsis Accession | Max fold change | Anova (p)          |
|--------------------|-------------------|-----------------------|-----------------|--------------------|
| hormone metabolism | cassava4.1_015449 | AT1G28200             | 1.407374243     | <b>0.010436829</b> |
| hormone metabolism | cassava4.1_013447 | AT1G52340             | 1.283231678     | <b>0.038862279</b> |
| hormone metabolism | cassava4.1_014697 | AT5G43830             | 1.499349065     | <b>0.011633709</b> |
| hormone metabolism | cassava4.1_015980 | AT5G42650             | 1.540189318     | <b>0.045488344</b> |
| hormone metabolism | cassava4.1_001259 | AT1G67560             | 1.196296357     | <b>0.013257182</b> |
| hormone metabolism | cassava4.1_005134 | AT4G32810             | -9.38927503     | <b>0.044837868</b> |
| hormone metabolism | cassava4.1_000306 | AT2G36910             | -2.828121082    | <b>0.040240115</b> |
| hormone metabolism | cassava4.1_005510 | AT2G26170             | -2.30123054     | <b>0.04271988</b>  |
| hormone metabolism | cassava4.1_006458 | AT1G11680             | -1.461856026    | <b>0.036350652</b> |
| hormone metabolism | cassava4.1_003527 | AT5G63120             | -2.073351598    | <b>0.002667439</b> |
| hormone metabolism | cassava4.1_023409 | AT1G79460             | -42.83336756    | <b>0.004069604</b> |

#### 4.4. Discussion

##### 4.4.1. Robustness of proteomics data analysis

Here, I show that shotgun proteome analysis proved helpful in elucidating the changes in protein abundance when cassava storage roots are transformed from a sink to a source and induced to mobilize their starch. The PCA analysis showed that one of the TDP biological replicates separated apart from the other two, while the ZDP control group clustered together closely. However, the treated samples were clearly separated from the controls along the main axis of the PCA analysis, suggesting that changes caused by pruning occurred in all three TDP samples. It is likely that after

pruning, the plants are more stressed than the control plants, and therefore in a less uniform state. It is also likely that, because of the TDP sample separation, the statistical significance of individual protein changes are less pronounced than they could be with further replication. Nevertheless, the data I present here are valuable, and their analysis provides insight into storage root signalling and metabolism.

When analysing my proteome data, it became apparent that cassava frequently has multiple homologs to a given Arabidopsis protein suggesting that gene duplication might have since the evolutionary divergence of the two species. However, for some cases it transpired that the cassava genome database is not correctly annotated. For example, an incorrect annotation was found for LDA, where the single gene locus is interrupted and incorrectly given as two loci in the database. Thus, in order to analyse protein families in more detail, it will be crucial to carefully verify that the gene annotation is correct.

#### **4.4.2. Changes in starch metabolism caused by pruning**

As expected, I could show that pruning off the cassava shoots induces starch remobilization in storage roots. The decrease in starch content, both at the site of stem pruning and in subterranean storage roots, was highly significant after 10 days (Figure 4.1 B, C). This is in accordance with previous studies of pre-harvest pruning experiments (van Oirschot et al., 2000). I could show using various native gels that there was an increase in amylolytic activity in storage roots accompanying the change in starch. However, with the exception of LDA, it was not possible to really say which enzymes are responsible for the activity bands I observed, hence the use of proteomics.

My data reveal that there are changes in starch metabolic proteins, but these changes are not as widespread as one might have expected. Furthermore, not all of the changes can be simply interpreted. The down-regulation of the root specific large subunit of AGPase (APL3) after pruning is in agreement with the idea that the rate of starch synthesis is decreased upon transition from sink-to-source. AGPase is widely accepted to be the step at which the flux into starch is controlled in plants. The down-regulation of PWD after pruning is more surprising as the enzyme has a designated role in starch degradation (Baunsgaard et al., 2005; Kotting et al., 2005). That said, it was shown that glucan phosphorylation not only occurs during starch degradation, but also during synthesis (Nielsen et al., 1994), explaining the presence of PWD in control storage roots. Furthermore, it should be pointed out firstly, that the change in PWD abundance was small and secondly, that it is not known whether phosphorylation is really required for the degradation of starch in cassava root, as it is in Arabidopsis leaves or potato tubers.

Despite the red-pullulan gels suggesting a higher LDA activity at TDP compared to ZDP (Figure 4.2), at the proteome level no significant change was observed. It is possible that the increase in activity was due to posttranslational modification rather than an increase in protein abundance. However, the semi-quantitative native gels also need to be backed up with accurate assays. In contrast, the proteomic analysis showed that AMY3 protein abundance is substantially increased in starch-mobilizing roots TDP. It is not clear whether this activity is reflected on the native gel analysis, and further work will be needed to determine if there is an increase in  $\alpha$ -amylase activity. In *Arabidopsis*, mutant analysis revealed that AMY3 is not essential for transitory starch degradation (Yu et al., 2005). However, for cereals it is well described that  $\alpha$ -amylases are the main hydrolytic enzymes involved in degrading storage starch for germination (Fincher, 1989). Furthermore, in other systems, an increase in  $\alpha$ -amylase activity correlates with re-mobilization of storage starch (e.g. in defoliated white-clover and poplar wood ray cells; Baur-Hösch et al., 1990; Witt and Sauter, 1994; Gallagher et al., 1997). This supports the idea that storage starch degradation in some heterotrophic tissues involves  $\alpha$ -amylases. If the main pathway to degrade cassava storage starch does involve AMY3, it implies that branched and linear maltooligosaccharides are released from the granule surface. Branched maltooligosaccharides will serve as substrates for debranching enzymes (LDA and ISA3) to generate more linear maltooligosaccharids (Delatte et al., 2006; Streb et al., 2008). Linear maltooligosaccharides can be further processed by stromal enzymes. In the pathway described in *Arabidopsis* leaves  $\beta$ -amylases would be one of the key stromal enzymes degrading both the granule surface and linear maltooligosaccharides. It is surprising that in the proteome analysis, I could not identify any  $\beta$ -amylase homologs. The reason for this might be that  $\beta$ -amylases are not involved in storage starch mobilization in cassava or the proteins were below the detection limit.

To strengthen my hypothesis that AMY3 is a key player in cassava root starch breakdown, functional analyses are needed. A transgenic cassava line with repressed AMY3 is in development, and will give allow the functionality of AMY3 in storage root to be tested directly (see Chapter 5). If it is involved, changing its expression may offer an opportunity to increase starch yields in cassava storage roots by preventing the post-harvest decline in starch levels.

#### 4.4.3. Changes in sucrose metabolism caused by pruning

Although I interpret the changes in proteins detected on the assumption that the sink-source transition occurred, it is interesting that the actual sucrose amount did not change (Figure 4.1). In sink tissues sucrose is unloaded from the phloem and transported to the cell, where it is hydrolysed by SUS or cytosolic INV depending on how sucrose is cleaved UDPG and fructose (SUS) or glucose and fructose (INV) are generated. The free hexoses are then phosphorylated either by HXK or FK in order to feed the hexose-P pool. In the proteomic analysis I observed more of FK in untreated samples

(sink). This suggests that fructose is phosphorylated predominantly by FK to increase F6P levels. In subsequent steps F6P can be equilibrated by PGM to G6P that then can be transported to the amyloplast by the pG6PT where it can be converted to starch. Interestingly, I could identify more of cytosolic F1,6BPase before pruning. This enzyme catalyses the dephosphorylation of F1,6BP to F6P by releasing Pi. As a result again F6P is produced that could be subjected towards the amyloplast, and hence starch synthesis. A recent screen for fructose insensitive mutants in Arabidopsis identified the cytosolic F1,6BPase as a sensor for fructose availability. It was shown that similar to HXK1 signalling this is not dependent on the catalytic activity. This finding suggests that potentially in heterotrophic tissues similar mechanism exists. On the other hand after pruning and thus during transition from sink to source metabolism, cINV2 increased suggesting that sucrose is hydrolysed in the cytosol to glucose and fructose. This might be beneficial as high sucrose export rate to the new emerging leaves might not be required yet. The concomitant increase of hexokinases (HXK1 and HXK3) suggests that potentially the demand for hexose-P increased in the root that can feed into diverse metabolic pathways. Furthermore, a plastidic fructose-1,6 bisphosphatealdolase (FBA) that catalyses the reversible reaction of F1,6BP to DHAP and triose-P. Further DHAP can enter the oxidative pentose phosphate pathway generating NADPH. Thus, these findings suggest that the sucrose metabolism has changed. There are good evidences that sucrose pathway generates F6P that is likely converted to G6P and hence starch in the amyloplast in untreated storage roots. On the other hand the high abundance of cINV suggests that there is an increased demand for hexose-Ps that can supply various metabolic pathways.

#### **4.4.4. Evidence for sugar signalling in cassava storage roots**

It might be that the concentration of sucrose is finely controlled and is kept constant despite large changes in fluxes. After pruning, the influx of sucrose must have stopped abruptly and, after a time, sucrose production in the root must occur to support regrowth. It is also possible that there were significant changes between ZDP and TDP but that after ten days, a new homeostasis has been reached. That said there were relatively few changes in sucrose metabolic proteins detected ten days after pruning compared to control plants, suggesting that perhaps the storage root has already the enzymatic capacity to synthesise sucrose.

In potato tubers, where transition of sink to source was studied upon tuber sprouting, a correlation between activity of either SUS or cINV and developmental stage of the tissue was observed. Thus, INV activity is predominant in the tuber initiation phase, where growth occurs. Sucrose cleavage by SUS on the other hand is the main sucrolytic pathway in developed tubers (Ross et al., 1994; Appeldoorn et al., 1997). The up-regulation of cINV2 TDP might also indicate a change in how sucrose is degraded in cassava roots. The concomitant up-regulation of two hexokinases, HXK1 and HXK3,

point into the direction that there is an increased capacity to feed hexoses (potentially generated by cINV2) into the hexose-P pool. Hexose-Ps are intermediates used for diverse catabolic and anabolic pathways. An increase in HXK activity and in biosynthetic activities associated with transition to a source tissue would also lead to an increased demand of ATP consistent with my findings of a significant increase of proteins involved in respiration (Table 4.3). Although not measured here, I anticipate that there would be an increase in respiration in the storage roots of pruned plants.

In roots TDP, there was a significant up-regulation of KING1, a regulatory subunit of SnRK1, while the catalytic  $\alpha$ -subunit remained unchanged. SnRK1, a homolog to AMPK in mammals and Snf1 in yeast, is reported to be involved in sucrose signalling and up-regulated upon starvation. Expression analysis showed that SnRK1 is up-regulated in growing potato stolons, whereas in mature tuber tissue fewer transcripts were detected suggesting that SnRK1 is active during tuber maturation (Man et al., 1997). In addition it was shown that the transcript of  $\gamma$ -subunit (KING1) is highly expressed in stems and root tissue of *Arabidopsis* (Bouly et al., 1999). SnRK1 is proposed to act in two ways; it directly inhibits target enzymes involved in isoprenoid synthesis, nitrogen assimilation and sucrose synthesis (Sugden et al., 1999) by phosphorylating them. For *Arabidopsis*, it was also shown that overexpression of the catalytic  $\alpha$ -subunits (*AtKIN10* and *AtKIN11*) increases the transcription of target enzymes involved in starvation and/or stress responses via a transcriptional cascade involving factors like GBF5 and bZIP (Sato et al., 2004; Baena-Gonzalez et al., 2007). Amongst up-regulated transcripts, were genes repressed by sugars. In addition, for wheat endosperm it was shown that the wheat *TaAMY2* promoter is induced by SnRK1, supporting the hypothesis that SnRK1 is involved in low sugar-signalling. In contrast, overexpression of SnRK1 in potato tubers as well as rice led to an increase in starch content as well as an increase in sucrose synthase and ADGPase activity (McKibbin et al., 2006). Given the strong data implicating SnRK1 in the response to starvation, the finding that the KING1 subunit is greatly up-regulated is exciting and makes it an excellent target for further experimental analysis.

Also in the context of sucrose signalling, it was shown that the sucrose signalling pathway of SnRK1 is linked to the signalling pathway via the metabolite trehalose 6-phosphate (T6P). It was shown in *Arabidopsis* that T6P increases after sucrose feeding and re-illumination at the end of the night (Schluepmann et al., 2004; Lunn et al., 2006). These findings have led to the suggestion that T6P serves as a signal for high carbon availability. It was further shown that T6P can stimulate redox activation of AGPase (Kolbe et al., 2005; Lunn et al., 2006) and that supplying exogenous trehalose increases APL3 transcription (Wingler et al., 2000). My proteomics data revealed the presence of four homologs of trehalose-6-phosphate synthase (TPS; Supplemental Table 4.1), but they were unchanged during the experiment.

The pruning of my cassava plants clearly had a big impact on other metabolic pathways that were not the primary focus of my work, with changes of proteins involved in lipid, protein and amino acid metabolism and in the biosynthesis of hormones such as strigolactones. Clearly such changes can be interpreted in the context of this study. For example, the fact that strigolactones act as inhibitors of apical dormancy release is consistent with re-sprouting of lateral buds (Figure 4.1 A, Table 4.6). Such findings suggest that in cassava, as in other plants, strigolactones are involved in regrowth and branching. My work therefore provides further gene targets for investigation that could ultimately be used to improve plant vigour or to alter plant architecture.

The extent of re-mobilization of starch after pruning however, cannot only be explained only by the transition of the storage root to a source tissue supplying newly-developing sinks (i.e. the new leaves). The strength of these sinks is not likely to be strong enough to require all the carbon lost from the starch pool. The decrease of 48% in starch content 10 days after pruning (Figure 4.1 E) additionally reflects the needs of storage roots to maintain its own cellular homeostasis through respiration. Without the support of the autotrophic leaf tissues, stored carbohydrates are converted into CO<sub>2</sub> through the OPPP and the TCA cycle. In addition to supplying energy (e.g. for housekeeping metabolism and membrane energization) these pathways will also supply various intermediates which are needed to feed other biochemical processes (i.e. protein metabolism, gene transcription, general metabolic pathways) throughout the day and night.

#### 4.4.5. General conclusion and outlook

Overall, the analysis of changes in the primary carbohydrate metabolism together with the overall proteome changes occurring after pruning lead me to the conclusion that a transition from sink to source did indeed occur. The finding that AMY3 levels correlated with starch remobilization, places it as a main candidate in the starch degradation pathway depends. Moreover, the absence of  $\beta$ -amylase homologs in the proteome data supports this idea. To confirm the hypothesis that AMY3 is the key enzyme in cassava to mobilize starch a functional analysis using an RNAi construct to suppress AMY3 expression is underway (Chapter 5).

It might be that some proteins (e.g. the  $\beta$ -amylases) are missing as their abundance is below detection limit. Our laboratory recently conducted a combined proteome and transcriptome study in the non-model plants *Cecropia peltata*. More detailed information was gained with a transcriptome analysis (Bischof et al., 2013) than with the proteome analysis, but the combination of both was optimal. In order to strengthen the data I present here, a whole transcriptome analysis using the RNAseq is underway. As transcript levels respond faster than protein levels, I performed a time-course harvest of storage roots after pruning. At the time of writing, storage roots were harvested 4h,

#### *4. Metabolic shift in cassava storage root from sink-to-source*

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24h, 2 days, 6 days and 10 days after pruning, with control samples (uncut plants) at time points 0h, 4h and 24h. The outcome of this experiment is still under investigation, but when completed, will be directly comparable with my proteome study.

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## 5. Increasing starch bound phosphate level: A transgenic approach

### 5.1. Introduction

Depending on the end use in food or non-food industries, starches with different properties are needed. Apart from processed food, confectionary and drinks, starch is used to make corrugated board and paper. For most of these processes, starches are modified post-extraction to obtain the required properties. Many modifications are gained by substitution of hydroxyl groups. Substitutions are gained through esterification (e.g. phosphate, acetate), oxidation, etherification (e.g. hydroxypropyl, hydroxyethyl) or cross-linking (e.g. phosphate diester). These chemical alterations confer properties such as increased water retention, better retrogradation, starch structure stabilisation, or improved pasting properties. In the paper industry, cationic starches are used for paper strengthening (Tharanathan, 2005). Native starches from various botanical sources differ in their physico-chemical properties. Several studies have shown that parameters like amylose:amylopectin ratio, granule size, protein and lipid content, and the degree of starch phosphorylation are responsible for influence key functional characteristics such as peak viscosity, paste clarity, or retrogradation (Tester and Morrison, 1990; Jobling et al., 2002). The degree of phosphorylation was reported to influence peak viscosity and gel formation. This was shown by the analysis of physico-chemical properties of potato wild-type starch compared to *Stgwd* suppressor lines which have low levels of starch-bound phosphate (see below and Chapter 1). This revealed that high maximum viscosity derives from starch phosphorylation (Vikso-Nielsen et al., 2001). Potato tuber starch, which has a high degree of phosphorylation (0.5%), shows a much higher viscosity peak compared to cassava root starch, which has only 0.05% starch phosphorylation (Blennow et al., 1998).

Early studies of GWD down-regulation in potato revealed a starch excess phenotype in leaves, while tubers exhibited a reduction in cold sweetening. These findings indicated that *StGWD* is involved in starch degradation in both tissues. As these studies also showed that the lack of *StGWD* leads to a reduced G6-phosphorylation (Lorberth et al., 1998; Vikso-Nielsen et al., 2001), they provided the first indication that phosphorylation is important for the initial step in starch degradation. Subsequent studies in the model plant *Arabidopsis* supported this finding. Thus, a model has emerged in which amylopectin on the starch granule surface is phosphorylated by GWD during degradation, rendering it more soluble. Phosphate-ester bound to the starch granule is suggested to open the packing of

double helical chain structures by altering the steric conformations. It may also destabilise the double helices themselves. This is proposed to make the glucan chains more accessible for hydrolysing enzymes like  $\alpha$ - and  $\beta$ - amylases, and the debranching enzymes (ISA3, LDA) involved in starch degradation (Edner et al., 2007). Through studies of Arabidopsis, another glucan dikinase (PWD, phosphoglucan, water dikinase) was subsequently discovered and shown to phosphorylate glucose units of amylopectin at the C3 position after pre-phosphorylation of GWD (Baunsgaard et al., 2005; Kotting et al., 2005). GWD was also shown to be specific for the 6-carbon of the individual glucose units (Ritte et al., 2002).

Limited proteolysis revealed that StGWD, a 155 kDa protein, consists of 5 stable fragments. Analysis of these fragments showed that GWD contains three major domains important for successful amylopectin phosphorylation. GWD contains two CBM domain that are involved in binding amylopectin and a pyruvate-phosphate dikinase-like domain is involved in phosphotransfer via an autocatalytic histidine residue (Mikkelsen et al., 2005; Mikkelsen et al., 2006). Investigation of the third domain through site-directed mutagenesis of cysteine residues revealed a redox regulation motif. Functional analysis revealed that GWD is active by reduction of a disulphide bridge in a reducing environment which is predominant during the light phase in chloroplasts. Although the main function of starch phosphorylation was shown to be important during starch mobilisation, Nielsen et al. (1994) reported that during starch biosynthesis phosphate is incorporated into starch. In potato tubers discs 0.5% of the glucosyl residues in newly synthesised starch are phosphorylated (Nielsen et al., 1994). This finding also explains why starch granules are phosphorylated not only at the surface but also in the inner layers. This was recently visualized by synchrotron X-ray microfluorescence mapping in potato starch granules (Buleon et al., 2014).

For efficient hydrolysis of starch granules to maltose and oligosaccharides, phosphate esters need to be removed. In higher plants two glucan phosphatases – SEX4 and LSF2 - have been described (Zeeman and Ap Rees, 1999; Niittyla et al., 2006; Kotting et al., 2009; Santelia et al., 2011). The characterisation of these phosphatases in Arabidopsis revealed that both enzymes are responsible for removing phosphate residues both from the starch granules and from soluble phospho-oligosaccharide released from the starch granule. Recombinant AtSEX4 protein efficiently removes phosphate residues from both the C-6 and C-3 position of the glucose units. The *Atsex4* mutant has increased starch levels as well as high phospho-oligosaccharide content. The accumulation of phospho-oligosaccharide revealed the importance of removing phosphate contents as some hydrolysing enzymes

(including the important maltose-producing exoamylase,  $\beta$ -amylase) are blocked by phosphate residues (Kotting et al., 2009). In contrast, AtLSF2 removes preferentially C3-bound phosphate (Santelia et al., 2012). In contrast to *Atsex4* mutants, *Atlsf2* mutants show no difference in starch levels to the wild type, and nor do phospho-oligosaccharides accumulate. This suggests that side-specificity of AtSEX4 for C3-bound phosphate may be sufficient to mediate its removal during starch breakdown. However, the loss of LSF2 was reflected at the level of total starch-bound phosphate, which was increased by 25%, specifically due to an increase at the C3 position. In addition, it was shown that although *Atlsf2* seems to have only little effect on starch metabolism, the double mutant *Atsex4lsf2* had a more severe starch-accumulating phenotype than the *Atsex4* single mutant. This showed that although AtLSF2 is dispensable for normal rates of starch degradation, its activity becomes important in the *Atsex4* mutant background (Santelia et al., 2011).

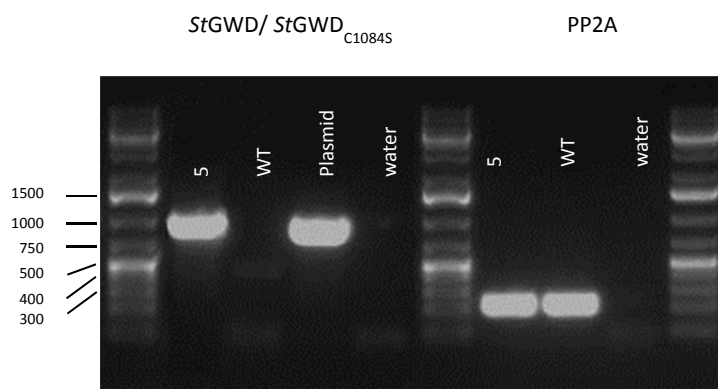
In recent studies, transgenic plants overexpressing *StGWD* were described and published in the scientific literature and in patent applications. *StGWD* was expressed in barley (Carciofi et al., 2011), wheat and corn (Sonnewald and Kossmann, 2013). Carciofi et al. (2011) showed that *StGWD* overexpression leads to more than a 7-fold increase of starch-bound phosphate. This corresponds to 3 times more phosphate than what is found for cassava native starch. Here, we aimed to increase the degree of starch-bound phosphate in cassava starch granules, as well as to increase starch yield. Therefore, three approaches were followed. Two aimed at increasing starch-bound phosphate and one at increasing starch levels in cassava storage root. Firstly, potato *StGWD* was overexpressed either in its wild-type form or as a modified, redox-insensitive form (*StGWD<sub>C108A</sub>*). This was done with the expectation that, if the wild type form was inactive *in vivo* through oxidation, the redox-insensitive form would be constitutively active. Secondly, RNAi constructs were designed against each of the two endogenous genes encoding glucan phosphatases *SEX4* and *LSF2* in order to reduce their activity. Both RNAi constructs are driven by a root-specific promoter to limit dephosphorylation activity specifically in cassava storage roots. Third, an RNAi construct was designed against the plastidial alpha amylase gene (*AMY3*) to decrease starch degradation in storage root and thereby increasing starch yields in an agricultural setting. As shown in the previous chapter, *AMY3* is highly induced at both gene and protein levels during starch remobilisation in cassava storage roots.

## 5.2. Transformation and regeneration of cassava

### 5.2.1. Overexpression of potato glucan, water dikinase in cassava tissue culture

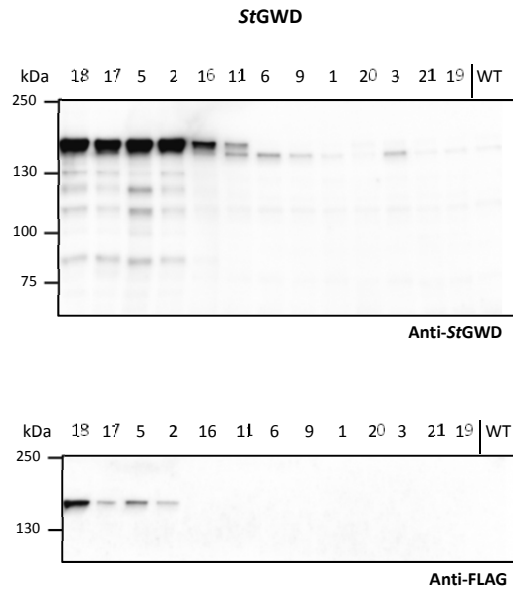
#### 5.2.2. Construct description, *in vitro* analysis

As described, potato starch is highly phosphorylated compared to other plant species. To investigate if the potato *StGWD* would be as efficient in phosphorylating starch granules in other species and to investigate the impact of the mutated, redox-insensitive *StGWD* *in vivo*, constructs for expression of *StGWD* or the redox-insensitive *StGWD*<sub>C1084S</sub> were transformed into cassava friable embryonic cell cultures (FECs; see Chapter 2.12; Figure 2.1). In subsequent steps, the transformed FECs were successfully regenerated on selective media, containing the antibiotic geneticin. The last steps for regenerating plantlets are performed on media without antibiotics. Thus, the regenerated plantlets were subsequently re-tested by growing them on selective rooting media containing the antibiotic geneticin. In this test, only transformed plantlets containing the construct produce roots. For the *StGWD* construct, 22 individual plant lines were isolated and for the *StGWD*<sub>C1084S</sub>, 43 plant lines were isolated. The insertion of the transformation construct in the cassava plantlets was further verified by insert-specific PCR (Figure 5.1, Chapter 2.12.1) and DNA sequencing of the products. For *StGWD* lines, this revealed an 81% positive transformation rate. For the *StGWD*<sub>C1084S</sub> lines, all lines selected in the rooting test were positive by PCR and sequencing. For further analysis, 13 individual *StGWD* lines and 35 individual *StGWD*<sub>C1084S</sub> were selected.



**Figure 5.1** Representative PCR reactions to test positive *StGWD* and *StGWD*<sub>C1084S</sub> transgene insertion. Primers annealing in the promoter and insert region were chosen for the PCR. Here the transgenic *StGWD* #5 line was loaded on a 1% Agarose gel together with wild-type, plasmid and water as template. For DNA control the *PP2A* was amplified.

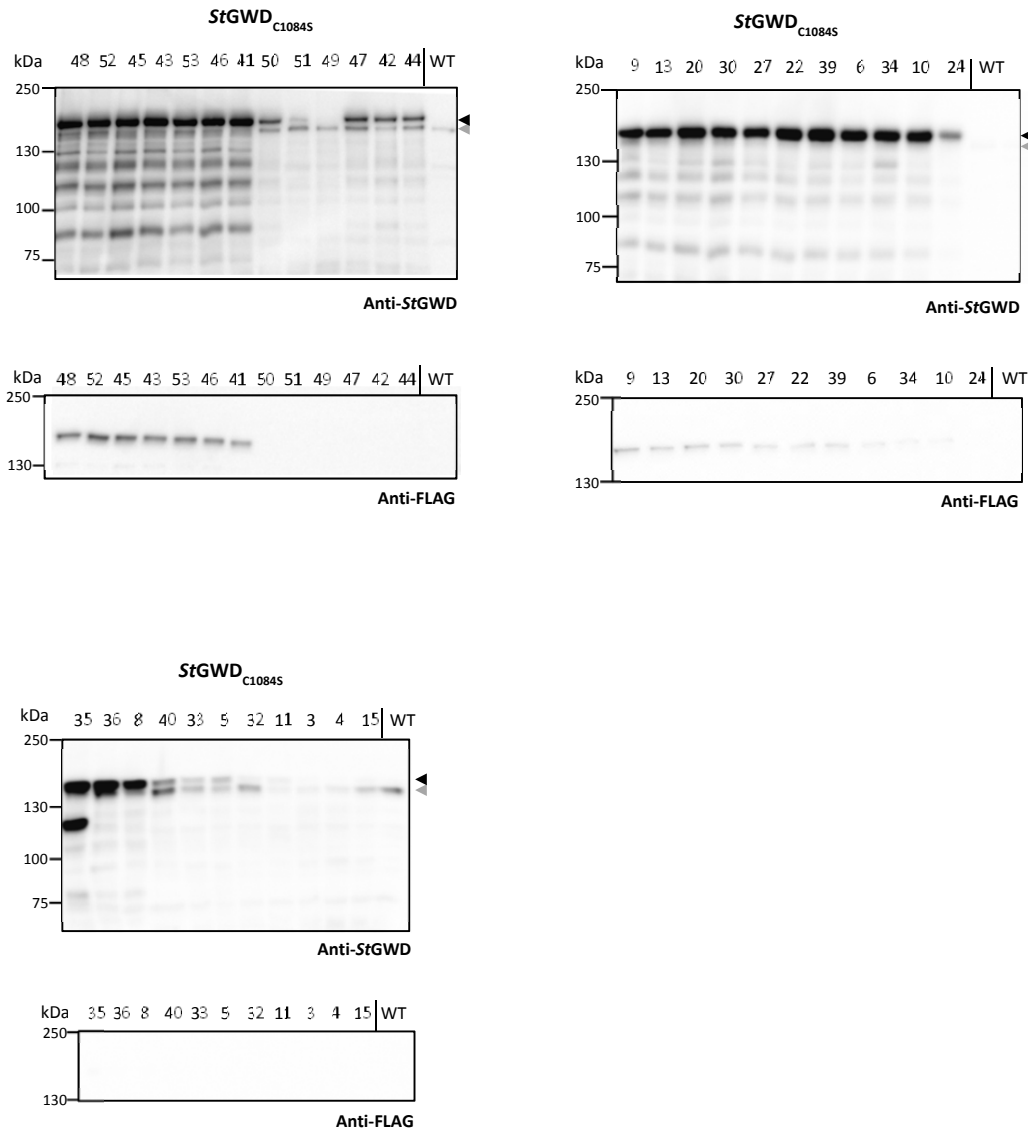
The expression levels of StGWD and StGWD<sub>C1084S</sub> proteins in the selected transgenic lines were investigated by immunoblot analysis, compared to untransformed wild-type plants. As both proteins were tagged with a FLAG-tag at the C-terminus, the protein level was investigated using two antibodies; an anti-GWD antiserum raised against recombinant StGWD protein (Eurogentec) and a commercially-available anti-FLAG antibody raised against the FLAG-tag ( $\alpha$ -M2, Sigma). The anti-GWD antibody recognised both the overexpressed StGWD/StGWD<sub>C1084S</sub> (162 kDa) protein and another protein of similar molecular weight – presumably the endogenous MeGWD (155 kDa). This second band was variable in intensity, but was observed in both transformed plants and the wild type. As shown in Figures 5.2 and 5.3, the expression level of the recombinant protein in leaf tissue varied from high to undetectable between the individual transformed lines. The antibody against the FLAG tag was used to discriminate between the endogenous and overexpressed StGWD protein. Immunoblots incubated with the anti-FLAG antibody showed that it was specific (i.e. the second band was not visible), but the overall sensitivity was much lower than for the anti-GWD antibodies. Considering both the anti-GWD and the anti-FLAG immunoblots, the transgenic lines were divided into three groups of high, intermediate and low/non-expressing lines. High expression refers to clear protein detection with both antibodies; intermediate when protein expression detected with the anti-GWD, but not the anti-FLAG antibodies; low when neither antibodies detected the overexpressed proteins. For the StGWD over-expressing lines, four (lines 18, 17, 5 and 2) were identified with high, two with intermediate (lines 16 and 11) and seven with low (lines 6, 9, 1, 20, 3, 21, and 19) protein expression levels (Figure 5.3). For the transgenic StGWD<sub>C1084S</sub> cassava lines tested, there were 18 with high (lines 48, 52, 45, 43, 53, 46, 41, 9, 13, 20, 30, 27, 22, 39, 6, 34, 10, and 24), 15 with intermediate (50, 51, 47, 42, 44, 35, 36, 8, 40, 33, 5, 32, and 11), and four with low (lines 3, 15, 4, and 49) protein expression (Figure 5.3).



**Figure 5.2 StGWD expression levels in transgenic Cassava lines**

Thirty  $\mu\text{g}$  of total leaf protein from individual transgenic Cassava lines were subjected to immunoblot analysis. Replicate blots were performed using anti-StGWD (top panel) or anti-FLAG (bottom panel) primary antibodies. The black arrow indicates the 162 kDa StGWD protein. The grey arrow may represent the endogenous 155 kDa MeGWD protein.

5. Increasing starch-bound phosphate level: A transgenic approach



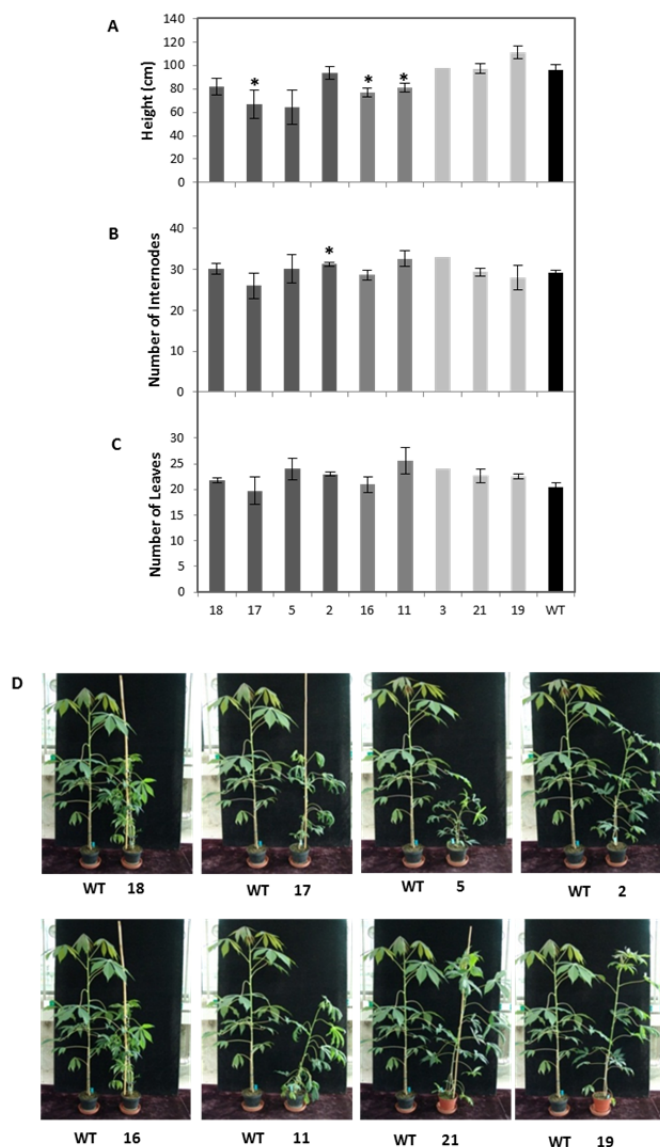
**Figure 5.3** *StGWD*<sub>C1084S</sub> expression levels in transgenic Cassava lines

Immunoblot analysis as in Figure 5.1 of individual transgenic cassava lines using anti-*StGWD* or anti-FLAG antibodies. The black arrow indicates the 162 kDa *StGWD*<sub>C1084S</sub> protein. The grey arrow may represent the endogenous 155 kDa *MeGWD* protein.



### 5.2.3. Growth analysis of transgenic StGWD and StGWD<sub>C1084S</sub> lines compared to wild-type plants

To describe any effects of expression of either the StGWD or the StGWD<sub>C1084S</sub> constructs on growth, yield and starch-bound phosphate, a set of transgenic lines were transferred to soil and grown under greenhouse conditions. The selection of lines for analysis was performed according to the protein levels detected in leaves. Transgenic lines were selected with either high or low expression, the latter serving as transgenic controls. Thus, for StGWD-9 overexpressing lines (18, 17, 5, 2, 16, 11, 3, 21 and 19) (Figure 5.2), and 12 for StGWD<sub>C1084S</sub> the lines (48, 52, 45, 46, 41, 49, 44, 9, 13, 33, 5, 3, 15) (Figure 5.3) were grown in the greenhouse for storage root formation. As an additional control, in-vitro grown wild type (cv. 60444) was transferred on soil. Four months after transplantation of the transgenic lines, plant height, leaf numbers and internode numbers were determined as described in Chapter 3.2.1. Figures 5.4 and 5.5 show the transgenic StGWD and StGWD<sub>C1084S</sub> lines (ordered according to transgene expression levels; see Figures 5.2 and 5.3), compared to wild-type plants. For the transgenic lines expressing the wild-type StGWD construct the number of internodes and leaves showed a tendency to be higher than in wild-type plants though, only for line 2 a significant difference in number of internode was observed (Figure 5.4 B-C). The analysis of leaf number shows a tendency that transgenic plants – StGWD and StGWD<sub>C1084S</sub> – retain the leaves compared to wild type plants, though only for the StGWD<sub>C1084S</sub> – lines 33, 41, 5, and 9 a significant difference was seen. Older leaves were curled and bent downwards, while the newly developing leaves at the apex were apparently unaffected. Although this seemed to be a general phenotype for transformed cassava plants, in StGWD lines 17 and 5, the effect was more severe and seemed to be consistent for all leaves as they aged. The other plants showed the leaf-phenotype for a certain period, but it then well as disappeared at a later stage in development. This leaf phenotype was not observed when the plants were grown *in vitro*. The StGWD lines, when compared to wild-type plants, grew less well shown by plant height (Figure 5.4 A). This reduced growth effect was statistically significant (*Student's t*-test  $\leq 0.05$ ) for the lines 11, 16 and 17. For these transgenic lines the protein expression levels were high. This suggests that the protein expression might influence growth of the internodes as the plants otherwise appear to be at the same developmental stage. The negative influence was obvious in two plant lines with high expression (17, 5), which showed a pronounced stunted growth (Figure 5.4 D). Three out of four lines with highest protein expression levels (18, 17 and 5) showed decreased growth as the two lines with intermediate protein expression (16 and 11) (Figure 5.4 A). However, the transgenic

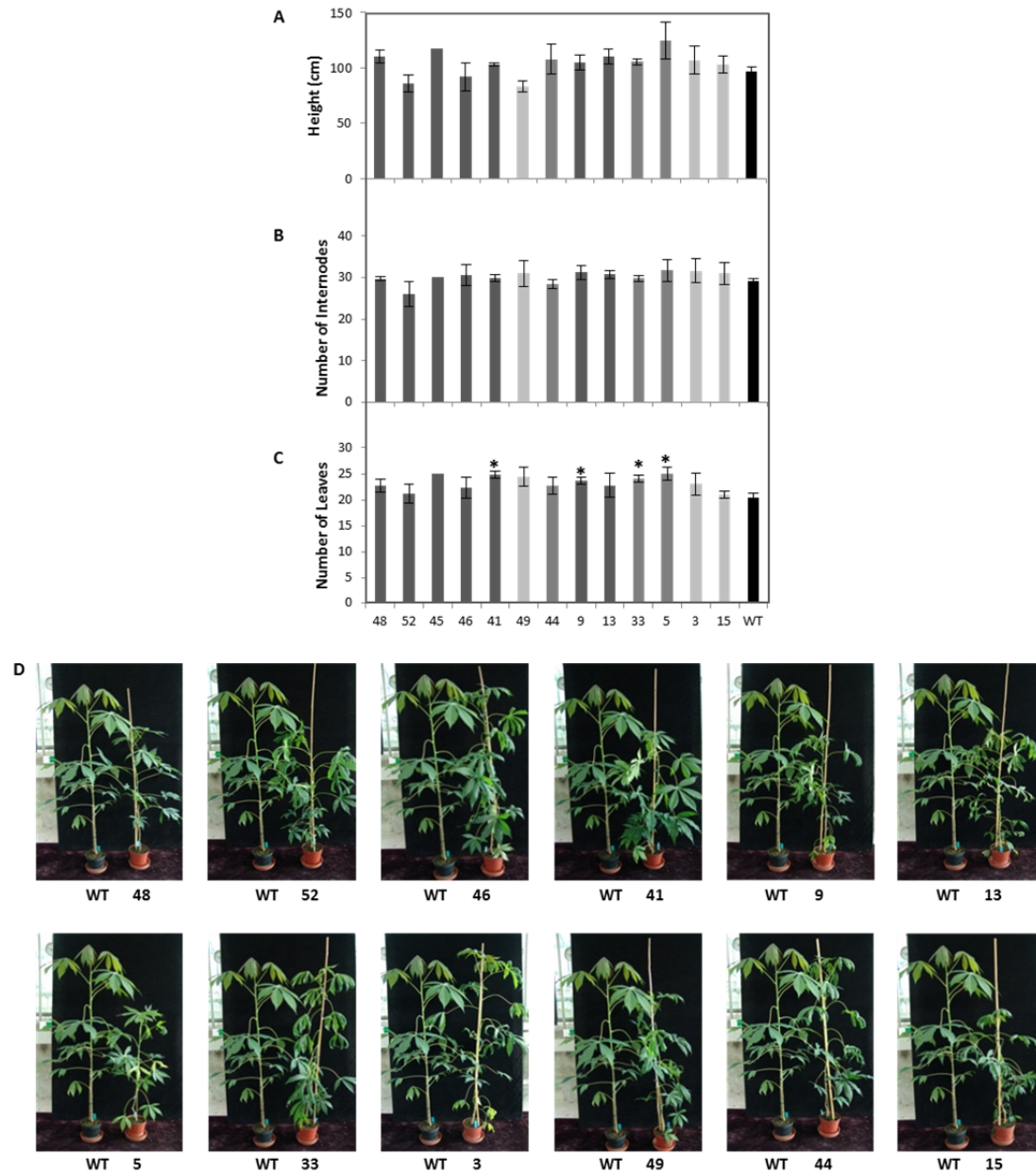


**Figure 5.4 Growth analyses of transgenic StGWD cassava lines**

*In vitro* grown plantlets were transferred into soil and grown in the greenhouse. The general growth parameters were determined after 4 months. The transgenic lines were arranged according the protein loading in Figure 5.1 (from high in dark grey to low in light grey; Figure 5.1). A) Height (cm), B) number of internodes, C) number of leaves. Transgenic lines (grey bars), cv. 60444 wild type (black bar). Mean ± SE (N=1-5). Asterisk shows significant difference (t-test < 0.05) D) Pictures of representative transgenic plants and a wild-type plant.

lines with low or non-detectable expressed protein (3, 21, and 19) grew comparably to wild-type plants. From the analysis of the redox-insensitive StGWD<sub>C1084S</sub> lines, no clear correlation between plant growth parameters and recombinant protein expression could be made. None of the lines showed a significant difference compared to wild-type plants (Figure 5.5).

## 5. Increasing starch-bound phosphate level: A transgenic approach

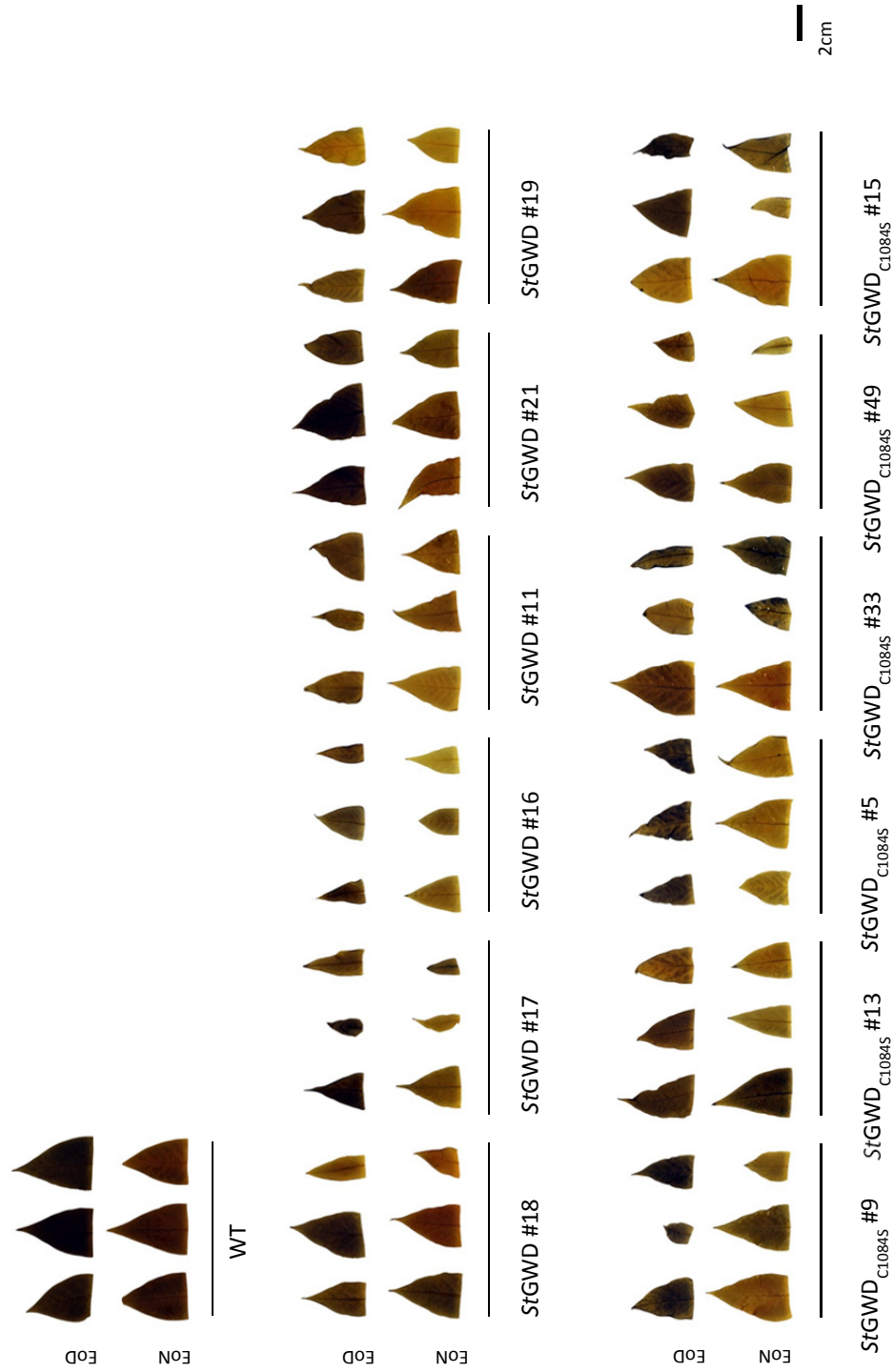


**Figure 5.5 Growth analyses of transgenic *StGWD*<sub>C10845</sub> cassava lines**

*In vitro* grown plantlets were transferred into soil and grown in the greenhouse. The general growth parameters were determined after 4 months. The transgenic lines were arranged according the protein loading in Figure 5.2 (from high in dark grey to low in light grey; Figure 5.2). A) Height (cm), B) number of internodes, C) number of leaves. Transgenic lines (grey bars), cv. 60444 wild type (black bar). Mean  $\pm$  SE (N=1-5). Asterisk shows significant difference (t-test < 0.05). D) Pictures of representative transgenic plants and a wild-type plant.

#### 5.2.4. Starch visualization in leaf and determination of storage root growth in the transgenic plants

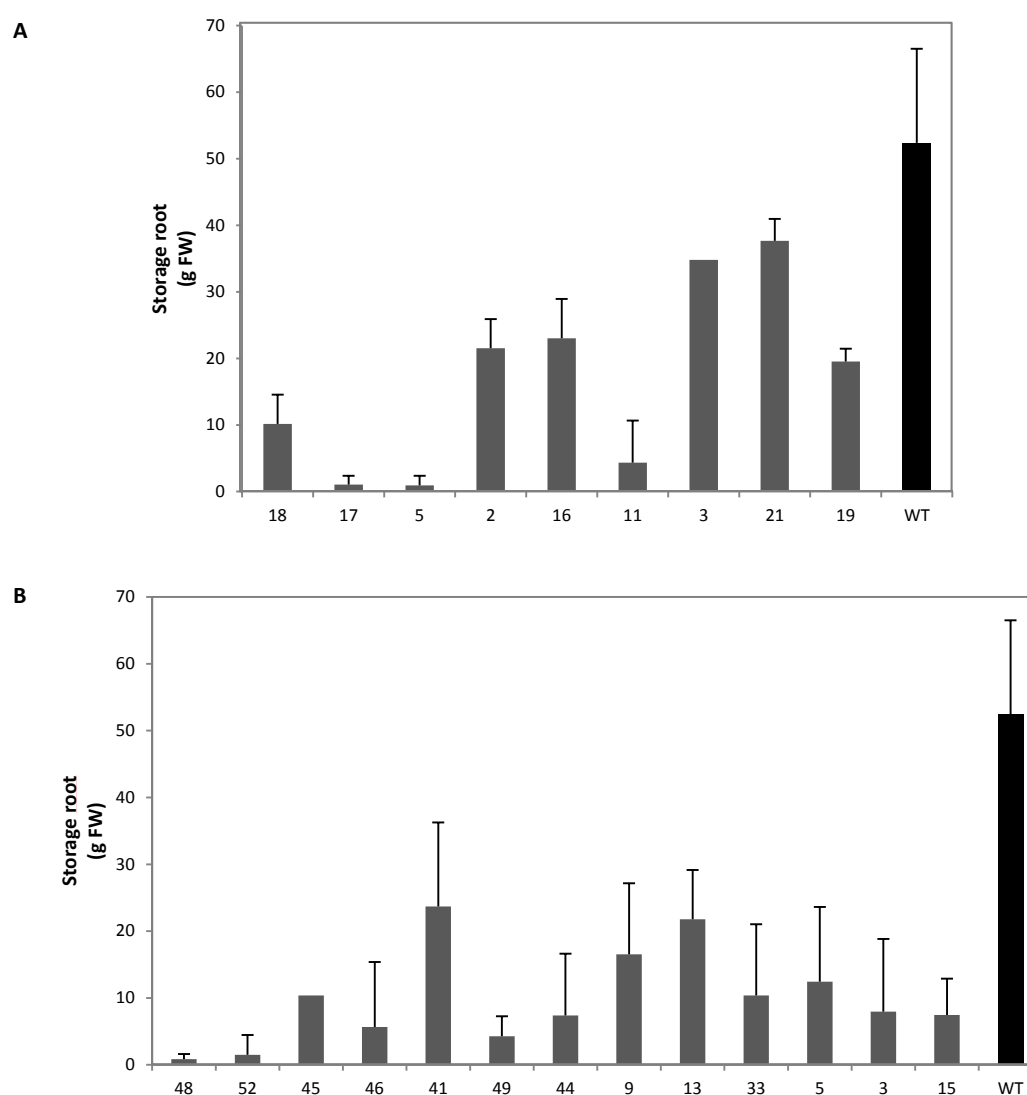
To provide a first insight in impact of *StGWD* or *StGWD*<sub>C1084S</sub> expression on starch metabolism, the tips of leaf 4 or 5 were stained with iodine at the end of the day and end of the night (Figure 5.6). As shown in Chapter 3 (Figure 3.5), leaf starch in the wild type is not fully degraded at the end of the night. Wild-type leaves stained in a brown-reddish colour at both time points. For the transgenic lines, leaf tissue was harvested from two plants representing high, intermediate and low protein expression levels. Starch stained a brown-reddish colour as in the wild type. For the transgenic *StGWD* lines with high protein expression (18, 17), starch had accumulated at the end of the day and a residual amount still seen at the end of the night. The two *StGWD* transgenic lines with intermediate protein expression level (16, 11) had starch at the end of the day, but less starch could be seen at the end of the night than in the wild type. For one low expressing line (21) starch was detected at both time points where for another (19) less starch was observed at the end of the night compared with the end of the day. Interestingly, the transgenic lines expressing the redox insensitive *StGWD*<sub>C1084S</sub> starch stained darker in contrast to the brown-reddish colour of the wild type and the *StGWD* overexpressing lines. The redox insensitive lines expressing high protein levels (9, 13) and intermediate protein level (5) had starch at the end of the day but did not stain at the end of the night. The leaves of a second line expressing intermediate protein levels (33) stained for starch at both time points. The two non-expressing *StGWD*<sub>C1084S</sub> lines stained for starch at the end of the day but not at the end of night (Figure 5.6). Overall preliminary results hint at a difference in the starch in the *StGWD*<sub>C1084S</sub> lines, but these differences (either qualitative or quantitative) need to be confirmed by further measurements and analyses.



**Figure 5.6 Iodine staining of transgenic StGWD/StGWD<sub>C1084S</sub> leaves at the end of the day (EoD) and end of the night (EoN).** Leaf tips from transgenic StGWD and StGWD<sub>C1084S</sub> lines, with different protein expression levels were harvested at EoD and EoN were stained with iodine.

## 5. Increasing starch-bound phosphate level: A transgenic approach

To investigate the phosphorylation activity of StGWD and StGWD<sub>C1084S</sub> on cassava starch, the storage roots of the transgenic plants were harvested after 6 months. The mean storage root fresh weight of the transgenic plants was in all cases lower than in wild-type plants (Figure 5.7). On average, the storage root fresh weight of StGWD lines was 68% lower than in the wild type, varying between 0.93 g and 37.67 g (Figure 5.7 A). On average, the storage root biomass production of StGWD<sub>C1084S</sub> lines was 81% less than in the wild-type, varying between 0.85 g and 10.37 g (Figure 5.7 B). In some of the individual transgenic line no storage roots had developed. No correlation was observed between storage root development and transgenic protein expression (Figure 5.7).

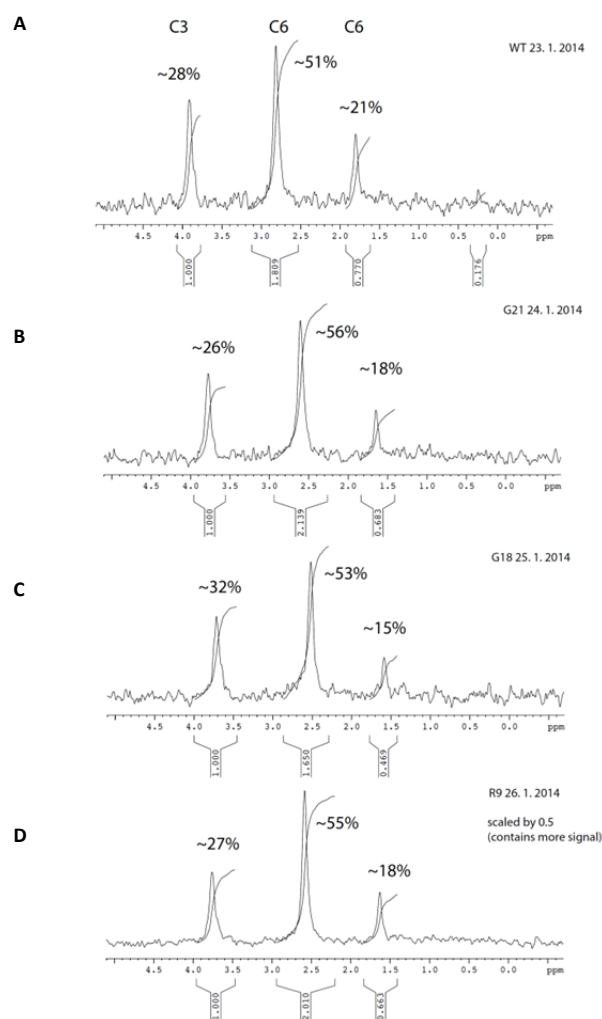


**Figure 5.7 Storage root of transgenic StGWD and StGWD<sub>C1084S</sub> cassava lines**

The fresh weight of the storage roots from transgenic (grey bars) and wild-type (black bars) plants was determined after 6 months of growth. A) StGWD lines B) StGWD<sub>C1084S</sub> lines. The transgenic lines are arranged according to the protein expression levels (high to low) given in Figures 5.1 and 5.2. Mean  $\pm$  SD. (N=1-5).

### 5.2.5. Storage starch: $^{31}\text{P}$ -NMR reveals increased phosphate bound to C6 and C3 position in *StGWD*<sub>C1084S</sub>

Starch was isolated from the *StGWD* and *StGWD*<sub>C1084S</sub> lines and subjected for  $^{31}\text{P}$ -NMR analysis to get information about the ratio of C3 and C6 phosphorylation, as was described by Santelia et al. (2011). For these measurements, starch was isolated from the storage roots of representative transgenic lines as well as from the wild type. The non-expressing *StGWD* line (21) was used as a transformation control (Figure 5.2). Representative lines with high protein expression for *StGWD* (#18) and *StGWD*<sub>C1084S</sub> (#9) were chosen (Figure 5.2, Figure 5.3).  $^{31}\text{P}$ -NMR gives a relative percentage of C3 and C6 phosphorylation within a sample, and is also semi-quantitative for phosphate abundance. All analysed samples revealed similar C3:C6 phosphorylation ratio between 2.1 and 2.7 (Figure 5.8). The peak intensity was similar for all samples except for the *StGWD*<sub>C1084S</sub> line 9 highly expressing the redox insensitive form. For this sample the peak was more intense (the y-axis scale in Figure 5.8 D is double that of the other spectra in A-C). This suggests that more total phosphate was present. However, for quantification of total bound phosphate other, more quantitative experimental approaches are needed.



**Figure 5.8 Evaluation of phosphate bound to C6 or C3 position in transgenic *StGWD* and *StGWD*<sub>C1084S</sub>**  
<sup>31</sup>P-NMR one-dimensional spectra of hydrolyzed root starch of wild-type and *StGWD* and *StGWD*<sub>C1084S</sub> transgenic lines. Recorded between 9216 and 16,384 transients at 303K, pH 6.0. Peak areas are proportional to the relative amount of glucan-bound phosphate and are given as a percentage on top of each peak. Chemical shifts are referenced to external H<sub>3</sub>PO<sub>4</sub> (85%) A) wild type B) transformation control *StGWD* #21, non-expressing (Figure 5.2) C) *StGWD* #18, a high expressing line D) *StGWD*<sub>C1084S</sub> #9, high protein expressing line. For D) the peak signal is half compared to the samples A-C). Figure and measurements conducted and adapted from Dr. M. Schubert, ETH Zürich.

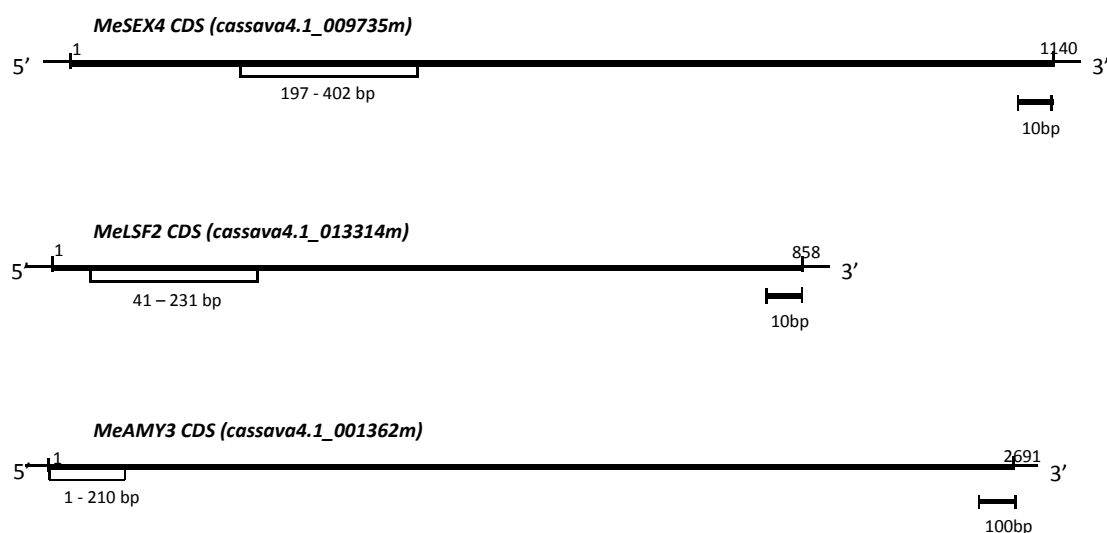
### 5.2.6. Increase in total starch phosphate: RNAi construct design against *MeSEX4* and *MeLSF2*

Another approach to increase phosphate content bound to starch is to knock-out the glucan phosphatases *SEX4* and *LSF2* using the RNAi technique. In Arabidopsis, it was shown that *Atsex4* mutants lead to stunted growth. This stunted growth is caused by the fact that *Atsex4* mutant plants accumulate starch that is not degraded during the night, thus the supply of carbon during the night is blocked (Zeeman et al., 1999; Kotting et al., 2005). Thus,



it is possible that down-regulation or knocking-out of *MeSEX4* transcript might lead to a comparable impact on whole plant growth in cassava, and negatively affect biomass production. Furthermore, the propagation of plants by stem cuttings might also be negatively affected when starch can't be degraded to fuel regrowth. Therefore, an RNAi construct was designed driven by a potato patatin class I promoter, which was shown to be tuber specific in potato and root-specific in *Arabidopsis* (Naumkina et al., 2007).

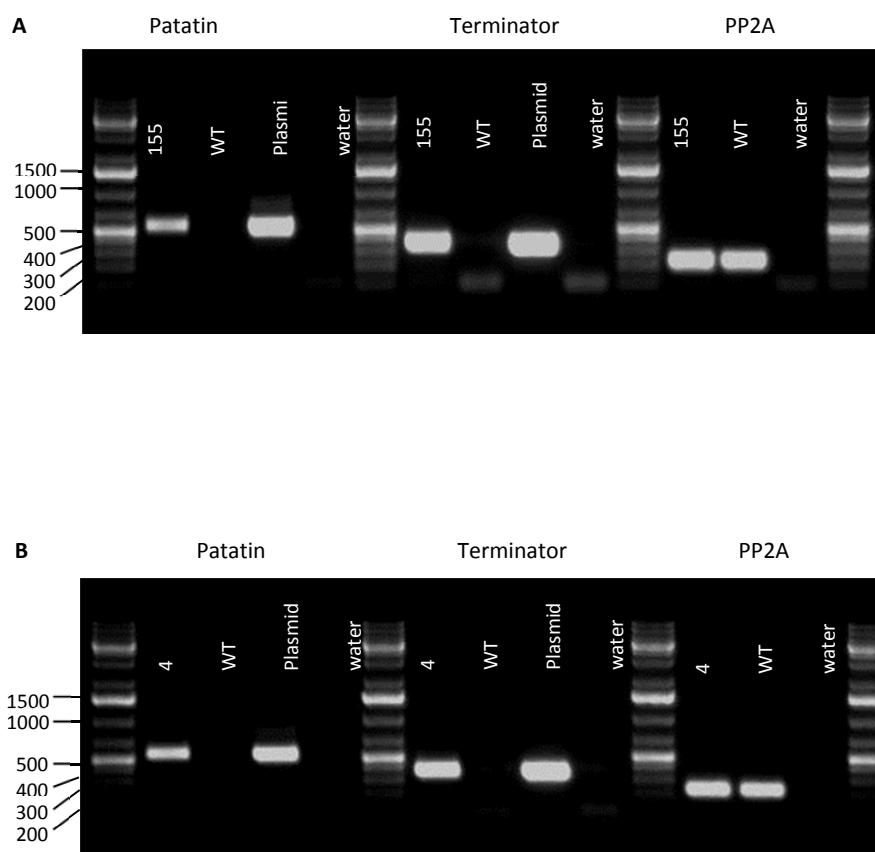
The protein sequences of *AtSEX4* and *AtLSF2* were used as a query to search for the orthologous proteins in cassava. A BLASTP search was performed against the cassava proteome database ([www.phytozome.net](http://www.phytozome.net); Prochnik et al., 2012). The translation product of gene accession number *cassava4.1\_009735m* showed highest homology to the *AtSEX4* protein sequence and the translation product of gene accession number *cassava4.1\_013314m* showed highest homology to the *LSF2* protein. For the production of the RNAi hairpin constructs, sequences unique to the individual transcripts (205 bp for *SEX4*-RNAi and 190 bp for *LSF2*-RNAi) were chosen (Figure 5.9 A, B). All RNAi constructs were cloned into a modified pCAMBIA1301 vector with a patatin class I promoter and Nos-terminator (Figure 2.1).



**Figure 5.9 Sequences used for RNAi construction**

The indicated sequence form the corresponding CDS was used as a template to design a hairpin construct

Both constructs were successfully cloned and transformed into cassava FECs according to the protocol described previously (Bull et al., 2009; see also Chapter 2). For the SEX4-RNAi lines 155 and for LSF2-RNAi lines 133 individual plants were grown on selective growth media containing hygromycin. Under these conditions, only transgenic plantlets produced roots. For the SEX4-RNAi construct 46 and for the LSF2-RNAi construct, 57 plantlets made roots (Table 5.1). Using PCR reactions with primer combinations specific for the promoter and terminator sequences present in the T-DNA insertion, integration of the construct into the genomic DNA was confirmed (Figure 5.10). The confirmation PCR analysis revealed that for SEX4-RNAi lines the majority (84%) were positive for both PCR reactions. Four lines were positive for only promoter or terminator sequence PCRs and one line showed no insertion. For the LSF2-RNAi lines, 86% were positive for both sequences, 17% were positive for either promoter or terminator sequence, and two lines showed no insertion. Preliminary observations suggest that the SEX4-RNAi lines have a pronounced slow-growth phenotype in tissue culture and that the leaves for several independent lines are abnormally narrow. In contrast, LSF2-RNAi lines are comparable to the wild type plants grown in tissue culture.



**Figure 5.10 Representative PCR reaction to test positive RNAi-lines**

Promoter (Patatin) and terminator (Terminator) sequence were amplified to verify the insertion of the hairpin construct. For each line a positive transgenic line, wild-type and plasmid containing the respective RNAi insert and water was loaded as indicated. For DNA control the *PP2A* was amplified. A) *SEX4*-RNAi, positive line #155 B) *LSF2*-RNAi line, positive control line #4.

### 5.2.7. RNAi construct design against *MeAMY3*

As described in Chapter 4, *AMY3* was identified as a likely candidate enzyme involved in starch degradation in heterotrophic storage root tissue. In order to test this biological role of *AMY3* in storage starch degradation and potentially increase storage root biomass by preventing unwanted starch breakdown, I designed a hairpin construct against the *MeAMY3* transcript. Although *AMY3* is dispensable for starch degradation in *Arabidopsis* leaves, we do not know if this is the case for cassava. Furthermore, down-regulation of *AMY3* could block starch degradation in heterotrophic tissue such as the stem. Thus, expressing a hairpin construct against the *MeAMY3* transcript on a whole plant level could potentially have a negative impact on plant growth and propagation, as described for *SEX4* down-regulation above. Thus, the *MeAMY3* RNAi hairpin was also designed to be driven by the patatin class I promoter (Naumkina et al., 2007).

The *AtAMY3* protein sequence was used as a query to search for the orthologous protein in cassava. A BLASTP search revealed that the protein encoded by the gene accession number cassava4.1\_001362m has the highest sequence homology to *AtAMY3*. For the RNAi hairpin construct, a unique 210-bp sequence matching the 5' end of the *MeAMY3* coding sequence was chosen (Figure 5.9 C). The RNAi construct was cloned into a modified pCAMBIA1301 vector (Figure 2.1).

At the time of writing, the *AMY3*-RNAi construct the plasmid has been transformed into FECs. The transformed FECs are growing on selection media (Figure 2.2, step 4 and 5) and 22 individual plantlets are currently regenerating on growth medium (Table 5.1). Next, these plants will be transferred and tested for rooting capacity on a medium containing hygromycin (Figure 2.2, step 6).

### 5.3. Discussion

#### 5.3.1. Analysis of Cassava plants expressing *StGWD* and *StGWD*<sub>C1084S</sub>

Here, I describe the successful isolation of multiple independent *StGWD* and *StGWD*<sub>C1084S</sub> overexpressing lines with different protein levels. Preliminary characterisation of growth parameters showed no persistent correlation between protein expression and growth behaviour. Though, three out of 4 high expressing *StGWD* lines were significantly smaller than wild-type plants (Figure 5.3 A). A reason might be that the insertion sites of the transgene disrupt endogenous genes affecting growth. However, this would be unlikely to occur at the relatively high frequency with which we observed reduced growth. In addition it might be that the plantlets coming from *in vitro* culture need some time to recover after changed conditions. However, transformed plants without transgene expression seemed not to be affected during this environmental change and grew as well as wild-type plants. The correlation between transgene expression and growth behaviour needs to be investigated in subsequent generations. In addition, the data for transgene protein expression level comes from leaf tissue. Although the transgene is driven by the ubiquitous 35S promoter it is possible that the protein expression in storage root differs. Thus, in order to correlate aspects of the phenotypes it will be crucial to determine transgenic protein expression level in storage roots as well. Then the overexpression of a key enzyme involved in starch metabolism could potentially influence the performance of any starch synthesising tissue, which further might have secondary effects on the whole plant.

It is known that repression of *AtGWD* leads to starch accumulation and stunted growth, caused by the reduced remobilization of starch during the dark period (Yu et al., 2001). Starch accumulation and hypo-phosphorylation was found in potato tubers with repressed *StGWD* (Lorberth et al., 1998). However, overexpressing *StGWD* in cassava could render starch more accessible to hydrolytic degradation because of phosphate-esters bound to starch solubilize the granule surface by disrupting the packing of double helical structure of amylopectin. In this case, the carbohydrate supply essential for growth would not be limited. Thus, I assume that overexpression of *StGWD* in cassava plants doesn't change growth phenotype substantially. As shown in Figure 5.4 the staining of transgenic *StGWD* and especially the *StGWD*<sub>C1084S</sub> lines degrade as much, if not more starch during the night compared to wild-type plants. An indication for facilitated starch remobilization caused by *GWD* overexpression was shown by Carciofi et al., (2011), where starch granules were visualized by Scanning Electron Microscopy (SEM). The starch granules from the endosperm of *StGWD* overexpressing barley lines had pores and irregularities on their surface that were not seen in the starch from the wild type. Similar pore like structures were shown for barley starch granules after partial  $\alpha$ -amylolytic hydrolysis (Li and Yeh, 2001). Thus, additional phosphate-esters on starch granules may indeed render the amylopectin more accessible to hydrolytic enzymes (Edner et al., 2007). Whether this occurs in my cassava lines and has an influence on starch levels will need to be tested. An increased phosphate-level bound to starch is also likely to change the starch properties as the granule architecture might be less densely packed and thus, exhibiting differences in physico-chemical behaviour. In addition, the staining of starch for the transgenic *StGWD*/*StGWD*<sub>C1084S</sub> shows differences in metabolizing starch efficiency indicating that starch hydrolysis is facilitated. However, a more detailed investigation with quantitative measurements of the starch content at the end of the day and end of the night will be needed to confirm the preliminary finding with iodine staining.

The aim of the transgenic lines was to increase total phosphate content on starch and potentially to modify starch properties. In barley, *StGWD* overexpression increased the total starch-bound phosphate content in the endosperm by 7-fold (Carciofi et al., 2011). Preliminary analysis of C3- and C6-bound phosphate by <sup>31</sup>P-NMR measurements revealed that although there was no shift in C3:C6 ratio, the high peak intensity in the starch sample from a plant highly expressing the redox insensitive form of *GWD* seems highly promising (Figure 5.8). As <sup>31</sup>P-NMR is not a quantitative method to determine total starch bound phosphate levels further experiments will need to support these preliminary analyses. At the

time of writing, analyses of total phosphate using assays based on the Malachite Green reagent seem to confirm a doubling of the phosphate content of the starch from this line (Wuyan Wang, unpublished results). Furthermore, the results I present here were gained from just one biological replicate. Thus, in order to unravel the functional effect of the transgenic lines these first results need to be corroborated by the analysis of other transgenic lines expressing the two versions of the GWD protein. However, taken together, the preliminary data of staining leaves with iodine and the  $^{31}\text{P}$ -NMR analysis of the redox-insensitive *StGWD*<sub>C1084S</sub> suggests that the starch in the transgenic line potentially has an altered structure (i.e. amylopectin) as visualized by iodine (Figure 5.6) and phosphate content.

It will now be essential to perform further experiments to reveal the full effect of *StGWD* and its redox-insensitive version on cassava root starch metabolism. The first thing will be to confirm the increase in total phosphate content, which can be measured using one of three different ways: Firstly, the aforementioned Malachite Green assay can be performed to determine total phosphate content after enzymatic glucan dephosphorylation. Secondly, it will be important to determine with additional NMR analyses whether there is a shift in the ratio of C3:C6 phosphorylation. On the one hand, one might expect an increase in C6 phosphorylation, given that GWD specifically mediates this reaction. However, as PWD depends on previous action of GWD, an increase in C3-bound phosphate might also occur as a consequence. A third method to discriminate between C6- and C3- bound phosphate quantitatively is by HPLC after acid-hydrolysis of the starch to release Glucose 6-P and Glucose 3-P. If an increase in phosphate is achieved, further structural analyses on the starches from the transgenic plants will be required to determine if starch granule morphology, composition (amylose:amylopectin ratio), or architecture (amylopectin chain length distribution) are affected. Furthermore, it will be crucial to analyse the physico-chemical properties to elucidate whether characteristics of commercial interest (i.e. paste clarity, viscosity, retrogradation) are improved.

### **5.3.2. RNAi-constructs transformed to Cassava cv. 60444 under investigation**

All three RNAi constructs described here have been transformed into cv. 60444 plantlets. For all three constructs SEX4-RNAi, LSF2-RNAi and AMY3-RNAi lines, plantlets could successfully be re-generated. For SEX4- and LSF2-RNAi genotyping revealed positive lines (Table 5.1). Whereas the phenotype of *in vitro* grown LSF2-RNAi lines is comparable to

wild-type plants SEX4-RNAi lines show growth retardation and reduced leaf area. This phenotype might be caused by the transformation process, as suggested for *StGWD/ StGWD<sub>C1084S</sub>* expressing plants grown on soil. However, the LSF2-RNAi lines would then also be expected to show this phenotype; the transformed plasmid is the same and the transformation process was done in parallel. This implies that the growth phenotype, if substantiated, is caused by the SEX4 RNAi construct. As shown for Arabidopsis, loss of SEX4 reduces growth as a consequence of being unable to degrade transitory starch at night, restricting carbohydrate supply (Kotting et al., 2009). The observation of retarded growth of my SEX4-RNAi lines, although not yet quantified, may reflect a similar metabolic perturbation. However, it is important to stress that the construct is driven by the class I patatin promoter which is supposedly root specific. The possibility exists that the promoter is leaky or that, in cassava, it is not exclusively expressed in roots. Furthermore, there are reports that the patatin promoter is induced by sucrose (Naumkina et al., 2007), which is supplied to the growth media at this stage in the transgenesis process. That said, it is questionable whether such a metabolic defect, as described above for Arabidopsis, would restrict growth in material grown in such sugar-rich culture medium.

Careful evaluation of promoter function is important when considering starch remobilization to avoid negatively influencing growth the propagation of material through stem cuttings. This will need to be investigated in further detail, for instance by transcript analysis in leaves and by analysis of transgenic plantlets transferred to soil (where no exogenous sucrose is supplied). Staining leaf tissue to detect a possible starch-excess phenotype will give a first indication as to whether the construct is expressed and functional in tissues other than storage roots.

Concerning the RNAi-LSF2 lines, after positive genotyping it will be crucial to analyse transcript levels in leaves and storage roots, once the lines are transferred on soil. *In vitro* grown LSF2-RNAi plantlets do not show any growth defects. This is unsurprising as this was also the case for *Atlsf2* mutants (Santelia et al., 2012). To confirm the functionality of this construct it will be critical to investigate storage starch directly to see whether there is a change in phosphate content and distribution like that seen in Arabidopsis *Atlsf2* mutants. This will be highly novel as the role of LSF2 in storage starch metabolism has never been studied. Furthermore, though it is well known that the level of starch phosphorylation has an impact on starch properties, it is not known what influence a changed ratio of C6:C3 bound phosphate has. The physico-chemical properties of Arabidopsis starches were not

investigated, presumably because of the difficulties in obtaining adequate amounts. This should not be a problem with cassava and if my transgenic plants exhibit the desired effects on starch bound phosphate, it may have real potential for future industrial applications.

Analysis of the AMY3-RNAi lines will give new information about starch mobilization in cassava storage roots. The finding that AMY3 has a minor role in Arabidopsis leaf starch metabolism (Yu et al., 2005) has dampened interest in this protein, but the high expression in cassava storage roots upon transition from sink to source tissue (Chapter 4) renews the question of how important AMY3 might be in this and other systems. The minor role in leaf starch metabolism, if also the case for cassava, makes AMY3 a good potential candidate for repression in that it may affect specifically root starch, though the aforementioned question about whether stem starch metabolism will also be affected remains. Thus, analysis of the AMY3-RNAi line will be important to reveal the importance of  $\alpha$ -amylolytic activity in heterotrophic tissue, and to what extent other hydrolysing enzymes are able to compensate for the loss of AMY3.

### 5.3.3. Outlook

In the longer term, there may be still more to gain by combining the modifications I have initiated here. In respect of generating phosphorylated starch in cassava I would think that generating a double transgenic overexpressing StGWD and repressing SEX4 and/or LSF2 could potentially increase starch yield as well as phosphorylation of starch further than any of the single modifications can.

In *Atsex4* mutant lines, glucan-bound phosphate accumulates as phospho-oligosaccharides rather than phosphorylated starch. It was shown that these phospho-oligosaccharides are released from the starch granule by the debranching enzyme ISA3 and by AMY3 (Kotting et al., 2009). The double mutant *Atsex4amy3* revealed a decrease in phospho-oligosaccharides and increase starch levels. Thus, repressing both AMY3- and SEX4/LSF2 might again increase starch yield and starch bound phosphate in cassava storage roots.

As described earlier, before the harvesting of cassava storage roots is performed, the above-ground plant part is cut off. This cutting was shown to increase shelf-life of the roots, as PPD response is delayed. However, during this process starch is degraded and thus yield is affected. It might be of interest to investigate if blocking starch degradation in SEX4-RNAi or AMY3-RNAi has any effect on the PPD response. If it does, such lines could be of substantial interest for cassava growers.



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## 6. General Discussion

In the first part of my PhD thesis I described growth analysis of greenhouse-grown cassava plants to gain information about experimental reproducibility and showed that the plants behaved in a comparable way. The absolute values of carbon assimilation and non-structural carbohydrates were lower than what has been described in the literature. This is likely because the previous measurements were mostly gained from agronomic cassava cultivars grown in the field (Angelov et al., 1993). It is not surprising that the absolute values differ. Unlike greenhouse experiments, growth in field is not limited in respect of soil space and the environmental conditions are not equally comparable. However, the advantage of our experimental design lies in the high reproducibility between experiments, which is important for this kind of basic research. I could show that under the given conditions, cassava accumulates similar starch levels in leaves during the day as does *Arabidopsis* (Gibon et al., 2004). Further, I could show that starch is synthesised during the day degraded during the night, as reported for *Arabidopsis* (Gibon et al., 2004; Fulton et al., 2008). Interestingly, in contrast to *Arabidopsis*, the synthesised starch is not fully remobilized at the end of the night and the residual starch content varies, depending on leaf age. (Figure 3.6 E). This is an important observation as it shows that single time point measurements or single leaf analysis have to be treated carefully. Further, the incomplete remobilisation suggests that the demand during the night is for less than the available stored carbohydrate. This would be an interesting point to follow up, because it could mean that cassava could grow faster with the available stored resources, which in turn opens possibilities for cassava varieties with more biomass production. Soluble sugar analysis revealed high levels of sucrose; far more than what is reported in *Arabidopsis* (Figure 3.6 F-H) (Gibon et al., 2004).

Combining the photosynthetic rate measurements and the levels of starch accumulated during a photo period in young leaves, a hypothetical carbon partitioning rate was calculated. This revealed that only 5-11% of the assimilated carbon is subjected into starch (Table 3.1 and Table 3.2). In contrast high levels of sucrose were measured. However, the photosynthetic rate and determination of carbohydrate levels were not performed with the same plant batch and not at the same time. This could potentially influence absolute values as suggested by comparing two carbohydrate measurements from two different plant batches analysed for the influence of leaf development and diurnal starch turnover (Figure 3.6). Thus the actual carbon flux is yet unknown. As mentioned in 'Chapter 3' <sup>14</sup>C-labelling experiments could substantiate this calculated value for carbon partitioning.

Nevertheless, in terms of source capacity (e.g. carbon assimilation and transport), the residual starch levels at the end of the night and high sucrose levels 9h into the day strongly indicate that cassava plants grown in the greenhouse are not restricted in carbohydrate availability (Figure 3.6 B,E). The carbon assimilated and stored throughout the day exceeds the demand of sink tissues. This is highly interesting in the context of biotechnological applications, where approaches are considered in order to increase source capacity. Hence, from the results I present here I conclude that the source capacity of cassava canopy is already high. As mentioned above for the carbohydrate analysis, plant batches used for analysis were grown at different times of the year. Comparing the absolute values and the pattern of carbohydrate allocation reveals that in terms of soluble sugars absolute levels differ whereas starch levels remain comparable amongst the plant batches (Figure 3.6). Therefore, sucrose and hexose pools could potentially serve as a buffering system to meet the needs for non-structural carbohydrates. It is likely that under stressful environmental conditions where low carbon assimilation occurs (i.e. scarce environmental conditions like water limitation, low light conditions, and high temperature), accumulated soluble sugars could compensate for the carbon deficiency. This hypothesis needs further investigation, for instance labelling experiments in combination with stress analysis might reveal changes in soluble carbohydrate availability and flux. It is also possible that the non-structural carbohydrate levels follow a species-specific pattern different to what has been described for *Arabidopsis* (Gibon et al., 2004). This could be tested with a 24h experiment where plant material is harvested throughout the day and night at different time points.

### **6.1. Integrating Proteomics and carbohydrate metabolism**

As cassava is an important starch crop, strategies to increase yield in storage roots could potentially be achieved by increasing source capacity or sink strength or by preventing starch mobilization.

An increase in source strength could potentially provide increased carbohydrates for transport to sink tissues. The finding of residual starch levels at the end of the night and high soluble sugar content in leaves (Figure 3.6) suggests that the carbohydrate available for transport exceeds the need during the night. Thus, a more promising approach to increase starch yield in heterotrophic tissue could be to increase sink strength in storage roots. In the past attempts to increase sink strength in various crop species was shown to be challenging, because sucrose or sugar availability revealed to be tightly regulated. This was shown by overexpression of yeast INV in the apoplast or cytosol of potato tubers, which increased

tuber water content, but not starch levels (Ferreira and Sonnewald, 2012). For the cytosolic INV expressing lines, increased glucose levels were observed, whereas sucrose and starch levels decreased. Further analysis revealed that high levels of hexose-phosphate were not imported to the amyloplast for starch synthesis but rather subjected to glycolysis (Sonnewald et al., 1997; Hajirezaei et al., 2000). This illustrated that the increase in hexoses derived from the hydrolytic activity of INV is channelled in an unexpected way. It is likely that the changes in hexose availability mimic the situation of high sugar availability. As mentioned before sucrose and hexoses are sensed by various proteins that trigger specific responses at the molecular level. Evidence for sugar signalling occurring in cassava storage root upon pruning (sugar starvation) are given by the identification of overrepresented key signalling proteins (e.g. SnRK1, HXK1). This suggests that a response to changes of metabolite availability does occur. Strategies to increase sink strength in cassava simply by increasing sucrose hydrolysis may also trigger a cellular response to carbohydrate availability as they did in potato.

It was shown that starch yield can be increased by SUS overexpression. Evidence for the correlation of SUS activity and starch synthesis came from transgenic potato plants where SUS was repressed. A lack of SUS led to decrease of starch levels, whereas *StSUS4* overexpressing lines accumulated up to double the starch amount detected in wild-type plants (Baroja-Fernandez et al., 2009). Thus, these findings suggest that SUS overexpression has a potential to increase starch yield in storage organs. Successful transformation of cassava lines were performed in another project, though the analysis is still under investigation (*Miyako Keller, unpublished*).

As an alternative to increasing yield, I decided to block starch degradation. As cassava storage roots accumulate up to 85% starch per dry weight, this yield already is high. However, as described, cassava plants are pruned before harvesting in order to minimize post-harvest physiological deterioration. To inhibit starch mobilization in a targeted way I performed a proteomic analysis to reveal important starch mobilizing enzymes in heterotrophic tissues.

My experimental setup showed that 10 days after pruning off the aerial parts of cassava there was a decrease in starch levels by half in storage roots to support regrowth of new leaves (Figure 4.1). The decreased starch levels correlated with higher total amylolytic activity as detected by native gels. Although the individual activity bands could not be

assigned to a defined enzymatic activity, proteomics provided a way to elucidate potential proteins responsible that would then be suitable targets for a transgenic approach.

The analysis of deregulated proteins between untreated control samples and roots 10 days after pruning revealed major changes in a wide range of metabolic pathways. Of the starch metabolizing enzymes, the high abundance of AMY3 protein after pruning leads me to conclude that starch remobilization may be highly dependent on this  $\alpha$ -amylase activity. This is quite novel as, thus far,  $\alpha$ -amylases were not clearly attributed to be involved in starch degradation of roots. Hopefully, the transgenic lines AMY3-RNAi I generated will provide definitive evidence for the importance of this  $\alpha$ -amylase in cassava starch mobilization one way or the other.

The activity of LDA on red-pullulan native PAGE also appeared to increase upon pruning (Figure 4.2). This enzyme was easy to assay because red-pullulan is a specific substrate it. However, although the protein was detected in the proteomics, an increase in abundance was not observed. Nevertheless, I propose a working model where the initial steps of starch mobilization in cassava storage root occurs by the interplay of AMY3 attacking the granule and LDA helping to debranch the limit dextrins it releases. According to the other proteins I found in the proteome, the glucosyl transferase DPE1 and the starch phosphorylase PHS1 could also be involved in subsequent metabolism of linear oligosaccharides. The resulting Glc and Glc1P could then enter metabolic pathways such as the oxidative pentose phosphate pathway (OPPP) or be transported to the cytosol for further down-stream metabolism (e.g. sucrose synthesis, glycolysis). Proteomics data give good broad evidence as to which pathways may have changed. However, to substantiate the findings it is crucial to perform further experiments. For example, the increased abundance of AMY3 does not necessarily mean that also the activity is also increased, although it is likely. Hence, specific enzyme assay is needed to substantiate the hypothesis. Moreover, the fact that I did not identify any  $\beta$ -amylases in the proteomics approach does not necessary lead to the conclusion that they are not present. Indeed, cassava, like Arabidopsis, has several  $\beta$ -amylase genes and some minor bands visible on the native gel with amylopectin could not be seen on the native gel with  $\beta$ -limit dextrin – a hallmark of a  $\beta$ -amylase. Thus, together with the analysis of  $\alpha$ -amylase activity it will be crucial to determine if there is any  $\beta$ -amylase activity present after all, and if pruning changes it.

Due to the sink-source transition where sucrose is exported rather than imported, a change in sucrose pathway was expected. The investigation of the sucrose pathway revealed

underrepresentation of F1,6BPase and FK after pruning. In contrast, a cytosolic INV2 and HXK1 and HXK3 were found to be more abundant after pruning compared to the control. The high abundance of both cINV and HXK1 in accordance with an increased abundance of the respiration pathway shows similarities to the finding that high cytosolic INV expression in potato tubers leads to increased respiration (Appeldoorn et al., 1997). In agreement with this hypothesis is the finding of proteins involved in glycolysis found to be highly abundant. Hence, while this strongly indicates that after sink-source transition cINV helps to meet the changed cellular demand for energetic compounds, it does not really provide insight into changes in sucrose biosynthesis.

The identification of a major component SnRK1 being increased after pruning is an exciting result. As discussed in chapter 4, this indicates that the metabolic reconfiguration in the storage roots of pruned plants is switched by starvation, potentially via this kinase. This also represents a good target for transgenic repression, as blocking its activity might keep the root in a dormant state and prevent unwanted regrowth.

The comparison of the proteomic analysis of storage roots before and after pruning has thus proved to be an excellent tool to identify major changes in metabolic pathways and revealed potential targets for future transgenic approaches. However, only 25 cassava proteins homologous to the 53 known Arabidopsis proteins involved in starch metabolism were identified. This is surely because protein detection is limited to the more abundant proteins. Previously it was shown that a combined analysis of proteome and transcriptome can be highly complementary (Bischof et al., 2012). Hence, I initiated an RNAseq analysis. Although these data are still under investigation, first results showed high induction of AMY3 transcript after pruning, consistent with the data presented in this thesis.

### **6.2. Potential of modified starch in industry**

In the third part of my PhD, three transgenic approaches were pursued to increase the value of cassava as starch crop. The main focus was to engineer starch with altered starch composition and architecture. Manipulating starch composition can confer improved physico-chemical properties. For the diverse industrial applications, both in the food and non-food branches, a diversity of starches raw materials is desired. Rather than modify native starches post-harvest, genetically modified plants could provide native improved starches directly to replace chemical treatments. For instance, it was shown that genetically modified amylose-free and short-chain potato amylopectin starch exhibits excellent freeze-

thaw stability (Jobling et al., 2002). This example reveals that genetic modification of starch crops have a great potential to engineer starches of industrial interest.

I designed transgenic cassava lines with the intention of increasing starch-bound phosphate. As described in 'Chapter 5' a key gene involved in phosphorylating starch is GWD. Cassava starch has only little starch bound phosphate (0.05%) – one tenth that of potato (0.5%). It was already reported that the expression of *StGWD* could increase total starch bound phosphate content in barley (Carciofi et al., 2011). Moreover *StGWD* is redox regulated, being inactive when oxidised, but can be rendered constitutively active with an amino acid substitution in the redox-motif (from a cysteine to serine; *StGWD*<sub>C1084S</sub>). Many positive, individual transformants with variation in *StGWD* and *StGWD*<sub>C1084</sub> expression levels were analysed. Preliminary analysis of the starch-bound phosphate of one plant per transgenic line revealed that expression of the redox-insensitive *StGWD*<sub>C1084</sub> appears to double phosphorylation levels compared to the wild type. This was seen for <sup>31</sup>P-NMR signal as well as in a Malachite Green assays (not in this thesis). This finding is very promising; the transformation was successful and more importantly, the overexpressed protein is functional. However, not everything is clear. It is surprising that the C3:C6 phosphorylation ratio was the same as for wild type, given that *StGWD* phosphorylates C6 position of the glucose residues (Ritte et al., 2006). However it is possible that the endogenous PWD, which phosphorylates at the C3 position, responds to the increase in prephosphorylated starch, and is more active as a consequence.

As discussed in Chapter 5, starch-bound phosphate potentially solubilizes amylopectin chains. Indications for an increased accessibility to hydrolytic enzymes were given by SEM pictures of starch granules in *StGWD* overexpressing lines of barley, showing more pores and irregularities (Carciofi et al., 2011). The iodine stained leaves of my transgenic cassava *StGWD/StGWD*<sub>C1084S</sub> showed that for most lines less starch was observed at the end of the night compared to wild-type leaves. Although very preliminary, this may suggest that starch mobilization is increased by *StGWD*. Alternatively, starch synthesis may be impaired or disturbed. To test this, it will be crucial to analyse the starch content of leaves at the end of day and end of night.

The preliminary data showing that starch-bound phosphate can be increased leads to further questions. The first results revealed that the one transgenic line had doubled starch-bound phosphate compared to wild type. This is still less than was reported for *StGWD* overexpression in barley endosperm, and less than for normal potato starch. This is

surprising as starch from different potato varieties are even more highly phosphorylated (8-33 nMol G6P mg<sup>-1</sup> starch, Carciofi et al., 2011). There are several reasons why phosphorylation activity of potato *StGWD* might be less efficient in another species. Firstly, the storage roots of transgenic lines were still small and not developed to the same extent as the wild-type control (Figure 5.7). This might be caused either by the transgenic event (a secondary effect of *StGWD/StGWD*<sub>C1084S</sub> overexpression) or simply because plants from this first generation (coming from culture media) develop their storage root more slowly than wild-type plants. However, it is likely that starch accumulation and storage capacity is not yet fully developed. Thus, it is possible that the degree of phosphorylation could change. Starch is phosphorylated during its synthesis (Nielsen et al., 1994). This can explain the phosphate residues within the starch granules. However, it was recently shown that the majority of starch-bound phosphate sits on the granule surface (Buleon et al., 2014), and could be subject to constant turnover (see below). The development of storage roots in the next generation is needed to rule out some of the more trivial explanations above.

Another reason for the lower phosphate content compared to potato starch might be that *StGWD* has a preference for a certain molecular architecture common in potato, but rare in cassava. This is not an unreasonable suggestion; it was reported that down-regulation of SBE in potato tubers change both the degree of polymerisation of linear glucan chains and, in addition, increased the degree of phosphorylation (Jobling, 1999; Blennow et al., 2000). In this respect, cassava starch has C-type architecture of the crystalline lamellae whereas potato has a B-type and barley A-type (reviewed in Damager et al., 2010). This means that the helices formed by the side chains of amylopectin are arranged in different patterns. Even minimal differences in granule surface could potentially affect the affinity and activity of the protein. This could be tested for instance *in vitro* with heterologous expressed *StGWD* protein - both wild type and redox-insensitive forms - using native starches isolated from different biological origins with different packing types (A-, B-, and C-type). This would show how efficiently *StGWD* phosphorylates different starch types.

Another reasonable explanation would be that increased phosphorylation of the cassava starch by *StGWD* occurs but is only transient and that a high proportion is again released by *SEX4* and *LSF2* before the phosphate can be buried and protected inside the starch granule. My other attempts to increase total starch bound phosphate should address this. As described in 'Chapter 5' I generated RNAi constructs against the two active glucan phosphatases *SEX4* and *LSF2*. Once the transgenic lines are growing in the greenhouse, one



could try to generate double or multiple transgenic crosses in order to generate cassava plants combining *StGWD/StGWD<sub>C10845</sub>* with the silencing constructs RNAi-SEX4 and RNAi-LSF2. The combination of high kinase activity and decreased phosphatase activity could potentially increase the phosphate content much more. Furthermore, *Arabidopsis sex4* mutants were shown to accumulate phosphooligosaccharides, released in part by AMY3 (Kotting et al., 2009). Hence, the double mutant lines RNAi-AMY3 and RNAi-SEX4 could also potentially lead to increased starch yield in combination with increased phosphate content. These generation and analyses of these combinations will need to be undertaken by a future PhD student or researcher.

Taking together all the results I gathered during my PhD, I believe that I could significantly contribute towards knowledge in cassava starch metabolism. Furthermore, I believe that the isolated transgenic lines have potential for valuable future research and maybe also for industrial applications.

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## 7. References

- Alves, A.A.C.** (2002). Cassava Botany and Physiology. Biology, Production and Utilization. R. Hillocks, J. Thresh, and A. Bellotti, eds (CBI Publishing, New York). Chapter 5, 67-89.
- Andriotis, V.M., Pike, M.J., Bunnewell, S., Hills, M.J., Smith, A.M.** (2010). The plastidial glucose-6-phosphate/phosphate antiporter GPT1 is essential for morphogenesis in *Arabidopsis* embryos. *Plant Journal* **64**, 128-139.
- Angelov, M.N., Sun, J.D., Byrd, G.T., Brown, R.H., Black, C.C.** (1993). Novel characteristics of cassava, *Manihot esculenta* Crantz, a reputed C-3-C4 intermediate photosynthesis species. *Photosynthesis Research* **38**, 61-72.
- Appeldoorn, N.J.G., deBruijn, S.M., KootGronsveld, E.A.M., Visser, R.G.F., Vreugdenhil, D., van der Plas, L.H.W.** (1997). Developmental changes of enzymes involved in conversion of sucrose to hexose-phosphate during early tuberisation of potato. *Planta* **202**, 220-226.
- Azimzadeh, O., Scherthan, H., Yentrapalli, R., Barjaktarovic, Z., Ueffing, M., Conrad, M., Neff, F., Calzada-Wack, J., Aubele, M., Buske, C., Atkinson, M.J., Hauck, S.M., Tapio, S.** (2012). Label-free protein profiling of formalin-fixed paraffin-embedded (FFPE) heart tissue reveals immediate mitochondrial impairment after ionising radiation. *Journal of Proteomics* **75**, 2384-2395.
- Baba, A.I., Nogueira, F.C.S., Pinheiro, C.B., Brasil, J.N., Jereissati, E.S., Juca, T.L., Soares, A.A., Santos, M.F., Domont, G.B., Campos, F.A.P.** (2008). Proteome analysis of secondary somatic embryogenesis in cassava (*Manihot esculenta*). *Plant Science* **175**, 717-723.
- Baena-Gonzalez, E., Rolland, F., Thevelein, J.M., and Sheen, J.** (2007). A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**, 938-910.
- Baguma, Y., Sun, C., Ahlandsberg, S., Mutisya, J., Palmqvist, S., Rubaihayo, P.R., Magambo, M.J., Egwng, T.G., Larsson, H., Jansson, C.** (2003). Expression patterns of the gene encoding starch branching enzyme II in the storage roots of cassava (*Manihot esculenta* Crantz). *Plant Science* **164**, 833-839.
- Baguma, Y.S., C., Boréén, M., Olsson, H., Rosenqvist, S., Mutisya, J., Rubaihayo, P. R., Jansson, C.** (2008). Sugar-mediated semidiurnal oscillation of gene expression in the cassava storage root regulates starch synthesis. *Plant Signaling & Behavior* **3**, 439-445.
- Ball, S.G., and Morell, M.K.** (2003). From bacterial glycogen to starch: Understanding the biogenesis of the plant starch granule. *Annual review of plant biology* **54**, 207-233.
- Baroja-Fernandez, E., Munoz, F.J., Montero, M., Etxeberria, E., Sesma, M.T., Ovecka, M., Bahaji, A., Ezquer, I., Li, J., Prat, S., Pozueta-Romero, J.** (2009). Enhancing sucrose synthase activity in transgenic potato (*Solanum tuberosum* L.) tubers results in increased levels of starch, ADPglucose and UDPglucose and total yield. *Plant Cell Physiology* **50**, 1651-1662.
- Baunsgaard, L., Lutken, H., Mikkelsen, R., Glaring, M.A., Pham, T.T., Blennow, A.** (2005). A novel isoform of glucan, water dikinase phosphorylates pre-phosphorylated alpha-glucans and is involved in starch degradation in *Arabidopsis*. *Plant Journal* **41**, 595-605.
- Baur-Höch, B., Machler, F., Nösberger, J.** (1990). Effect of carbohydrate demand on the remobilization of starch in stolons and roots of white clover (*Trifolium repens* L.) after defoliation. *Journal of Experimental Botany* **41**, 573-578.
- Bischof, S., Baerenfaller, K., Wildhaber, T., Troesch, R., Vidi, P.-A., Roschitzki, B., Hirsch-Hofmann, M., Hennig, L., Kessler, F., Gruissem, W., Baginsky, S.** (2011). Plastid proteome assembly without Toc159: photosynthetic protein import and accumulation of *N*-acetylated plastid precursor proteins. *Plant Cell* **11**, 3911-3928.

- Bischof, S., Umhang, M., Eicke, S., Streb, S., Qi, W.H., Zeeman, S.C.** (2013). *Cecropia peltata* accumulates starch or soluble glycogen by differentially regulating starch biosynthetic genes. *Plant Cell* **25**, 1400-1415.
- Blennow, A., Bay-Smidt, A.M., Wischmann, B., Olsen, C.E., Moller, B.L.** (1998). The degree of starch phosphorylation is related to the chain length distribution of the neutral and the phosphorylated chains of amylopectin. *Carbohydrate Research* **307**, 45-54.
- Blennow, A., Engelsens, S.B., Munck, L., Moller, B.L.** (2000). Starch molecular structure and phosphorylation investigated by a combined chromatographic and chemometric approach. *Carbohydrate Polymers* **41**, 163-174.
- Blennow, A., and Engelsens, S.B.** (2010). Helix-breaking news: fighting crystalline starch energy deposits in the cell. *Trends in Plant Science* **15**, 236-240.
- Booker, J., Sieberer, T., Wright, W., Williamson, L., Willett, B., Stirnberg, P., Turnbull, C., Srinivasan, M., Goddard, P., Leyser, O.** (2005). MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. *Developmental Cell* **8**, 443-449.
- Bouly, J.P., Gissot, L., Lessard, P., Kreis, M., Thomas, M.** (1999). *Arabidopsis thaliana* proteins related to the yeast SIP and SNF4 interact with AKIN alpha 1, an SNF1-like protein kinase. *Plant Journal* **18**, 541-550.
- Buleon, A., Cotte, M., Putaux, J.L., d'Hulst, C., Susini, J.** (2014). Tracking sulfur and phosphorus within single starch granules using synchrotron X-ray microfluorescence mapping. *Biochimica et Biophysica Acta* **1840**, 113-119.
- Bull, S.E., Owiti, J.A., Niklaus, M., Beeching, J.R., Gruissem, W., Vanderschuren, H.** (2009). Agrobacterium-mediated transformation of friable embryogenic calli and regeneration of transgenic cassava. *Nature protocols* **4**, 1845-1854.
- Busse, J.S., and Evert, R.F.** (1999). Pattern of differentiation of the first vascular elements in the embryo and seedling of *Arabidopsis thaliana*. *International Journal of Plant Sciences* **160**, 1-13.
- Calatayud, P.A., Llovera, E., Bois, J.F., Lamaze, T.** (2000). Photosynthesis in drought-adapted cassava. *Photosynthetica* **38**, 97-104.
- Carciofi, M., Shaif, S.S., Jensen, S.L., Blennow, A., Svensson, J.T., Vincze, E., Hebelstrup, K.H.** (2011). Hyperphosphorylation of cereal starch. *Journal of Cereal Science* **54**, 339-346.
- Carvalho, L.J.C.B., de Souza C.R.B., de Mattos Cascardo, J. C., Bloch Junior, C., Campos, L.** (2004). Identification and characterization of a novel cassava (*Manihot esculenta* Crantz) clone with high free sugar content and novel starch. *Plant Molecular Biology* **56**, 643-659.
- Caspar, T., Huber, S.C., Somerville, C.** (1985). Alterations in growth, photosynthesis, and respiration in a starchless mutant of *Arabidopsis thaliana* (L) deficient in chloroplast phosphoglucosyltransferase activity. *Plant Physiology* **79**, 11-17.
- Ceballos, H., Iglesias, C.A., Perez, J.C., and Dixon, A.G.O.** (2004). Cassava breeding: opportunities and challenges. *Plant Molecular Biology* **56**, 503-516.
- Ceballos, H., Sanchez, T., Morante, N., Fregene, M., Dufour, D., Smith, A.M., Denyer, K., Perez, J.C., Calle, F., Mestres, C.** (2007). Discovery of an amylose-free starch mutant in cassava (*Manihot esculenta* Crantz). *Journal of Agricultural and Food Chemistry* **55**, 7469-7476.
- Ceballos, H., Sanchez, T., Denyer, K., Tofino, A.P., Rosero, E.A., Dufour, D., Smith, A., Morante, N., Perez, J.C., Fahy, B.** (2008). Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). *Journal of Agricultural and Food Chemistry* **56**, 7215-7222.
- Ceballos, H., Iglesias, C.A., Pérez J.C., Dixon A.G.O.** (2004). Cassava breeding: opportunities and challenges. *Plant Molecular Biology* **56**, 503-516.

- Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R., Frommer, W.B.** (2012). Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* **335**, 207-211.
- Chia, T., Thorneycroft, D., Chapple, A., Messerli, G., Chen, J., Zeeman, S.C., Smith, S.M., Smith, A.M.** (2004). A cytosolic glucosyltransferase is required for conversion of starch to sucrose in *Arabidopsis* leaves at night. *Plant Journal* **37**, 853-863.
- Cho, M.H., Lim, H., Shin, D.H., Jeon, J.S., Bhoo, S.H., Park, Y.I., Hahn, T.R.** (2011). Role of the plastidic glucose translocator in the export of starch degradation products from the chloroplasts in *Arabidopsis thaliana*. *New Phytologist* **190**, 101-112.
- Cho, Y.H., and Yoo, S.D.** (2011). Signaling role of fructose mediated by FINS1/FBP in *Arabidopsis thaliana*. *PLoS Genetics* **7**.
- Claeyssen, E., and Rivoal, J.** (2007). Isozymes of plant hexokinase: occurrence, properties and functions. *Phytochemistry* **68**, 709-731.
- Craig, J., Lloyd, J.R., Tomlinson, K., Barber, L., Edwards, A., Wang, T.L., Martin, C., Hedley, C.L., Smith, A.M.** (1998). Mutations in the gene encoding starch synthase II profoundly alter amylopectin structure in pea embryos. *Plant Cell* **10**, 413-426.
- Crevillen, P., Ventriglia, T., Pinto, F., Orea, A., Merida, A., Romero, J.M.** (2005). Differential pattern of expression and sugar regulation of *Arabidopsis thaliana* ADP-glucose pyrophosphorylase-encoding genes. *Journal of Biological Chemistry* **280**, 8143-8149.
- Critchley, J.H., Zeeman, S.C., Takaha, T., Smith, A.M., Smith, S.M.** (2001). A critical role for disproportionating enzyme in starch breakdown is revealed by a knock-out mutation in *Arabidopsis*. *Plant Journal* **26**, 89-100.
- Cruz, J.L., Mosquim, P.R., Pelacani, C.R., Araújo, W.L., DaMatta, F.M.** (2003). Carbon partitioning and assimilation as affected by nitrogen deficiency in cassava. *Photosynthetica* **41**, 201-207.
- Damager, I., Engelsen, S.B., Blennow, A., Moller, B.L., Motawia, M.S.** (2010). First principles insight into the alpha-glucan structures of starch: their synthesis, conformation, and hydration. *Chemical Reviews* **110**, 2049-2080.
- De Schepper, V., De Swaef, T., Bauweraerts, I., Steppe, K.** (2013). Phloem transport: a review of mechanisms and controls. *Journal of Experimental Botany* **64**, 4839-4850.
- Delatte, T., Umhang, M., Trevisan, M., Eicke, S., Thorneycroft, D., Smith, S.M., Zeeman, S.C.** (2006). Evidence for distinct mechanisms of starch granule breakdown in plants. *Journal of Biological Chemistry* **281**, 12050-12059.
- Delvalle, D., Dumez, S., Wattebled, F., Roldan, I., Planchot, V., Berbezy, P., Colonna, P., Vyas, D., Chatterjee, M., Ball, S., Merida, A., D'Hulst, C.** (2005). Soluble starch synthase I: a major determinant for the synthesis of amylopectin in *Arabidopsis thaliana* leaves. *Plant Journal* **43**, 398-412.
- Domon, B., and Aebersold, R.** (2010). Options and considerations when selecting a quantitative proteomics strategy. *Nature Biotechnology* **28**, 710-721.
- Du, L.C., Bokanga, M., Moller, B.L., Halkier, B.A.** (1995). The biosynthesis of cyanogenic glucosides in roots of cassava. *Phytochemistry* **39**, 323-326.
- Edner, C., Li, J., Albrecht, T., Mahlow, S., Hejazi, M., Hussain, H., Kaplan, F., Guy, C., Smith, S.M., Steup, M., Ritte, G.** (2007). Glucan, water dikinase activity stimulates breakdown of starch granules by plastidial beta-amylases. *Plant Physiology* **145**, 17-28.
- Edwards, G.E., Sheta, E., Moore, B.D., Dai, Z., Franceschi, V.R., Cheng, S.H., Lin, C.H., Ku, M.S.B.** (1990). Photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), a C3 species with chlorenchymatous bundle sheath cells. *Plant Cell Physiology* **31**, 1199-1206.

- Einhauser, A., and Jungbauer, A.** (2001). The FLAG (TM) peptide, a versatile fusion tag for the purification of recombinant proteins. *Journal of Biochemical and Biophysical Methods* **49**, 455-465.
- El-Sharkawy, M.A.** (2004). Cassava biology and physiology. *Plant Molecular Biology* **56**, 481-501.
- El-Sharkawy, M.A., Cock J.H.** (1990). Photosynthesis of cassava (*Manihot esculenta*). *Experimental Agriculture* **26**, 325-340.
- El-Sharkawy, M.A., Cock, J.H., Held, A.A.** (1984). Photosynthetic responses of cassava cultivars (*Manihot esculenta* Crantz) from different habitats to temperature. *Photosynthesis Research* **5**, 243-250.
- Fernie, A.R., Swiedrych, A., Tauberger, E., Lytovchenko, A., Trethewey, R.N., Willmitzer, L.** (2002). Potato plants exhibiting combined antisense repression of cytosolic and plastidial isoforms of phosphoglucomutase surprisingly approximate wild type with respect to the rate of starch synthesis. *Plant Physiology and Biochemistry* **40**, 921-927.
- Ferreira, S.J., and Sonnewald, U.** (2012). The mode of sucrose degradation in potato tubers determines the fate of assimilate utilization. *Frontiers in Plant Science* **3**, 23,1-18
- Fettke, J., Eckermann, N., Kotting, O., Ritte, G., Steup, M.** (2006). Novel starch-related enzymes and carbohydrates. *Cellular and Molecular Biology* **52**, 883-904.
- Fincher, G.B.** (1989). Molecular and cellular biology associated with endosperm mobilization in germinating cereal-grains. *Annual Review Plant Physiology Plant Molecular Biology* **40**, 305-346.
- Fischer, R., Trudgian, D.C., Wright, C., Thomas, G., Bradbury, L.A., Brown, M.A., Bowness, P., Kessler, B.M.** (2012). Discovery of candidate serum proteomic and metabolomic biomarkers in ankylosing spondylitis. *Molecular Cell Proteomics* **11**.
- Fulton, D.C., Stettler, M., Mettler, T., Vaughan, C.K., Li, J., Francisco, P., Gil, D., Reinhold, H., Eicke, S., Messerli, G., Dorken, G., Halliday, K., Smith, A.M., Smith, S.M., Zeeman, S.C.** (2008). beta-AMYLASE4, a noncatalytic protein required for starch breakdown, acts upstream of three active beta-amylases in Arabidopsis chloroplasts. *Plant Cell* **20**, 1040-1058.
- Gallagher, J.A., Volenec, J.J., Turner, L.B., Pollock, C.J.** (1997). Starch hydrolytic enzyme activities following defoliation white clover. *Crop Science* **37**, 1812-1818.
- Geigenberger, P.** (2003). Regulation of sucrose to starch conversion in growing potato tubers. *Journal of Experimental Botany* **54**, 457-465.
- Geigenberger, P., Fernie, A.R., Gibon, Y., Christ, M., Stitt, M.** (2000). Metabolic activity decreases as an adaptive response to low internal oxygen in growing potato tubers. *Biological Chemistry* **381**, 723-740.
- Gibon, Y., Vigeolas, H., Tiessen, A., Geigenberger, P., Stitt, M.** (2002). Sensitive and high throughput metabolite assays for inorganic pyrophosphate, ADPGlc, nucleotide phosphates, and glycolytic intermediates based on a novel enzymic cycling system. *Plant Journal* **30**, 221-235.
- Gibon, Y., Blasing, O.E., Palacios-Rojas, N., Pankovic, D., Hendriks, J.H., Fisahn, J., Hohne, M., Gunther, M., Stitt, M.** (2004). Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period. *Plant Journal* **39**, 847-862.
- Goodall, G.J., and Filipowicz, W.** (1989). The Au-rich sequences present in the introns of plant nuclear pre-messenger RNAs are required for splicing. *Cell* **58**, 473-483.

- Greer, E., Martin, A.C., Pendle, A., Colas, I., Jones, A.M.E., Moore, G., Shaw, P.** (2012). The Ph1 locus suppresses Cdk2-type activity during premeiosis and meiosis in wheat. *Plant Cell* **24**, 152-162.
- Hardie, G., Carling, D., Carlson, M.** (1998). The AMP-activated/SNF1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? *Annual Review Biochemistry* **67**, 821-855.
- Harrison, C.J., Hedley, C.L., Wang, T.L.** (1998). Evidence that the rug3 locus of pea (*Pisum sativum* L.) encodes plastidial phosphoglucomutase confirms that the imported substrate for starch synthesis in pea amyloplasts is glucose-6-phosphate. *Plant Journal* **13**, 753-762.
- Hejazi, M., Fettke, J., Kotting, O., Zeeman, S.C., Steup, M.** (2010). The laforin-like dual-specificity phosphatase SEX4 from Arabidopsis hydrolyzes both C6-and C3-phosphate esters introduced by starch-related dikinases and thereby affects phase transition of alpha-glucans. *Plant Physiology* **152**, 711-722.
- Hendriks, J.H.M., Kolbe, A., Gibon, Y., Stitt, M., Geigenberger, P.** (2003). ADP-glucose pyrophosphorylase is activated by posttranslational redox-modification in response to light and to sugars in leaves of Arabidopsis and other plant species. *Plant Physiology* **133**, 838-849.
- Hill, L.M., and Smith, A.M.** (1991). Evidence that glucose-6-phosphate is imported as the substrate for starch synthesis by the plastids of developing pea embryos. *Planta* **185**, 91-96.
- Huber, J.L.A., Huber, S.C., Nielsen, T.H.** (1989). Protein-phosphorylation as a mechanism for regulation of spinach leaf sucrose-phosphate synthase activity. *Archives of Biochemistry and Biophysics* **270**, 681-690.
- Hussain, H., Mant, A., Seale, R., Zeeman, S., Hinchliffe, E., Edwards, A., Hylton, C., Bornemann, S., Smith, A.M., Martin, C., Bustos, R.** (2003). Three isoforms of isoamylase contribute different catalytic properties for the debranching of potato glucans. *Plant Cell* **15**, 133-149.
- Jang, J.-C., Leon, P., Zhou, L., Sheen, J.** (1997). Hexokinase as a sugar sensor in higher plants. *Plant Cell* **9**, 5-19.
- Jennings, D., and Iglesias, C.** (2002). Breeding for crop improvement. Cassava: Biology, Production and Utilization. R. Hillocks, J. Thresh, and A. Bellotti, eds (CBI Publishing, New York). Chapter 8, 149-166.
- Jobling, S.** (2004). Improving starch for food and industrial applications. *Current Opinion of Plant Biology* **7**, 210-218.
- Jobling, S., Schwall, G.P., Westcott, R.J., Sidebottom, C.M., Debet, M., Gidley, M.J., Jeffcoat, R., Safford, R.** (1999). A minor form of starch branching enzyme in potato (*Solanum tuberosum* L.) tubers has a major effect on starch structure: cloning and characterisation of multiple forms of SBE A. *Plant Journal* **18**, 163-171.
- Jobling, S., Westcott, R.J., Tayal, A., Schwall, G.P.** (2002). Production of a freeze-thaw-stable potato starch by antisense inhibition of three starch synthase genes. *Nature Biotechnology* **20**, 295-299.
- Kammerer, B., Fischer, K., Hilpert, B., Schubert, S., Gutensohn, M., Weber, A., Flugge, U.I.** (1998). Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: The glucose 6-phosphate phosphate antiporter. *Plant Cell* **10**, 105-117.
- Koch, K.** (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion Plant Biology* **7**, 235-246.



- Kolbe, A., Tiessen, A., Schluepmann, H., Paul, M., Ulrich, S., Geigenberger, P.** (2005). Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. *Proceedings of the National Academy of Sciences USA* **102**, 11118-11123.
- Kosegarten, H., and Mengel, K.** (1994). Evidence for a glucose 1-phosphate translocator in storage tissue amyloplasts of potato (*Solanum tuberosum*) suspension-cultured cells. *Physiologia Plantarum* **91**, 111-120.
- Kotting, O., Pusch, K., Tiessen, A., Geigenberger, P., Steup, M., Ritte, G.** (2005). Identification of a novel enzyme required for starch metabolism in Arabidopsis leaves. The phosphoglucan, water dikinase. *Plant Physiology* **137**, 242-252.
- Kotting, O., Santelia, D., Edner, C., Eicke, S., Marthaler, T., Gentry, M.S., Comparot-Moss, S., Chen, J., Smith, A.M., Steup, M., Ritte, G., Zeeman, S.C.** (2009). STARCH-EXCESS4 is a laforin-like phosphoglucan phosphatase required for starch degradation in *Arabidopsis thaliana*. *Plant Cell* **21**, 334-346.
- Kretzschmar, T., Kohlen, W., Sasse, J., Borghi, L., Schlegel, M., Bachelier, J.B., Reinhardt, D., Bours, R., Bouwmeester, H.J., Martinoia, E.** (2012). A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature* **483**, 341-344.
- Li, J.-Y., Yeh, A.-I.** (2001). Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Food Engineering* **50**, 141-148.
- Li, Y.Z., Pan, Y.H., Sun, C.B., Dong, H.T., Luo, X.L., Wang, Z.Q., Tang, J.L., Chen, B.** (2010). An ordered EST catalogue and gene expression profiles of cassava (*Manihot esculenta*) at key growth stages. *Plant Molecular Biology* **74**, 573-590.
- Lin, T.P., Caspar, T., Somerville, C.R., Preiss, J.** (1988). A starch deficient mutant of *Arabidopsis thaliana* with low ADPglucose pyrophosphorylase activity lacks one of the 2 subunits of the enzyme. *Plant Physiology* **88**, 1175-1181.
- Lohmeier-Vogel, E.M., Kerk, D., Nimick, M., Wrobel, S., Vickerman, L., Muench, D.G., Moorhead, G.B.** (2008). Arabidopsis At5g39790 encodes a chloroplast-localized, carbohydrate-binding, coiled-coil domain-containing putative scaffold protein. *BMC Plant Biology* **8**, 120.
- Lopez, C., Jorge, V., Piegue, B., Mba, C., Cortes, D., Restrepo, S., Soto, M., Laudie, M., Berger, C., Cooke, R., Delseny, M., Tohme, J., Verdier, V.** (2004). A unigene catalogue of 5700 expressed genes in cassava. *Plant Molecular Biology* **56**, 541-554.
- Lorberth, R., Ritte, G., Willmitzer, L., Kossmann, J.** (1998). Inhibition of a starch-granule-bound protein leads to modified starch and repression of cold sweetening. *Nature Biotechnology* **16**, 473-477.
- Lu, P., Vogel, C., Wang, R., Yao, X., Marcotte, E.M.** (2007). Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation. *Nature Biotechnology* **25**, 117-124.
- Lu, Y., Steichen, J.M., Weise, S.E., Sharkey, T.D.** (2006). Cellular and organ level localization of maltose in maltose-excess Arabidopsis mutants. *Planta* **224**, 935-943.
- Lunn, J.E., Feil, R., Hendriks, J.H., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W.R., Carillo, P., Hajirezaei, M.R., Stitt, M.** (2006). Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. *Biochemical Journal* **397**, 139-148.
- Man, A.L., Purcell, P.C., Hannappel, U., Halford, N.G.** (1997). Potato SNF1-related protein kinase: Molecular cloning, expression analysis and peptide kinase activity measurements. *Plant Molecular Biology* **34**, 31-43.

- McKibbin, R.S., Muttucumar, N., Paul, M.J., Powers, S.J., Burrell, M.M., Coates, S., Purcell, P.C., Tiessen, A., Geigenberger, P., Halford, N.G.** (2006). Production of high-starch, low-glucose potatoes through over-expression of the metabolic regulator SnRK1. *Plant Biotechnology Journal* **4**, 409-418.
- McMahon, J.M., White, W.L.B., Sayre, R.T.** (1995). Cyanogenesis in Cassava (*Manihot esculenta* Crantz). *Journal of Experimental Botany* **46**, 731-741.
- Mikkelsen, R., Suszkiewicz, K., Blennow, A.** (2006). A novel type carbohydrate-binding module identified in alpha-glucan, water dikinases is specific for regulated plastidial starch metabolism. *Biochemistry* **45**, 4674-4682.
- Mikkelsen, R., Mutenda, K.E., Mant, A., Schurmann, P., Blennow, A.** (2005). alpha-Glucan, water dikinase (GWD): A plastidic enzyme with redox-regulated and coordinated catalytic activity and binding affinity. *Proceeding of the National Academy of Sciences USA*. **102**, 1785-1790.
- Mitprasat, M., Roytrakul, S., Jiemsup, S., Boonseng, O., and Yokthongwattana, K.** (2011). Leaf proteomic analysis in cassava (*Manihot esculenta* Crantz) during plant development, from planting of stem cutting to storage root formation. *Planta* **233**, 1209-1221.
- Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., Sheen, J.** (2003). Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* **300**, 332-336.
- Naumkina, E.M., Bolyakina, Y.P., Romanov, G.A.** (2007). Organ-specificity and inducibility of patatin class I promoter from potato in transgenic arabidopsis plants. *Russian Journal of Plant Physiology* **54**, 350-359.
- Neuhaus, H.E., Thom, E., Mohlmann, T., Steup, M., Kampfenkel, K.** (1997). Characterization of a novel eukaryotic ATP/ADP translocator located in the plastid envelope of *Arabidopsis thaliana* L. *Plant Journal* **11**, 73-82.
- Nielsen, T.H., Deiting, U., Stitt, M.** (1997). A beta-amylase in potato tubers is induced by storage at low temperature. *Plant Physiology* **113**, 503-510.
- Nielsen, T.H., Wischmann, B., Enevoldsen, K., Moller, B.L.** (1994). Starch phosphorylation in potato tubers proceeds concurrently with *de novo* biosynthesis of starch. *Plant Physiology* **105**, 111-117.
- Niittyla, T., Messerli, G., Trevisan, M., Chen, J., Smith, A.M., Zeeman, S.C.** (2004). A previously unknown maltose transporter essential for starch degradation in leaves. *Science* **303**, 87-89.
- Niittyla, T., Comparot-Moss, S., Lue, W.L., Messerli, G., Trevisan, M., Seymour, M.D.J., Gatehouse, J.A., Villadsen, D., Smith, S.M., Chen, J.C., Zeeman, S.C., Alison, M.S.** (2006). Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. *Journal of Biological Chemistry* **281**, 11815-11818.
- Niklaus, M., Gruissem, W., Vanderschuren, H.** (2011). Efficient transformation and regeneration of transgenic cassava using the neomycin phosphotransferase gene as aminoglycoside resistance marker gene. *GM crops* **2**, 193-200.
- Owiti, J., Grossmann, J., Gehrig, P., Dessimoz, C., Laloi, C., Hansen, M.B., Gruissem, W., Vanderschuren, H.** (2011). iTRAQ-based analysis of changes in the cassava root proteome reveals pathways associated with post-harvest physiological deterioration. *Plant Journal* **67**, 145-156.
- Perez, S., and Bertoft, E.** (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch-Starke* **62**, 389-420.
- Pracharoenwattana, I., Cornah, J.E., Smith, S.M.** (2005). Arabidopsis peroxisomal citrate synthase is required for fatty acid respiration and seed germination. *Plant Cell* **17**, 2037-2048.

- Preiss, J., Kumar, A., Ghosh, P.** (1988). A study of the catalytic and regulatory functions of a sugar nucleotide synthetase using *in vitro* and *in vivo* mutagenesis techniques. *Glycoconjugate Journal* **5**, 365-365.
- Prochnik, S., Marri, P.R., Desany, B., Rabinowicz, P.D., Kodira, C., Mohiuddin, M., Rodriguez, F., Fauquet, C., Tohme, J., Harkins, T., Rokhsar, D.S., Rounsley, S.** (2012). The cassava genome: Current progress, future directions. *Tropical Plant Biology* **5**, 88-94.
- Raemakers, K., Schreuder, M., Suurs, L., Furrer-Verhorst, H., Vincken, J.-P., de Vetten, N., Jacobsen, E., Visser, R.G.F.** (2005). Improved cassava starch by antisense inhibition of granule-bound starch synthase I. *Molecular Breeding* **16**, 163-172.
- Reilly, K., Bernal, D., Cortes, D.F., Gomez-Vasquez, R., Tohme, J., Beeching, J.R.** (2007). Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. *Plant Molecular Biology* **64**, 187-203.
- Ritte, G., Lloyd, J.R., Eckermann, N., Rottmann, A., Kossmann, J., Steup, M.** (2002). The starch-related R1 protein is an alpha -glucan, water dikinase. *Proceeding of the National Academy of Sciences USA* **99**, 7166-7171.
- Ritte, G., Heydenreich, M., Mahlow, S., Haebel, S., Kotting, O., Steup, M.** (2006). Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. *FEBS Letter* **580**, 4872-4876.
- Roldan, I., Wattebled, F., Lucas, M.M., Delvalle, D., Planchot, V., Jimenez, S., Perez, R., Ball, S., D'Hulst, C., Merida, A.** (2007). The phenotype of soluble starch synthase IV defective mutants of *Arabidopsis thaliana* suggests a novel function of elongation enzymes in the control of starch granule formation. *Plant Journal* **49**, 492-504.
- Ross, H.A., Davies, H.V., Burch, L.R., Viola, R., Mcrae, D.** (1994). Developmental-changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). *Physiolgia Plantarum* **90**, 748-756.
- Ryan, E., and Fottrell, P.F.** (1974). Subcellular-localization of enzymes involved in assimilation of ammonia by soybean root nodules. *Phytochemistry* **13**, 2647-2652.
- Sanchez, T., Chavez, A.L., Ceballos, H., Rodriguez-Amaya, D., Nestel, P., Ishitani, M.** (2006). Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content. *Journal of the Science of Food and Agriculture* **86**, 634-639.
- Santelia, D., Kotting, O., Seung, D., Schubert, M., Thalmann, M., Bischof, S., Meekins, D.A., Lutz, A., Patron, N., Gentry, M.S., Allain, F.H.T., and Zeeman, S.C.** (2011). The phosphoglucan phosphatase like Sex Four2 dephosphorylates starch at the C3-Position in Arabidopsis. *Plant Cell* **23**, 4096-4111.
- Saravanan, R.S., and Rose, J.K.C.** (2004). A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics* **4**, 2522-2532.
- Satoh, R., Fujita, Y., Nakashima, K., Shinozaki, K., Yamaguchi-Shinozaki, K.** (2004). Analysis of bZIP transcription factors involved in hypoosmolarity-responsive expression of the ProDH gene in Arabidopsis. *Plant Cell Physiology* **45**, S228-S228.
- Sauer, N.** (2007). Molecular physiology of higher plant sucrose transporters. *FEBS Letter* **581**, 2309-2317.
- Schluepmann, H., van Dijken, A., Aghdasi, M., Wobbes, B., Paul, M., Smeekens, S.** (2004). Trehalose mediated growth inhibition of Arabidopsis seedlings is due to trehalose-6-phosphate accumulation. *Plant Physiology* **135**, 879-890.
- Schulze, W.X., and Usadel, B.** (2010). Quantitation in Mass-Spectrometry-Based Proteomics. *Annual Review of Plant Biology* **61**, 491-516.

- Sheffield, J., Taylor, N., Fauquet, C., Chen, S.X.** (2006). The cassava (*Manihot esculenta* Crantz) root proteome: Protein identification and differential expression. *Proteomics* **6**, 1588-1598.
- Shimomura, S., Nagai, M., Fukui, T.** (1982). Comparative glucan specificities of 2 types of spinach leaf phosphorylase. *Journal of Biochemistry* **91**, 703-717.
- Shure, M., Wessler, S., Fedoroff, N.** (1983). Molecular-identification and isolation of the *waxy* locus in maize. *Cell* **35**, 225-233.
- Smalle, J., and Vierstra, R.D.** (2004). The ubiquitin 26S proteasome proteolytic pathway. *Annual review of plant biology* **55**, 555-590.
- Smith, A.M., and Stitt, M.** (2007). Coordination of carbon supply and plant growth. *Plant Cell and Environment* **30**, 1126-1149.
- Sonnewald, U., and Kossmann, J.** (2013). Starches-from current models to genetic engineering. *Plant Biotechnology Journal* **11**, 223-232.
- Sorefan, K., Booker, J., Haurogne, K., Goussot, M., Bainbridge, K., Foo, E., Chatfield, S., Ward, S., Beveridge, C., Rameau, C., Leyser, O.** (2003). MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Gene Development* **17**, 1469-1474.
- Stadler, R., and Sauer, N.** (1996). The *Arabidopsis thaliana* *AtSUC2* gene is specifically expressed in companion cells. *Botanica Acta* **109**, 299-306.
- Steup, M., and Schachtele, C.** (1981). Mode of glucan degradation by purified phosphorylase forms from spinach leaves. *Planta* **153**, 351-361.
- Stirnberg, P., van de Sande, K., Leyser, H.M.O.** (2002). MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development* **129**, 1131-1141.
- Stitt, M., and Zeeman, S.C.** (2012). Starch turnover: pathways, regulation and role in growth. *Current Opinion of Plant Biology* **15**, 282-292.
- Streb, S., and Zeeman, S.C.** (2012). Starch metabolism in Arabidopsis. *The Arabidopsis book*. American Society of Plant Biologists **10**, e0160.
- Streb, S., Delatte, T., Umhang, M., Eicke, S., Schorderet, M., Reinhardt, D., Zeeman, S.C.** (2008). Starch granule biosynthesis in Arabidopsis is abolished by removal of all debranching enzymes but restored by the subsequent removal of an endoamylase. *Plant Cell* **20**, 3448-3466.
- Sugden, C., Donaghy, P.G., Halford, N.G., Hardie, D.G.** (1999). Two SNF1-Related protein kinases from spinach leaf phosphorylate and inactivate 3-hydroxy-3-methylglutaryl-coenzyme A reductase, nitrate reductase, and sucrose phosphate synthase *in vitro*. *Plant Physiology* **120**, 257-274.
- Szydlowski, N., Ragel, P., Raynaud, S., Lucas, M.M., Roldan, I., Montero, M., Munoz, F.J., Ovecka, M., Bahaji, A., Planchot, V., Pozueta-Romero, J., D'Hulst, C., Merida, A.** (2009). Starch granule initiation in Arabidopsis requires the presence of either class IV or class III starch synthases. *Plant Cell* **21**, 2443-2457.
- Takeda, Y., and Hizukuri, S.** (1981). Re-examination of the action of sweet-potato beta-amylase on phosphorylated (1-4)-alpha-D-glucan. *Carbohydrate Research* **89**, 174-178.
- Takeda, Y., Guan, H.P., Preiss, J.** (1993). Branching of amylose by the branching isoenzymes of maize endosperm. *Carbohydrate Research* **240**, 253-263.
- Tangphatsornruang, S., Naconsie, M., Thammarongtham, C., Narangajavana, J.** (2005). Isolation and characterization of an alpha-amylase gene in cassava (*Manihot esculenta*). *Plant Physiology Biochemistry* **43**, 821-827.
- Teerawanichpan, P., Lertpanyasampatha, M., Netrphan, S., Varavinit, S., Boonseng, O., Narangajavana, J.** (2008). Influence of cassava storage root development and environmental conditions on starch granule an size distribution. *Starch-Starke* **60**, 696-705.

- Tester, R.F., and Morrison, W.R.** (1990). Swelling and gelatinization of cereal starches .1. effects of amylopectin, amylose, and lipids. *Cereal Chemistry* **67**, 551-557.
- Tharanathan, R.N.** (2005). Starch-value addition by modification. *Critical Reviews in Food Science and Nutrition* **45**, 371-384.
- Usadel, B., Poree, F., Nagel, A., Lohse, M., Czedik-Eysenberg, A., Stitt, M.** (2009). A guide to using MapMan to visualize and compare Omics data in plants: a case study in the crop species, Maize. *Plant Cell Environment* **32**, 1211-1229.
- van Oirschot, Q.E.A., O'Brien, G.M., Dufour, D., El-Sharkawy, M.A., Mesa, E.** (2000). The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. *Journal of Science in Food and Agriculture* **80**, 1866-1873.
- Vaudel, M., Sickmann, A., Martens, L.** (2010). Peptide and protein quantification: A map of the minefield. *Proteomics* **10**, 650-670.
- Vikso-Nielsen, A., Blennow, A., Jorgensen, K., Kristensen, K.H., Jensen, A., Moller, B.L.** (2001). Structural, physicochemical, and pasting properties of starches from potato plants with repressed r1-gene. *Biomacromolecules* **2**, 836-843.
- Viola, R., Pelloux, J., van der Ploeg, A., Gillespie, T., Marquis, N., Roberts, A.G., Hancock, R.D.** (2007). Symplastic connection is required for bud outgrowth following dormancy in potato (*Solanum tuberosum* L.) tubers. *Plant Cell Environment* **30**, 973-983.
- Viola, R., Roberts, A.G., Haupt, S., Gazzani, S., Hancock, R.D., Marmioli, N., Machray, G.C., Oparka, K.J.** (2001). Tuberization in potato involves a switch from apoplastic to symplastic phloem unloading. *Plant Cell* **13**, 385-398.
- Weise, S.E., Weber, A.P.M., Sharkey, T.D.** (2004). Maltose is the major form of carbon exported from the chloroplast at night. *Planta* **218**, 474-482.
- Wingler, A., Fritzius, T., Wiemken, A., Boller, T., Aeschbacher, R.A.** (2000). Trehalose induces the ADP-glucose pyrophosphorylase gene, *ApL3*, and starch synthesis in Arabidopsis. *Plant Physiology* **124**, 105-114.
- Witt, W., and Sauter J. J.** (1996). Purification and properties of a starch granule-degrading  $\alpha$ -amylase from potato tubers. *Journal of Experimental Botany* **47**, 1789-1795.
- Witt, W., and Sauter J. J.** (1994). Starch metabolism in poplar wood ray cells during spring mobilization and summer deposition. *Physiologia Plantarum* **92**, 9-16.
- Yoo, S.H., and Jane, J.L.** (2002). Molecular weights and gyration radii of amylopectins determined by high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors. *Carbohydrate Polymers* **49**, 307-314.
- Yu, T.-S.K., H., Häusler, R.E., Hille, D., Flügge, U.-I., Zeeman S.C., Smith, A.M., Kossmann, J., Lloyd, J., Ritte, G., Steup, M., Lue, W.-L., Chen, J., Weber, A.** (2001). The Arabidopsis *sex1* mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. *Plant Cell* **13**, 1907-1918.
- Yu, T.S., Lue, W.L., Wang, S.M., and Chen, J.C.** (2000). Mutation of Arabidopsis plastid phosphoglucose isomerase affects leaf starch synthesis and floral initiation. *Plant Physiology* **123**, 319-325.
- Yu, T.S., Zeeman, S.C., Thorneycroft, D., Fulton, D.C., Dunstan, H., Lue, W.L., Hegemann, B., Tung, S.Y., Umemoto, T., Chapple, A., Tsai, D.L., Wang, S.M., Smith, A.M., Chen, J., Smith, S.M.** (2005).  $\alpha$ -Amylase is not required for breakdown of transitory starch in Arabidopsis leaves. *Journal of Biological Chemistry* **280**, 9773-9779.
- Yuen, C.Y.L., Leelapon, O., Chanvivattana, Y., Warakanont, J., Narangajavana, J.** (2009). Molecular characterization of two genes encoding plastidic ATP/ADP transport proteins in cassava. *Biologia Plantarum* **53**, 37-44.

- Zeeman, S.C., and Ap Rees, T.** (1999). Changes in carbohydrate metabolism and assimilate export in starch-excess mutants of *Arabidopsis*. *Plant Cell and Environment* **22**, 1445-1453.
- Zeeman, S.C., Kossmann, J., Smith, A.M.** (2010). Starch: Its Metabolism, Evolution, and Biotechnological Modification in Plants. *Annual Review of Plant Biology*, **61**, 209-234.
- Zeeman, S.C., Delatte, T., Messerli, G., Umhang, M., Stettler, M., Mettler, T., Streb, S., Reinhold, H., Kotting, O.** (2007). Starch breakdown: recent discoveries suggest distinct pathways and novel mechanisms. *Functional Plant Biology* **34**, 465-473.
- Zeeman, S.C., Thorneycroft, D., Schupp, N., Chapple, A., Weck, M., Dunstan, H., Haldimann, P., Bechtold, N., Smith, A.M., Smith, S.M.** (2004). Plastidial alpha-glucan phosphorylase is not required for starch degradation in *Arabidopsis* leaves but has a role in the tolerance of abiotic stress. *Plant Physiology* **135**, 849-858.
- Zhang, X.L., Myers, A.M., James, M.G.** (2005). Mutations affecting starch synthase III in *Arabidopsis* alter leaf starch structure and increase the rate of starch synthesis. *Plant Physiology* **138**, 663-674.
- Zhang, X.L., Szydlowski, N., Delvalle, D., D'Hulst, C., James, M.G., Myers, A.M.** (2008). Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in *Arabidopsis*. *BMC Plant Biology* **8**, 96.

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## 8. Curriculum vitae

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## EDUCATION

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|                       |                                                                                                                                                                                                         |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| June 2009 – present   | <b>PhD in Plant Biochemistry</b> , ETH Zürich. Investigation of starch metabolism in Cassava ( <i>Manihot esculenta</i> Crantz) Supervisor: Prof. Dr S. Zeeman                                          |
| Oct 2006 – April 2008 | <b>MSc in Plant Biology</b> , ETH Zürich. Physiological and Biochemical Characterization of a Putative mutant impaired in Glucose Signalling in Carbohydrate Metabolism. Supervisor: Prof. Dr S. Zeeman |
| Oct 2005 – Oct 2008   | <b>Studies in Integrative Biology</b> , ETH Zürich                                                                                                                                                      |
| Oct 2001 – Oct 2005   | <b>Studies in Pharmaceutical Sciences</b> , ETH Zürich                                                                                                                                                  |
| Aug 1998 – Aug 2001   | Gymnasium Bern – Köniz, modern languages (Center subject: Spanish, English, complementary subject: History)                                                                                             |



WORK EXPERIENCE

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May 2008 – May 2009                    **Research and personal assistant** at ETH Zürich, in the group of Plant Biochemistry, Prof. S. Zeeman.

April 2005 – Sept 2007                **Assistant**, Belfa AG, Glattbrugg

PUBLICATIONS

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**Hostettler, C.**, Kolling, K., Santelia, D., Streb, S., Kotting, O., and Zeeman, S.C. (2011). Analysis of starch metabolism in chloroplasts. *Methods Mol Biol* 775, 387-410.

Reinhold, H., Soyk, S., Simkova, K., **Hostettler, C.**, Marafino, J., Mainiero, S., Vaughan, C.K., Monroe, J.D., and Zeeman, S.C. (2011). beta-Amylase-Like Proteins Function as Transcription Factors in Arabidopsis, Controlling Shoot Growth and Development. *Plant Cell* 23, 1391-1403.

LANGUAGE SKILLS

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|          |                                     |
|----------|-------------------------------------|
| German:  | mother tongue                       |
| English: | proficient (C1), written and spoken |
| French:  | independent (B1) written and spoken |
| Spanish: | basic (A2) written and spoken       |

**Supplemental Table 4.1. Quantitative proteome map of cassava roots during pruning.** Root proteins were extracted by phenol/methanol precipitation (Saravanan and Rose, 2004) and equivalent amounts of protein were separated by SDS-PAGE followed by in-gel tryptic digestion. Peptides were analyzed by mass spectrometry (MS) using an Orbitrap mass spectrometer. Three biological replicates including sample collection, protein extraction and separation were analyzed by MS for each time point. Measured spectra were analyzed by Mascot using the the cassava genomic database *Mesculenta\_147\_peptide.fa* (download from [ftp://ftp.jgi-psf.org/pub/JGI\\_data/phytozome/v7.0/Mesculenta/](ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v7.0/Mesculenta/)) and quantitative information for each identified peptide and protein was obtained by data analysis using the software Progenesis LC-MS ([www.nonlinear.com](http://www.nonlinear.com)). Information about Arabidopsis AGI-homologues and protein descriptions were downloaded from [ftp://ftp.jgi-psf.org/pub/JGI\\_data/phytozome/v7.0/Mesculenta](ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v7.0/Mesculenta). SUB-cellular protein localization was based on the database SUBA (Heazlewood et al., 2007; <http://suba.plantenergy.uwa.edu.au>). DAP: days after pruning.

| Cassava accession     | Arabidopsis Accession | Anova (p)   | Max fold change | Progenesis             |                                |                | Mapman                |
|-----------------------|-----------------------|-------------|-----------------|------------------------|--------------------------------|----------------|-----------------------|
|                       |                       |             |                 | Highest mean condition | Peptides used for quantitation | Bincode        | Name                  |
| cassava4.1_007929m PA | AT1G51710             | 0.000184834 | 1.247724727     | Ten DAP                | 7                              | 29.5.11.05     | protein               |
| cassava4.1_009485m PA | AT5G10770             | 0.000331509 | 1.54793695      | Ten DAP                | 2                              | 27.3.99        | RNA                   |
| cassava4.1_006320m PA | AT4G35090             | 0.000423785 | 2.90455018      | Ten DAP                | 21                             | 21.6           | redox                 |
| cassava4.1_003566m PA | AT1G09420             | 0.000655903 | 1.233848973     | Ten DAP                | 9                              | 7.1.01         | OPP                   |
| cassava4.1_008547m PA | AT1G49820             | 0.0011878   | 2.376973953     | Ten DAP                | 3                              | 35.1           | not assigned          |
| cassava4.1_028937m PA | AT5G35360             | 0.001626041 | 1.636998465     | Ten DAP                | 3                              | 11.1.01        | lipid metabolism      |
| cassava4.1_012839m PA | AT3G24170             | 0.001630858 | 1.41248156      | Ten DAP                | 3                              | 21.2.2         | redox                 |
| cassava4.1_010570m PA | AT1G07750             | 0.00165494  | 1.904043819     | Ten DAP                | 4                              | 33.1           | development           |
| cassava4.1_006656m PA | AT5G57655             | 0.001667931 | 1.989665603     | Ten DAP                | 12                             | 35.1           | not assigned          |
| cassava4.1_013350m PA | AT3G45600             | 0.001742257 | 1.687943839     | Ten DAP                | 4                              | 33.99          | development           |
| cassava4.1_013267m PA | AT3G54820             | 0.00177835  | 1.936787885     | Ten DAP                | 9                              | 34.19.1        | transport             |
| cassava4.1_006459m PA | AT1G06110             | 0.001786115 | 1.752486433     | Ten DAP                | 2                              | 29.5.11.4.3.02 | protein               |
| cassava4.1_008713m PA | AT5G18170             | 0.002006845 | 2.03320298      | Ten DAP                | 8                              | 12.3.01        | N-metabolism          |
| cassava4.1_015379m PA | AT4G11150             | 0.002298373 | 1.346622159     | Ten DAP                | 10                             | 34.1.01        | transport             |
| cassava4.1_011655m PA | AT4G13010             | 0.002317828 | 2.695500686     | Ten DAP                | 9                              | 26.07          | misc                  |
| cassava4.1_010105m PA | AT5G43330             | 0.002399012 | 1.203658368     | Ten DAP                | 17                             | 8.2.09         | TCA / org             |
| cassava4.1_006188m PA | AT3G24170             | 0.002824254 | 1.206469929     | Ten DAP                | 11                             | 21.2.2         | redox                 |
| cassava4.1_006148m PA | AT4G35230             | 0.002996077 | 1.945108137     | Ten DAP                | 2                              | 29.4.1.52      | protein               |
| cassava4.1_001385m PA | AT1G47550             | 0.003194085 | 1.328324206     | Ten DAP                | 4                              | 35.2           | not assigned          |
| cassava4.1_001602m PA | AT3G26720             | 0.003244191 | 1.615893902     | Ten DAP                | 11                             | 26.03          | misc                  |
| cassava4.1_002966m PA | AT1G06290             | 0.003248717 | 2.249492405     | Ten DAP                | 5                              | 11.9.4.02      | lipid metabolism      |
| cassava4.1_004339m PA | AT3G14310             | 0.003386575 | 1.481605442     | Ten DAP                | 16                             | 10.8.01        | cell wall             |
| cassava4.1_011670m PA | AT4G27585             | 0.003651751 | 1.356094422     | Ten DAP                | 8                              | 35.1           | not assigned          |
| cassava4.1_009672m PA | AT3G54360             | 0.00386717  | 1.46021094      | Ten DAP                | 6                              | 29.5.11.04.02  | protein               |
| cassava4.1_008396m PA | AT1G12050             | 0.004113184 | 1.602166851     | Ten DAP                | 3                              | 13.2.6.2       | amino acid metabolism |
| cassava4.1_009839m PA | AT1G12840             | 0.004195739 | 1.388372798     | Ten DAP                | 16                             | 34.1           | transport             |
| cassava4.1_003845m PA | AT1G72160             | 0.004723487 | 1.345255193     | Ten DAP                | 35                             | 28.99          | DNA                   |
| cassava4.1_003845m PA | AT1G72160             | 0.004723487 | 1.345255193     | Ten DAP                | 35                             | 34.99          | transport             |
| cassava4.1_005081m PA | AT1G36280             | 0.004856757 | 1.772631821     | Ten DAP                | 11                             | 23.1.2.08      | nucleotide metabolism |
| cassava4.1_000890m PA | AT1G22610             | 0.004928974 | 2.923968357     | Ten DAP                | 3                              | 35.1.19        | not assigned          |

|                       |           |             |             |         |    |               |                       |
|-----------------------|-----------|-------------|-------------|---------|----|---------------|-----------------------|
| cassava4.1_022803m PA | AT3G13235 | 0.005124596 | 1.252930263 | Ten DAP | 8  | 29.5.11.01    | protein               |
| cassava4.1_018093m PA | AT1G53540 | 0.005343764 | 8.023552477 | Ten DAP | 4  | 20.2.1        | stress                |
| cassava4.1_019208m PA | AT2G43750 | 0.005888425 | 1.541881836 | Ten DAP | 3  | 13.1.5.3.01   | amino acid metabolism |
| cassava4.1_003278m PA | AT1G62440 | 0.005963094 | 1.599930163 | Ten DAP | 3  | 10.5.3        | cell wall             |
| cassava4.1_033294m PA | AT5G44640 | 0.006099166 | 1.123323949 | Ten DAP | 2  | 35.1          | not assigned          |
| cassava4.1_008949m PA | AT5G08370 | 0.006160509 | 1.484106938 | Ten DAP | 11 | 3.8.2         | minor CHO metabolism  |
| cassava4.1_032921m PA | AT3G48690 | 0.006820393 | 6.305755438 | Ten DAP | 3  | 35.1          | not assigned          |
| cassava4.1_029528m PA | AT5G10770 | 0.00716048  | 1.780161588 | Ten DAP | 6  | 27.3.99       | RNA                   |
| cassava4.1_001252m PA | AT1G58370 | 0.007256631 | 1.475983414 | Ten DAP | 7  | 10.6.2        | cell wall             |
| cassava4.1_006617m PA | AT1G70580 | 0.007351459 | 1.926825688 | Ten DAP | 6  | 1.2.3         | PS                    |
| cassava4.1_006617m PA | AT1G70580 | 0.007351459 | 1.926825688 | Ten DAP | 6  | 13.1.1.3.01   | amino acid metabolism |
| cassava4.1_008799m PA | AT2G26560 | 0.007481419 | 1.641450843 | Ten DAP | 6  | 33.1          | development           |
| cassava4.1_014036m PA | AT4G16210 | 0.007485635 | 1.643172336 | Ten DAP | 8  | 11.9.4.04     | lipid metabolism      |
| cassava4.1_014036m PA | AT4G16210 | 0.007485635 | 1.643172336 | Ten DAP | 8  | 13.2.3.5      | amino acid metabolism |
| cassava4.1_001604m PA | AT3G50950 | 0.007588512 | 1.948000233 | Ten DAP | 3  | 20.1          | stress                |
| cassava4.1_006302m PA | AT4G35090 | 0.007777746 | 3.291850587 | Ten DAP | 30 | 21.6          | redox                 |
| cassava4.1_005123m PA | AT3G48000 | 0.008100567 | 1.519328287 | Ten DAP | 22 | 5.10          | fermentation          |
| cassava4.1_012571m PA | AT5G54080 | 0.008195887 | 3.560328754 | Ten DAP | 2  | 13.2.6.2      | amino acid metabolism |
| cassava4.1_005575m PA | AT4G29010 | 0.008383851 | 1.577977396 | Ten DAP | 8  | 11.9.4.09     | lipid metabolism      |
| cassava4.1_009237m PA | AT1G52290 | 0.009068043 | 1.582418007 | Ten DAP | 6  | 30.2.22       | signalling            |
| cassava4.1_006596m PA | AT3G12780 | 0.009172008 | 1.745902602 | Ten DAP | 3  | 1.3.03        | PS                    |
| cassava4.1_006596m PA | AT3G12780 | 0.009172008 | 1.745902602 | Ten DAP | 3  | 4.010         | glycolysis            |
| cassava4.1_007889m PA | AT5G58330 | 0.009206196 | 2.283125624 | Ten DAP | 3  | 8.2.09        | TCA / org             |
| cassava4.1_016819m PA | AT2G45820 | 0.009305408 | 1.662800685 | Ten DAP | 15 | 27.3.67       | RNA                   |
| cassava4.1_014834m PA | AT5G63400 | 0.009597616 | 1.394046695 | Ten DAP | 4  | 23.4.01       | nucleotide metabolism |
| cassava4.1_015449m PA | AT1G28200 | 0.010436829 | 1.407374243 | Ten DAP | 3  | 17.1.3        | hormone metabolism    |
| cassava4.1_022406m PA | AT4G34030 | 0.010464068 | 1.115867703 | Ten DAP | 2  | 13.2.4.4      | amino acid metabolism |
| cassava4.1_010150m PA | AT4G17520 | 0.011073163 | 1.452526425 | Ten DAP | 4  | 27.4          | RNA                   |
| cassava4.1_010180m PA | AT2G17265 | 0.011336394 | 1.355471482 | Ten DAP | 5  | 13.1.3.6.1.04 | amino acid metabolism |
| cassava4.1_007540m PA | AT1G59900 | 0.011352076 | 1.398320386 | Ten DAP | 5  | 8.1.01.01     | TCA / org             |
| cassava4.1_011138m PA | AT3G22740 | 0.011499745 | 9.158163652 | Ten DAP | 7  | 13.1.3.4.012  | amino acid metabolism |
| cassava4.1_012276m PA | AT2G38750 | 0.011587371 | 1.127961248 | Ten DAP | 18 | 31.1          | cell                  |
| cassava4.1_014697m PA | AT5G43830 | 0.011633709 | 1.499349065 | Ten DAP | 6  | 15            | metal handling        |
| cassava4.1_014697m PA | AT5G43830 | 0.011633709 | 1.499349065 | Ten DAP | 6  | 17.2.3        | hormone metabolism    |
| cassava4.1_021183m PA | AT1G35720 | 0.011709466 | 1.169080202 | Ten DAP | 29 | 31.1          | cell                  |
| cassava4.1_011779m PA | AT5G05340 | 0.012357838 | 1.393003633 | Ten DAP | 9  | 26.12         | misc                  |
| cassava4.1_004831m PA | AT5G62530 | 0.012721537 | 1.369684373 | Ten DAP | 15 | 13.2.2.2      | amino acid metabolism |
| cassava4.1_007130m PA | AT1G04170 | 0.012777074 | 1.411027139 | Ten DAP | 11 | 29.2.3        | protein               |
| cassava4.1_032853m PA | AT3G60140 | 0.012963094 | 3.99794269  | Ten DAP | 2  | 26.03         | misc                  |
| cassava4.1_011775m PA | AT1G05260 | 0.013026138 | 1.752926694 | Ten DAP | 8  | 20.2.2        | stress                |
| cassava4.1_001259m PA | AT1G67560 | 0.013257182 | 1.196296357 | Ten DAP | 13 | 17.7.1.02     | hormone metabolism    |
| cassava4.1_006560m PA | AT5G27380 | 0.013390276 | 1.818096767 | Ten DAP | 2  | 21.2.2        | redox                 |
| cassava4.1_011934m PA | AT2G40010 | 0.013602982 | 1.172392775 | Ten DAP | 16 | 29.2.2        | protein               |
| cassava4.1_003090m PA | AT3G10740 | 0.013648187 | 1.783904242 | Ten DAP | 7  | 35.1          | not assigned          |

|                       |           |             |             |         |    |             |                                   |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|-----------------------------------|
| cassava4.1_006689m PA | AT1G64760 | 0.014038146 | 1.233673092 | Ten DAP | 5  | 35.1        | not assigned                      |
| cassava4.1_015319m PA | AT2G18110 | 0.01445178  | 1.568825174 | Ten DAP | 10 | 29.2.4      | protein                           |
| cassava4.1_004362m PA | AT4G37870 | 0.015794126 | 2.842536127 | Ten DAP | 17 | 6.04        | gluconeogenesis/ glyoxylate cycle |
| cassava4.1_006415m PA | AT1G63500 | 0.015797169 | 1.310031239 | Ten DAP | 5  | 29.4        | protein                           |
| cassava4.1_015242m PA | AT5G63620 | 0.015933251 | 2.085665939 | Ten DAP | 8  | 35.1        | not assigned                      |
| cassava4.1_015966m PA | AT3G62020 | 0.01599747  | 1.865450268 | Ten DAP | 5  | 20.2.99     | stress                            |
| cassava4.1_013284m PA | AT5G60660 | 0.016435053 | 1.4258252   | Ten DAP | 5  | 34.19.1     | transport                         |
| cassava4.1_001300m PA | AT1G74310 | 0.016472509 | 2.158408629 | Ten DAP | 53 | 20.2.1      | stress                            |
| cassava4.1_009233m PA | AT2G01140 | 0.016611607 | 1.31375782  | Ten DAP | 21 | 1.3.06      | PS                                |
| cassava4.1_008188m PA | AT3G48530 | 0.017106935 | 11.3332787  | Ten DAP | 6  | 35.1        | not assigned                      |
| cassava4.1_011051m PA | AT5G22330 | 0.017210329 | 1.579219012 | Ten DAP | 4  | 35.1        | not assigned                      |
| cassava4.1_029504m PA | AT4G39230 | 0.017316378 | 1.468246346 | Ten DAP | 10 | 16.8.5      | secondary metabolism              |
| cassava4.1_000903m PA | AT2G05710 | 0.017624705 | 1.241465409 | Ten DAP | 23 | 8.1.03      | TCA / org                         |
| cassava4.1_013205m PA | AT4G21320 | 0.017768663 | 2.769392266 | Ten DAP | 3  | 20.2.1      | stress                            |
| cassava4.1_003839m PA | AT3G03060 | 0.018067829 | 1.337480979 | Ten DAP | 6  | 29.5.11.20  | protein                           |
| cassava4.1_001530m PA | AT5G06350 | 0.018268656 | 1.962559571 | Ten DAP | 2  | 35.2        | not assigned                      |
| cassava4.1_001538m PA | AT4G35790 | 0.018376305 | 1.58413125  | Ten DAP | 11 | 11.9.3.01   | lipid metabolism                  |
| cassava4.1_010825m PA | AT5G08540 | 0.019285698 | 1.541150533 | Ten DAP | 7  | 35.2        | not assigned                      |
| cassava4.1_007524m PA | AT1G80600 | 0.01966984  | 1.692282687 | Ten DAP | 4  | 13.1.2.3.04 | amino acid metabolism             |
| cassava4.1_008175m PA | AT3G17810 | 0.020121655 | 1.346053171 | Ten DAP | 13 | 23.2        | nucleotide metabolism             |
| cassava4.1_023189m PA | AT3G42050 | 0.020321894 | 1.4106215   | Ten DAP | 15 | 34.1.01     | transport                         |
| cassava4.1_010559m PA | AT5G25770 | 0.020379347 | 1.271911282 | Ten DAP | 4  | 35.2        | not assigned                      |
| cassava4.1_011202m PA | AT1G50510 | 0.020600799 | 1.938611586 | Ten DAP | 3  | 35.1        | not assigned                      |
| cassava4.1_003404m PA | AT3G06510 | 0.021541596 | 2.038064428 | Ten DAP | 2  | 26.03       | misc                              |
| cassava4.1_004771m PA | AT5G04360 | 0.022131553 | 1.490585736 | Ten DAP | 7  | 2.1.2.04    | major CHO metabolism              |
| cassava4.1_008933m PA | AT2G43790 | 0.02218486  | 1.476349168 | Ten DAP | 9  | 30.6        | signalling                        |
| cassava4.1_008257m PA | AT2G38000 | 0.022194642 | 2.281747913 | Ten DAP | 11 | 20.2.1      | stress                            |
| cassava4.1_008257m PA | AT2G38000 | 0.022194642 | 2.281747913 | Ten DAP | 11 | 29.6        | protein                           |
| cassava4.1_008374m PA | AT5G09900 | 0.022289753 | 1.165663759 | Ten DAP | 16 | 29.5.11.20  | protein                           |
| cassava4.1_015645m PA | AT3G60820 | 0.022298921 | 1.17945286  | Ten DAP | 5  | 29.5.11.20  | protein                           |
| cassava4.1_006432m PA | AT5G11880 | 0.022360409 | 1.417256807 | Ten DAP | 18 | 13.1.3.5.05 | amino acid metabolism             |
| cassava4.1_008771m PA | AT1G09795 | 0.02236232  | 1.348388951 | Ten DAP | 6  | 13.1.7.01   | amino acid metabolism             |
| cassava4.1_011460m PA | AT5G42800 | 0.022640606 | 1.520848984 | Ten DAP | 5  | 16.8.3.01   | secondary metabolism              |
| cassava4.1_010140m PA | AT4G37970 | 0.022721325 | 1.327226671 | Ten DAP | 22 | 16.2.1.010  | secondary metabolism              |
| cassava4.1_002951m PA | AT5G13640 | 0.023079062 | 1.178699477 | Ten DAP | 2  | 11.8.10     | lipid metabolism                  |
| cassava4.1_021615m PA | AT3G55260 | 0.023288961 | 1.648389028 | Ten DAP | 3  | 35.1        | not assigned                      |
| cassava4.1_005844m PA | AT5G14220 | 0.023298414 | 1.544615719 | Ten DAP | 8  | 19.09       | tetrapyrrole synthesis            |
| cassava4.1_007980m PA | AT3G52880 | 0.023824769 | 1.420397426 | Ten DAP | 24 | 21.2        | redox                             |
| cassava4.1_001362m PA | AT1G69830 | 0.023873795 | 6.271784075 | Ten DAP | 7  | 2.2.2.1     | major CHO metabolism              |
| cassava4.1_004506m PA | AT1G60420 | 0.023916742 | 1.678195678 | Ten DAP | 19 | 35.1        | not assigned                      |
| cassava4.1_008421m PA | AT5G58290 | 0.023993294 | 1.485892869 | Ten DAP | 15 | 29.5.11.20  | protein                           |
| cassava4.1_010513m PA | AT2G28680 | 0.024262984 | 3.009674099 | Ten DAP | 10 | 33.1        | development                       |
| cassava4.1_011708m PA | AT2G45400 | 0.024337862 | 1.423547767 | Ten DAP | 5  | 16.8.4.01   | secondary metabolism              |
| cassava4.1_013730m PA | AT3G27430 | 0.02456257  | 1.842292617 | Ten DAP | 4  | 29.5.11.20  | protein                           |

|                       |           |             |             |         |    |              |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_006605m PA | AT3G12780 | 0.024595073 | 1.61206826  | Ten DAP | 26 | 1.3.03       | PS                                               |
| cassava4.1_006605m PA | AT3G12780 | 0.024595073 | 1.61206826  | Ten DAP | 26 | 4.010        | glycolysis                                       |
| cassava4.1_007181m PA | AT2G33150 | 0.024956686 | 2.286929361 | Ten DAP | 10 | 11.9.4.05    | lipid metabolism                                 |
| cassava4.1_007181m PA | AT2G33150 | 0.024956686 | 2.286929361 | Ten DAP | 10 | 13.2.4.1     | amino acid metabolism                            |
| cassava4.1_013823m PA | AT1G19580 | 0.025608326 | 1.192395482 | Ten DAP | 13 | 35.1         | not assigned                                     |
| cassava4.1_004359m PA | AT4G14210 | 0.025714328 | 1.388460969 | Ten DAP | 5  | 16.1.4.02    | secondary metabolism                             |
| cassava4.1_009976m PA | AT1G74640 | 0.025768111 | 1.406039938 | Ten DAP | 4  | 35.2         | not assigned                                     |
| cassava4.1_007163m PA | AT4G38220 | 0.025997167 | 1.607520884 | Ten DAP | 3  | 29.5         | protein                                          |
| cassava4.1_008563m PA | AT2G24580 | 0.026408252 | 1.497246038 | Ten DAP | 3  | 13.1.5.2.041 | amino acid metabolism                            |
| cassava4.1_002479m PA | AT3G06860 | 0.026417829 | 1.959534388 | Ten DAP | 21 | 11.9.4.09    | lipid metabolism                                 |
| cassava4.1_012423m PA | AT2G28680 | 0.026822532 | 3.966860939 | Ten DAP | 5  | 33.1         | development                                      |
| cassava4.1_006938m PA | AT3G06960 | 0.026921797 | 1.810813641 | Ten DAP | 4  | 35.2         | not assigned                                     |
| cassava4.1_006859m PA | AT5G46180 | 0.0271597   | 1.838310247 | Ten DAP | 5  | 13.2.2.3     | amino acid metabolism                            |
| cassava4.1_004619m PA | AT5G24300 | 0.027163229 | 1.244623178 | Ten DAP | 10 | 2.1.2.02     | major CHO metabolism                             |
| cassava4.1_012172m PA | AT4G24340 | 0.02779684  | 2.232144911 | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_010585m PA | AT2G22780 | 0.028130428 | 1.768182403 | Ten DAP | 2  | 6.03         | gluconeogenesis                                  |
| cassava4.1_001079m PA | AT3G08840 | 0.028145441 | 1.125596694 | Ten DAP | 25 | 35.1         | not assigned                                     |
| cassava4.1_004821m PA | AT4G02320 | 0.028879098 | 1.689617201 | Ten DAP | 13 | 10.8.99      | cell wall                                        |
| cassava4.1_011574m PA | AT5G66390 | 0.029161634 | 1.388266508 | Ten DAP | 9  | 26.12        | misc                                             |
| cassava4.1_009175m PA | AT2G20360 | 0.029255982 | 1.374081152 | Ten DAP | 15 | 9.1.2        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_030131m PA | AT4G37870 | 0.029497669 | 2.560662975 | Ten DAP | 2  | 6.04         | gluconeogene/ glyoxylate cycle                   |
| cassava4.1_013229m PA | AT3G23400 | 0.030211562 | 1.774555917 | Ten DAP | 5  | 31.1         | cell                                             |
| cassava4.1_006252m PA | AT1G44170 | 0.030252118 | 1.392279242 | Ten DAP | 19 | 5.10         | fermentation                                     |
| cassava4.1_014185m PA | AT3G11050 | 0.030807941 | 2.039471953 | Ten DAP | 14 | 15.2         | metal handling                                   |
| cassava4.1_009061m PA | AT1G45000 | 0.031655988 | 1.202475005 | Ten DAP | 21 | 29.5.11.20   | protein                                          |
| cassava4.1_034124m PA | AT2G38610 | 0.031898734 | 1.463435978 | Ten DAP | 5  | 35.1         | not assigned                                     |
| cassava4.1_005703m PA | AT3G22200 | 0.032529975 | 1.768579566 | Ten DAP | 17 | 13.1.1.1.02  | amino acid metabolism                            |
| cassava4.1_006367m PA | AT1G63940 | 0.03266988  | 1.746078082 | Ten DAP | 17 | 21.2.1       | redox                                            |
| cassava4.1_014262m PA | AT3G45970 | 0.032980867 | 1.465024207 | Ten DAP | 5  | 10.7         | cell wall                                        |
| cassava4.1_009693m PA | AT3G17880 | 0.033098781 | 1.637245385 | Ten DAP | 7  | 21.01        | redox                                            |
| cassava4.1_029420m PA | AT5G42260 | 0.033247131 | 5.52076836  | Ten DAP | 4  | 26.03        | misc                                             |
| cassava4.1_017520m PA | AT3G24540 | 0.033431781 | 1.416408678 | Ten DAP | 6  | 30.2.22      | signalling                                       |
| cassava4.1_010343m PA | AT1G17020 | 0.033740587 | 1.772202731 | Ten DAP | 3  | 16.8.4       | secondary metabolism                             |
| cassava4.1_006853m PA | AT2G44350 | 0.033774052 | 1.613845419 | Ten DAP | 4  | 8.1.02       | TCA / org                                        |
| cassava4.1_009779m PA | AT5G09810 | 0.034029813 | 1.185250403 | Ten DAP | 39 | 31.1         | cell                                             |
| cassava4.1_003490m PA | AT3G52200 | 0.034907379 | 1.263526843 | Ten DAP | 18 | 8.1.01.02    | TCA / org                                        |
| cassava4.1_009991m PA | AT4G17370 | 0.035048704 | 1.72928579  | Ten DAP | 2  | 35.1         | not assigned                                     |
| cassava4.1_009469m PA | AT5G62390 | 0.035367764 | 1.561448059 | Ten DAP | 13 | 30.3         | signalling                                       |
| cassava4.1_007251m PA | AT4G38220 | 0.035387818 | 1.539019794 | Ten DAP | 15 | 29.5         | protein                                          |
| cassava4.1_007931m PA | AT1G53280 | 0.0355971   | 1.584043363 | Ten DAP | 20 | 35.1         | not assigned                                     |
| cassava4.1_005201m PA | AT4G09510 | 0.035931392 | 1.279162588 | Ten DAP | 6  | 2.2.1.03.01  | major CHO metabolism                             |
| cassava4.1_008212m PA | AT1G53750 | 0.037236273 | 1.524920926 | Ten DAP | 15 | 29.5.11.20   | protein                                          |
| cassava4.1_008528m PA | AT1G49820 | 0.037640945 | 1.716446498 | Ten DAP | 13 | 35.1         | not assigned                                     |
| cassava4.1_027841m PA | AT4G19880 | 0.03792572  | 1.689813809 | Ten DAP | 2  | 26.09        | misc                                             |

|                       |           |             |             |         |    |              |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_007221m PA | AT1G47840 | 0.038045423 | 1.512944971 | Ten DAP | 17 | 2.2.1.04     | major CHO metabolism                             |
| cassava4.1_006559m PA | AT2G18730 | 0.038275462 | 1.306196433 | Ten DAP | 7  | 11.3.05      | lipid metabolism                                 |
| cassava4.1_007094m PA | AT4G31990 | 0.038616693 | 1.344153267 | Ten DAP | 22 | 13.1.1.2.01  | amino acid metabolism                            |
| cassava4.1_013447m PA | AT1G52340 | 0.038862279 | 1.283231678 | Ten DAP | 8  | 17.1.1.1.011 | hormone metabolism                               |
| cassava4.1_006138m PA | AT4G29130 | 0.039299266 | 1.559017046 | Ten DAP | 15 | 2.2.1.04     | major CHO metabolism                             |
| cassava4.1_006303m PA | AT4G35090 | 0.039559359 | 3.329616518 | Ten DAP | 3  | 21.6         | redox                                            |
| cassava4.1_003427m PA | AT5G57580 | 0.039731649 | 2.126304302 | Ten DAP | 3  | 30.3         | signalling                                       |
| cassava4.1_001233m PA | AT1G68560 | 0.039750451 | 1.79339563  | Ten DAP | 2  | 2.2.2.1      | major CHO metabolism                             |
| cassava4.1_001233m PA | AT1G68560 | 0.039750451 | 1.79339563  | Ten DAP | 2  | 26.03        | misc                                             |
| cassava4.1_002552m PA | AT1G50380 | 0.039766223 | 1.485492241 | Ten DAP | 15 | 29.5         | protein                                          |
| cassava4.1_015763m PA | AT5G63880 | 0.040025265 | 1.232033827 | Ten DAP | 2  | 27.3.71      | RNA                                              |
| cassava4.1_002871m PA | AT5G04590 | 0.040264369 | 1.20751879  | Ten DAP | 9  | 14.03        | S-assimilation                                   |
| cassava4.1_019325m PA | AT5G42890 | 0.04040195  | 1.561863456 | Ten DAP | 2  | 11.8         | lipid metabolism                                 |
| cassava4.1_001514m PA | AT5G12950 | 0.040475663 | 1.331413898 | Ten DAP | 9  | 35.2         | not assigned                                     |
| cassava4.1_011289m PA | AT5G65550 | 0.04073648  | 1.380319881 | Ten DAP | 6  | 16.8.1.012   | secondary metabolism                             |
| cassava4.1_008844m PA | AT5G19550 | 0.04100868  | 1.260339511 | Ten DAP | 22 | 13.1.1.2.01  | amino acid metabolism                            |
| cassava4.1_018274m PA | -         | 0.041254303 | 2.271361357 | Ten DAP | 8  | -            | -                                                |
| cassava4.1_005935m PA | AT3G18080 | 0.041369304 | 1.68803424  | Ten DAP | 4  | 26.03        | misc                                             |
| cassava4.1_006286m PA | AT4G24830 | 0.041827029 | 1.460609283 | Ten DAP | 21 | 13.1.2.3.022 | amino acid metabolism                            |
| cassava4.1_009614m PA | AT4G32400 | 0.041859795 | 1.204114911 | Ten DAP | 4  | 2.1.2.05     | major CHO metabolism                             |
| cassava4.1_009614m PA | AT4G32400 | 0.041859795 | 1.204114911 | Ten DAP | 4  | 34.8         | transport                                        |
| cassava4.1_004864m PA | AT3G13930 | 0.042369153 | 1.298227513 | Ten DAP | 24 | 8.1.01.02    | TCA / org                                        |
| cassava4.1_010126m PA | AT3G14420 | 0.042417068 | 1.750614151 | Ten DAP | 3  | 1.2.02       | PS                                               |
| cassava4.1_001244m PA | AT1G68560 | 0.042873595 | 1.296483626 | Ten DAP | 18 | 2.2.2.1      | major CHO metabolism                             |
| cassava4.1_001244m PA | AT1G68560 | 0.042873595 | 1.296483626 | Ten DAP | 18 | 26.03        | misc                                             |
| cassava4.1_008387m PA | AT2G20420 | 0.043278571 | 1.300115518 | Ten DAP | 29 | 8.1.06       | TCA / org                                        |
| cassava4.1_004238m PA | AT3G25800 | 0.044526673 | 1.170059726 | Ten DAP | 6  | 29.4         | protein                                          |
| cassava4.1_013231m PA | AT4G00430 | 0.044991767 | 1.724545394 | Ten DAP | 6  | 34.19.1      | transport                                        |
| cassava4.1_012932m PA | AT5G39790 | 0.045000512 | 1.769509262 | Ten DAP | 3  | 30.1         | signalling                                       |
| cassava4.1_024858m PA | AT5G07090 | 0.0453126   | 1.360660378 | Ten DAP | 11 | 29.2.2       | protein                                          |
| cassava4.1_015980m PA | AT5G42650 | 0.045488344 | 1.540189318 | Ten DAP | 2  | 17.7.1.03    | hormone metabolism                               |
| cassava4.1_022125m PA | AT4G20360 | 0.04550581  | 2.152845168 | Ten DAP | 2  | 29.2.4       | protein                                          |
| cassava4.1_012203m PA | AT3G12070 | 0.046365439 | 1.254214436 | Ten DAP | 3  | 16.1.1       | secondary metabolism                             |
| cassava4.1_007019m PA | AT3G57050 | 0.047772756 | 1.5727444   | Ten DAP | 9  | 13.1.3.4.02  | amino acid metabolism                            |
| cassava4.1_013108m PA | AT4G28510 | 0.047867372 | 1.095548426 | Ten DAP | 15 | 35.1         | not assigned                                     |
| cassava4.1_009952m PA | AT4G35650 | 0.04786744  | 1.431034228 | Ten DAP | 14 | 8.2.04       | TCA / org                                        |
| cassava4.1_029754m PA | -         | 0.048235431 | 2.005286887 | Ten DAP | 12 | -            | -                                                |
| cassava4.1_016410m PA | AT4G20260 | 0.048320706 | 1.827661574 | Ten DAP | 15 | 35.1         | not assigned                                     |
| cassava4.1_006146m PA | AT5G24318 | 0.049024098 | 1.605479466 | Ten DAP | 2  |              |                                                  |
| cassava4.1_009232m PA | AT1G53880 | 0.049159598 | 1.323855844 | Ten DAP | 2  | 29.2.3       | protein                                          |
| cassava4.1_006167m PA | AT2G20710 | 0.049873837 | 1.601900133 | Ten DAP | 2  | 27.3.67      | RNA                                              |
| cassava4.1_014405m PA | AT4G05530 | 0.050516088 | 1.513458331 | Ten DAP | 4  | 26.22        | misc                                             |
| cassava4.1_012350m PA | AT5G40810 | 0.051180451 | 1.333333982 | Ten DAP | 10 | 9.6          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_009774m PA | AT4G19810 | 0.051297032 | 2.477640027 | Ten DAP | 2  | 20.1         | stress                                           |

|                       |           |             |             |         |    |             |                       |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|-----------------------|
| cassava4.1_013146m PA | AT3G25530 | 0.051384993 | 2.293879567 | Ten DAP | 9  | 7.1.03      | OPP                   |
| cassava4.1_004242m PA | AT4G34740 | 0.05201263  | 1.463150813 | Ten DAP | 3  | 23.1.2.01   | nucleotide metabolism |
| cassava4.1_025314m PA | AT5G05340 | 0.05239306  | 1.681763208 | Ten DAP | 6  | 26.12       | misc                  |
| cassava4.1_007958m PA | AT2G37860 | 0.052532176 | 1.327618316 | Ten DAP | 4  | 33.99       | development           |
| cassava4.1_033681m PA | AT5G52640 | 0.052579521 | 2.219789608 | Ten DAP | 8  | 20.2.1      | stress                |
| cassava4.1_003547m PA | AT3G22520 | 0.053450028 | 1.424501163 | Ten DAP | 12 | 35.2        | not assigned          |
| cassava4.1_012822m PA | AT3G19450 | 0.053613244 | 1.339313346 | Ten DAP | 8  | 16.2.1.010  | secondary metabolism  |
| cassava4.1_015781m PA | AT5G17710 | 0.053680231 | 1.914582244 | Ten DAP | 5  | 29.6        | protein               |
| cassava4.1_009957m PA | AT4G01370 | 0.05461617  | 2.199295373 | Ten DAP | 2  | 30.6        | signalling            |
| cassava4.1_008595m PA | AT1G79340 | 0.055298181 | 2.241940642 | Ten DAP | 3  | 29.5        | protein               |
| cassava4.1_007758m PA | AT5G13420 | 0.055953372 | 1.564594557 | Ten DAP | 17 | 7.2.02      | OPP                   |
| cassava4.1_013928m PA | AT2G40300 | 0.056077517 | 2.096646531 | Ten DAP | 2  | 15.2        | metal handling        |
| cassava4.1_010112m PA | AT4G34660 | 0.056201116 | 2.572800448 | Ten DAP | 2  | 35.1        | not assigned          |
| cassava4.1_004646m PA | AT3G57890 | 0.057087088 | 1.600495507 | Ten DAP | 4  | 31.1        | cell                  |
| cassava4.1_012124m PA | AT5G05340 | 0.057482353 | 1.294967875 | Ten DAP | 16 | 26.12       | misc                  |
| cassava4.1_015076m PA | AT3G23600 | 0.057643451 | 2.518995587 | Ten DAP | 6  | 26.01       | misc                  |
| cassava4.1_008470m PA | AT2G30970 | 0.057728064 | 1.299925515 | Ten DAP | 8  | 13.1.1.2.01 | amino acid metabolism |
| cassava4.1_015518m PA | AT1G52560 | 0.057826935 | 5.765906962 | Ten DAP | 3  | 20.2.1      | stress                |
| cassava4.1_011348m PA | AT2G38550 | 0.058066603 | 2.12048969  | Ten DAP | 8  | 35.2        | not assigned          |
| cassava4.1_016053m PA | AT4G17870 | 0.058304653 | 1.453804348 | Ten DAP | 2  | 35.2        | not assigned          |
| cassava4.1_007767m PA | AT3G15000 | 0.058402951 | 1.475471216 | Ten DAP | 2  | 35.1        | not assigned          |
| cassava4.1_015693m PA | AT3G23790 | 0.05866846  | 1.214305238 | Ten DAP | 3  | 11.1.08     | lipid metabolism      |
| cassava4.1_005434m PA | AT3G02090 | 0.05916803  | 1.143925682 | Ten DAP | 26 | 29.3.2      | protein               |
| cassava4.1_008899m PA | AT4G37560 | 0.059230945 | 2.478273535 | Ten DAP | 3  | 26.01       | misc                  |
| cassava4.1_006239m PA | AT5G55530 | 0.059323751 | 1.716334891 | Ten DAP | 2  | 20.2.2      | stress                |
| cassava4.1_013192m PA | AT2G37170 | 0.059604376 | 1.405645798 | Ten DAP | 2  | 34.19.1     | transport             |
| cassava4.1_003884m PA | AT1G32900 | 0.059838338 | 1.766510779 | Ten DAP | 22 | 2.1.2.02    | major CHO metabolism  |
| cassava4.1_009342m PA | AT1G23740 | 0.060365659 | 1.883058797 | Ten DAP | 3  | 26.07       | misc                  |
| cassava4.1_003800m PA | AT4G18240 | 0.06039722  | 1.388017137 | Ten DAP | 13 | 2.1.2.02    | major CHO metabolism  |
| cassava4.1_003755m PA | AT1G80300 | 0.060462505 | 1.398461807 | Ten DAP | 7  | 34.99       | transport             |
| cassava4.1_005238m PA | AT5G12200 | 0.060578289 | 1.51697375  | Ten DAP | 7  | 23.2        | nucleotide metabolism |
| cassava4.1_010524m PA | AT5G62740 | 0.060582809 | 1.324566012 | Ten DAP | 8  | 35.1        | not assigned          |
| cassava4.1_006893m PA | AT5G01930 | 0.060823333 | 1.859860797 | Ten DAP | 5  | 10.6.2      | cell wall             |
| cassava4.1_011816m PA | AT4G21580 | 0.060938874 | 1.3633373   | Ten DAP | 13 | 26.01       | misc                  |
| cassava4.1_012665m PA | AT1G34750 | 0.061007351 | 1.772860503 | Ten DAP | 2  | 29.4        | protein               |
| cassava4.1_014374m PA | AT1G23730 | 0.061351853 | 3.988592007 | Ten DAP | 3  | 8.03        | TCA / org             |
| cassava4.1_014374m PA | AT1G23730 | 0.061351853 | 3.988592007 | Ten DAP | 3  | 16.99       | secondary metabolism  |
| cassava4.1_014300m PA | AT1G11360 | 0.061895336 | 1.30200224  | Ten DAP | 9  | 20.2.99     | stress                |
| cassava4.1_009634m PA | AT2G05830 | 0.062010836 | 1.314886414 | Ten DAP | 12 | 29.2.3      | protein               |
| cassava4.1_014556m PA | AT5G65430 | 0.062325234 | 2.620329253 | Ten DAP | 5  | 30.7        | signalling            |
| cassava4.1_000993m PA | AT3G26720 | 0.062798181 | 1.47073049  | Ten DAP | 10 | 26.03       | misc                  |
| cassava4.1_005204m PA | AT5G63190 | 0.063137994 | 1.876195606 | Ten DAP | 6  | 28.99       | DNA                   |
| cassava4.1_008812m PA | AT3G09630 | 0.063667245 | 1.209794454 | Ten DAP | 28 | 29.2.2      | protein               |
| cassava4.1_022124m PA | AT5G46180 | 0.064380903 | 1.660399724 | Ten DAP | 8  | 13.2.2.3    | amino acid metabolism |

|                       |           |             |             |         |    |              |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_004784m PA | AT1G23010 | 0.064509475 | 1.995783063 | Ten DAP | 4  | 35.1         | not assigned                                     |
| cassava4.1_005834m PA | AT3G06850 | 0.064513066 | 1.738671063 | Ten DAP | 5  | 13.2.4.1     | amino acid metabolism                            |
| cassava4.1_014362m PA | AT1G76010 | 0.06456237  | 1.666145104 | Ten DAP | 3  | 27.3.67      | RNA                                              |
| cassava4.1_015081m PA | AT4G39120 | 0.064596342 | 3.292903674 | Ten DAP | 2  | 3.4.05       | minor CHO metabolism                             |
| cassava4.1_007300m PA | AT1G15000 | 0.064658902 | 1.594007354 | Ten DAP | 6  | 29.5.05      | protein                                          |
| cassava4.1_005513m PA | AT4G23100 | 0.064935838 | 1.731188063 | Ten DAP | 10 | 21.2.2       | redox                                            |
| cassava4.1_012863m PA | AT5G40450 | 0.065291969 | 2.333114682 | Ten DAP | 4  | 35.2         | not assigned                                     |
| cassava4.1_028771m PA | AT2G44470 | 0.065772681 | 5.377251633 | Ten DAP | 5  | 26.03        | misc                                             |
| cassava4.1_013905m PA | AT1G53990 | 0.066075431 | 2.4408933   | Ten DAP | 2  | 26.28        | misc                                             |
| cassava4.1_014450m PA | AT4G35220 | 0.066128776 | 1.551299072 | Ten DAP | 2  | 28.99        | DNA                                              |
| cassava4.1_015185m PA | AT1G16470 | 0.066160414 | 1.476539204 | Ten DAP | 9  | 29.5         | protein                                          |
| cassava4.1_005478m PA | AT3G15180 | 0.066163812 | 1.038889796 | Ten DAP | 4  | 29.5.11.20   | protein                                          |
| cassava4.1_011093m PA | AT2G34460 | 0.066175915 | 1.54684509  | Ten DAP | 6  | 35.1         | not assigned                                     |
| cassava4.1_005072m PA | AT5G07830 | 0.066891806 | 1.33476902  | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_009222m PA | AT3G51800 | 0.067106988 | 1.481433002 | Ten DAP | 17 | 27.3.67      | RNA                                              |
| cassava4.1_010413m PA | AT3G20790 | 0.067108396 | 1.804225249 | Ten DAP | 4  | 35.1         | not assigned                                     |
| cassava4.1_025830m PA | AT4G13360 | 0.067457245 | 1.302273849 | Ten DAP | 9  | 11.9.4.03    | lipid metabolism                                 |
| cassava4.1_025830m PA | AT4G13360 | 0.067457245 | 1.302273849 | Ten DAP | 9  | 13.2.3.5     | amino acid metabolism                            |
| cassava4.1_004654m PA | AT1G74960 | 0.067936275 | 2.119408602 | Ten DAP | 5  | 11.1.03      | lipid metabolism                                 |
| cassava4.1_004749m PA | AT3G54660 | 0.068054203 | 1.342478493 | Ten DAP | 13 | 21.2.2       | redox                                            |
| cassava4.1_006422m PA | AT1G63500 | 0.068439156 | 1.561000762 | Ten DAP | 9  | 29.4         | protein                                          |
| cassava4.1_006514m PA | AT3G02360 | 0.068515089 | 1.157948949 | Ten DAP | 24 | 7.1.03       | OPP                                              |
| cassava4.1_011834m PA | AT5G20080 | 0.069813724 | 2.735159814 | Ten DAP | 4  | 21.99        | redox                                            |
| cassava4.1_018310m PA | AT1G70830 | 0.070129725 | 1.326049479 | Ten DAP | 5  | 20.2.99      | stress                                           |
| cassava4.1_010796m PA | AT1G71695 | 0.070223875 | 1.68419489  | Ten DAP | 14 | 26.12        | misc                                             |
| cassava4.1_006541m PA | AT5G13700 | 0.070271449 | 1.434290406 | Ten DAP | 6  | 22.2.1       | polyamine metabolism                             |
| cassava4.1_010793m PA | AT3G28715 | 0.070803684 | 1.594073253 | Ten DAP | 6  | 34.1.01      | transport                                        |
| cassava4.1_009230m PA | AT1G30580 | 0.071021211 | 1.396539902 | Ten DAP | 14 | 35.2         | not assigned                                     |
| cassava4.1_008265m PA | AT5G14780 | 0.071294825 | 1.526066796 | Ten DAP | 21 | 25.10        | C1-metabolism                                    |
| cassava4.1_004581m PA | AT5G42740 | 0.071848769 | 1.095756629 | Ten DAP | 16 | 4.03         | glycolysis                                       |
| cassava4.1_029773m PA | -         | 0.072573514 | 3.529971943 | Ten DAP | 4  | -            | -                                                |
| cassava4.1_011685m PA | AT3G14130 | 0.072577913 | 1.881728673 | Ten DAP | 3  | 1.2.02       | PS                                               |
| cassava4.1_006534m PA | AT4G39660 | 0.073111024 | 1.53209489  | Ten DAP | 4  | 13.1.1.3.011 | amino acid metabolism                            |
| cassava4.1_013157m PA | AT2G32520 | 0.073329733 | 2.407094125 | Ten DAP | 5  | 26.01        | misc                                             |
| cassava4.1_005717m PA | AT5G01590 | 0.073330691 | 1.767087109 | Ten DAP | 4  | 35.2         | not assigned                                     |
| cassava4.1_013158m PA | AT1G11840 | 0.073713737 | 1.465869143 | Ten DAP | 17 | 13.2.3.2     | amino acid metabolism                            |
| cassava4.1_013158m PA | AT1G11840 | 0.073713737 | 1.465869143 | Ten DAP | 17 | 24.02        | Biodegradation of Xenobiotics                    |
| cassava4.1_007696m PA | AT4G05390 | 0.07422721  | 1.242594337 | Ten DAP | 9  | 7.3          | OPP                                              |
| cassava4.1_000971m PA | AT3G26720 | 0.075131762 | 1.746442693 | Ten DAP | 2  | 26.03        | misc                                             |
| cassava4.1_014824m PA | AT3G54110 | 0.075192815 | 2.108727377 | Ten DAP | 5  | 9.8          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_003459m PA | AT1G48480 | 0.075341077 | 1.478632347 | Ten DAP | 5  | 30.2.3       | signalling                                       |
| cassava4.1_015142m PA | AT1G50380 | 0.075596309 | 1.628853185 | Ten DAP | 2  | 29.5         | protein                                          |
| cassava4.1_007371m PA | AT5G23300 | 0.075944318 | 1.662588682 | Ten DAP | 7  | 23.1.1.04    | nucleotide metabolism                            |
| cassava4.1_010564m PA | AT2G47470 | 0.076002899 | 1.158256843 | Ten DAP | 12 | 21.01        | redox                                            |



|                       |           |             |             |         |    |             |                                   |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|-----------------------------------|
| cassava4.1_007825m PA | AT3G51840 | 0.076044277 | 1.456671513 | Ten DAP | 7  | 11.9.4.02   | lipid metabolism                  |
| cassava4.1_009435m PA | AT1G67830 | 0.076232759 | 1.875391751 | Ten DAP | 7  | 26.28       | misc                              |
| cassava4.1_001583m PA | AT1G68020 | 0.076347475 | 1.661357559 | Ten DAP | 10 | 3.2.3       | minor CHO metabolism              |
| cassava4.1_017275m PA | AT1G27450 | 0.076350311 | 1.775992811 | Ten DAP | 6  | 23.3.1.01   | nucleotide metabolism             |
| cassava4.1_004722m PA | AT2G12550 | 0.076713491 | 1.564076367 | Ten DAP | 7  | 29.5.11     | protein                           |
| cassava4.1_016679m PA | AT4G02450 | 0.077528949 | 1.913315701 | Ten DAP | 3  | 35.1.40     | not assigned                      |
| cassava4.1_004909m PA | AT1G20510 | 0.077832438 | 1.79561715  | Ten DAP | 11 | 16.2        | secondary metabolism              |
| cassava4.1_010152m PA | AT1G24090 | 0.078300659 | 1.568456912 | Ten DAP | 2  | 27.1.19     | RNA                               |
| cassava4.1_006963m PA | AT4G29010 | 0.078731416 | 1.39991261  | Ten DAP | 4  | 11.9.4.09   | lipid metabolism                  |
| cassava4.1_009577m PA | AT3G20320 | 0.079683254 | 1.382229445 | Ten DAP | 7  | 35.1        | not assigned                      |
| cassava4.1_012353m PA | AT5G35360 | 0.079896684 | 1.253456069 | Ten DAP | 16 | 11.1.01     | lipid metabolism                  |
| cassava4.1_009867m PA | AT4G08390 | 0.080470099 | 1.346612793 | Ten DAP | 13 | 21.2.1      | redox                             |
| cassava4.1_000229m PA | AT1G15520 | 0.080774004 | 1.739864434 | Ten DAP | 4  | 34.16       | transport                         |
| cassava4.1_010609m PA | AT2G22780 | 0.081508672 | 1.630146593 | Ten DAP | 9  | 6.03        | gluconeogenesis                   |
| cassava4.1_011797m PA | AT3G12500 | 0.081958189 | 1.505522867 | Ten DAP | 4  | 20.1        | stress                            |
| cassava4.1_007775m PA | AT1G51760 | 0.082856187 | 1.636460263 | Ten DAP | 9  | 17.2.1      | hormone metabolism                |
| cassava4.1_005856m PA | AT1G48030 | 0.083034992 | 1.115108049 | Ten DAP | 28 | 8.1.01.03   | TCA / org                         |
| cassava4.1_005713m PA | AT1G20200 | 0.083222592 | 1.237560229 | Ten DAP | 23 | 29.5.11.20  | protein                           |
| cassava4.1_025703m PA | AT1G17160 | 0.083268321 | 1.843801599 | Ten DAP | 4  | 3.5         | minor CHO metabolism              |
| cassava4.1_008310m PA | AT3G05530 | 0.08333596  | 1.190981454 | Ten DAP | 21 | 29.5.11.20  | protein                           |
| cassava4.1_016682m PA | AT3G21110 | 0.083977953 | 2.063122047 | Ten DAP | 3  | 23.1.2.07   | nucleotide metabolism             |
| cassava4.1_025834m PA | AT5G58590 | 0.083994416 | 1.135409662 | Ten DAP | 8  | 30.5        | signalling                        |
| cassava4.1_005305m PA | AT1G52260 | 0.08415807  | 1.181996761 | Ten DAP | 14 | 21.01       | redox                             |
| cassava4.1_004173m PA | AT1G72160 | 0.084369128 | 1.387906865 | Ten DAP | 27 | 28.99       | DNA                               |
| cassava4.1_004173m PA | AT1G72160 | 0.084369128 | 1.387906865 | Ten DAP | 27 | 34.99       | transport                         |
| cassava4.1_008246m PA | AT4G35630 | 0.084469505 | 1.274305843 | Ten DAP | 18 | 13.1.5.1.02 | amino acid metabolism             |
| cassava4.1_031450m PA | AT3G02090 | 0.084526198 | 1.312059731 | Ten DAP | 5  | 29.3.2      | protein                           |
| cassava4.1_001250m PA | AT1G58370 | 0.085756528 | 1.692607178 | Ten DAP | 9  | 10.6.2      | cell wall                         |
| cassava4.1_014862m PA | AT3G12490 | 0.086067278 | 1.835124189 | Ten DAP | 8  | 29.5.03     | protein                           |
| cassava4.1_009795m PA | AT5G12040 | 0.086437944 | 1.573525459 | Ten DAP | 9  | 35.1        | not assigned                      |
| cassava4.1_008822m PA | AT1G11860 | 0.087461873 | 1.444836627 | Ten DAP | 13 | 13.2.5.2    | amino acid metabolism             |
| cassava4.1_009217m PA | AT2G01140 | 0.088076604 | 1.711619311 | Ten DAP | 5  | 1.3.06      | PS                                |
| cassava4.1_014519m PA | AT5G65430 | 0.088374287 | 2.340842327 | Ten DAP | 2  | 30.7        | signalling                        |
| cassava4.1_015372m PA | AT2G18050 | 0.089306229 | 20.78379869 | Ten DAP | 3  | 28.1.3      | DNA                               |
| cassava4.1_015148m PA | AT5G35530 | 0.089423011 | 1.175309963 | Ten DAP | 19 | 29.2.2      | protein                           |
| cassava4.1_027209m PA | AT5G50950 | 0.089605968 | 1.31052149  | Ten DAP | 19 | 8.1.08      | TCA / org                         |
| cassava4.1_007687m PA | AT4G29040 | 0.090152619 | 1.458370165 | Ten DAP | 19 | 29.5.11.20  | protein                           |
| cassava4.1_007682m PA | AT5G66120 | 0.090178485 | 1.26512224  | Ten DAP | 14 | 13.1.6.1.02 | amino acid metabolism             |
| cassava4.1_007682m PA | AT5G66120 | 0.090178485 | 1.26512224  | Ten DAP | 14 | 18.5        | Co-factor and vitamine metabolism |
| cassava4.1_013197m PA | AT4G10960 | 0.090683565 | 1.779109445 | Ten DAP | 2  | 10.1.02     | cell wall                         |
| cassava4.1_005338m PA | AT1G79440 | 0.092137298 | 1.325675898 | Ten DAP | 15 | 8.2.99      | TCA / org                         |
| cassava4.1_005338m PA | AT1G79440 | 0.092137298 | 1.325675898 | Ten DAP | 15 | 13.1.1.1.03 | amino acid metabolism             |
| cassava4.1_018127m PA | AT1G07400 | 0.092940795 | 2.246755149 | Ten DAP | 2  | 20.2.1      | stress                            |
| cassava4.1_028027m PA | AT1G05150 | 0.09335274  | 1.313032591 | Ten DAP | 13 | 30.3        | signalling                        |

|                       |           |             |             |         |    |             |                        |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|------------------------|
| cassava4.1_033243m PA | AT1G29670 | 0.093570108 | 1.372641654 | Ten DAP | 3  | 26.28       | misc                   |
| cassava4.1_012577m PA | AT4G04210 | 0.09366112  | 1.131417713 | Ten DAP | 3  | 29.5        | protein                |
| cassava4.1_011945m PA | AT5G39580 | 0.093767439 | 1.520976492 | Ten DAP | 14 | 26.12       | misc                   |
| cassava4.1_012175m PA | AT1G72370 | 0.093878715 | 1.43518343  | Ten DAP | 11 | 29.2.2      | protein                |
| cassava4.1_012123m PA | AT5G65020 | 0.094034705 | 1.185072511 | Ten DAP | 23 | 31.1        | cell                   |
| cassava4.1_015937m PA | AT1G08360 | 0.09491255  | 1.463014366 | Ten DAP | 5  | 29.2.2      | protein                |
| cassava4.1_014785m PA | AT2G05840 | 0.095075691 | 1.879268117 | Ten DAP | 2  | 29.5.11.20  | protein                |
| cassava4.1_010625m PA | AT1G72680 | 0.095350359 | 1.155570461 | Ten DAP | 6  | 16.2.1.010  | secondary metabolism   |
| cassava4.1_004357m PA | AT3G14310 | 0.095743875 | 1.240568708 | Ten DAP | 20 | 10.8.01     | cell wall              |
| cassava4.1_014530m PA | AT5G65430 | 0.096514632 | 1.909766122 | Ten DAP | 20 | 30.7        | signalling             |
| cassava4.1_025303m PA | AT1G34760 | 0.096807014 | 1.39546415  | Ten DAP | 4  | 30.7        | signalling             |
| cassava4.1_016186m PA | AT3G01280 | 0.097118226 | 1.55317116  | Ten DAP | 3  | 34.20       | transport              |
| cassava4.1_016265m PA | AT1G50670 | 0.097774385 | 1.463405664 | Ten DAP | 3  | 29.5.03     | protein                |
| cassava4.1_003715m PA | AT2G42520 | 0.098027506 | 1.751151739 | Ten DAP | 6  | 27.1.2      | RNA                    |
| cassava4.1_010901m PA | AT2G37400 | 0.09827619  | 1.616095121 | Ten DAP | 7  | 35.1        | not assigned           |
| cassava4.1_006016m PA | AT5G38530 | 0.098580554 | 1.533754741 | Ten DAP | 2  | 13.1.6.5.05 | amino acid metabolism  |
| cassava4.1_005135m PA | AT3G48000 | 0.098895426 | 1.459693801 | Ten DAP | 12 | 5.10        | fermentation           |
| cassava4.1_012377m PA | AT5G05780 | 0.09921274  | 1.146935279 | Ten DAP | 7  | 29.5.11.20  | protein                |
| cassava4.1_014880m PA | AT4G35220 | 0.099505094 | 1.241651867 | Ten DAP | 3  | 28.99       | DNA                    |
| cassava4.1_003947m PA | AT3G03250 | 0.099628734 | 1.973510332 | Ten DAP | 5  | 4.01        | glycolysis             |
| cassava4.1_007378m PA | AT4G02930 | 0.099637493 | 1.422604815 | Ten DAP | 19 | 29.2.4      | protein                |
| cassava4.1_031851m PA | AT2G28470 | 0.100173729 | 1.824336403 | Ten DAP | 2  | 10.6.2      | cell wall              |
| cassava4.1_031851m PA | AT2G28470 | 0.100173729 | 1.824336403 | Ten DAP | 2  | 26.03       | misc                   |
| cassava4.1_004631m PA | AT3G16910 | 0.100232505 | 1.900017914 | Ten DAP | 7  | 11.1.08     | lipid metabolism       |
| cassava4.1_002296m PA | AT5G66420 | 0.100338059 | 1.256878759 | Ten DAP | 9  | 35.2        | not assigned           |
| cassava4.1_014189m PA | AT3G58730 | 0.100433077 | 1.928489194 | Ten DAP | 14 | 34.1.01     | transport              |
| cassava4.1_009858m PA | AT5G41970 | 0.100711165 | 1.221749119 | Ten DAP | 2  | 35.2        | not assigned           |
| cassava4.1_016080m PA | AT4G32770 | 0.102437178 | 1.181698428 | Ten DAP | 2  | 16.1.3.04   | secondary metabolism   |
| cassava4.1_013758m PA | AT1G79690 | 0.102708689 | 1.519950738 | Ten DAP | 8  | 35.1        | not assigned           |
| cassava4.1_015360m PA | AT1G74050 | 0.102916491 | 1.811506317 | Ten DAP | 12 | 29.2.2      | protein                |
| cassava4.1_013127m PA | AT2G26670 | 0.102936095 | 1.947373893 | Ten DAP | 2  | 19.021      | tetrapyrrole synthesis |
| cassava4.1_005518m PA | AT5G48300 | 0.102984919 | 1.399319996 | Ten DAP | 41 | 2.1.2.01    | major CHO metabolism   |
| cassava4.1_009995m PA | AT5G14040 | 0.103680728 | 1.24008339  | Ten DAP | 4  | 34.9        | transport              |
| cassava4.1_008398m PA | AT2G47960 | 0.104158069 | 1.774534008 | Ten DAP | 4  | 35.2        | not assigned           |
| cassava4.1_007756m PA | AT5G13420 | 0.104546175 | 1.435539489 | Ten DAP | 12 | 7.2.02      | OPP                    |
| cassava4.1_010500m PA | AT2G17390 | 0.104635787 | 1.422041907 | Ten DAP | 10 | 27.3.39     | RNA                    |
| cassava4.1_011898m PA | AT2G45960 | 0.104671447 | 1.305293364 | Ten DAP | 4  | 34.19.1     | transport              |
| cassava4.1_014340m PA | AT3G07030 | 0.104703753 | 1.449146193 | Ten DAP | 2  | 35.2        | not assigned           |
| cassava4.1_009119m PA | AT2G06050 | 0.105660151 | 1.960366965 | Ten DAP | 4  | 17.7.1.05   | hormone metabolism     |
| cassava4.1_014361m PA | AT5G44730 | 0.105799638 | 1.559748533 | Ten DAP | 2  | 33.99       | development            |
| cassava4.1_007070m PA | AT3G07320 | 0.106068623 | 1.272388897 | Ten DAP | 4  | 26.04       | misc                   |
| cassava4.1_006601m PA | AT2G22250 | 0.106390049 | 1.48077009  | Ten DAP | 15 | 13.1.1.2.01 | amino acid metabolism  |
| cassava4.1_007828m PA | AT1G51760 | 0.106848206 | 1.333445294 | Ten DAP | 4  | 17.2.1      | hormone metabolism     |
| cassava4.1_006819m PA | AT4G27070 | 0.107220011 | 1.444489587 | Ten DAP | 9  | 13.1.6.5.05 | amino acid metabolism  |

|                       |           |             |             |         |    |             |                       |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|-----------------------|
| cassava4.1_008272m PA | AT3G61540 | 0.107274725 | 1.429869844 | Ten DAP | 2  | 29.5        | protein               |
| cassava4.1_016592m PA | AT4G10430 | 0.107393213 | 1.786402396 | Ten DAP | 4  | 35.2        | not assigned          |
| cassava4.1_007668m PA | AT4G31340 | 0.107440936 | 1.652766391 | Ten DAP | 4  | 31.1        | cell                  |
| cassava4.1_022815m PA | -         | 0.107459891 | 3.588931611 | Ten DAP | 4  | -           | -                     |
| cassava4.1_032980m PA | AT3G07040 | 0.108332888 | 1.717412787 | Ten DAP | 2  | 20.1        | stress                |
| cassava4.1_001840m PA | AT4G02280 | 0.108474525 | 1.601670806 | Ten DAP | 37 | 2.2.1.05    | major CHO metabolism  |
| cassava4.1_010916m PA | AT5G50960 | 0.109771979 | 1.289702027 | Ten DAP | 2  | 35.1        | not assigned          |
| cassava4.1_008357m PA | AT1G29150 | 0.109788784 | 1.288257706 | Ten DAP | 3  | 29.5.11.20  | protein               |
| cassava4.1_017844m PA | AT1G19910 | 0.110222168 | 1.400984234 | Ten DAP | 2  | 34.1.01     | transport             |
| cassava4.1_034037m PA | AT4G39230 | 0.11036905  | 1.691425069 | Ten DAP | 7  | 16.8.5      | secondary metabolism  |
| cassava4.1_009367m PA | AT4G24220 | 0.110531344 | 1.500818316 | Ten DAP | 12 | 35.2        | not assigned          |
| cassava4.1_012695m PA | AT3G61220 | 0.110671344 | 1.564297867 | Ten DAP | 7  | 26.22       | misc                  |
| cassava4.1_011955m PA | AT1G44835 | 0.111332268 | 1.372309489 | Ten DAP | 2  | 29.1.015    | protein               |
| cassava4.1_010415m PA | AT5G50850 | 0.112275193 | 1.392954128 | Ten DAP | 15 | 8.1.01.01   | TCA / org             |
| cassava4.1_009388m PA | AT3G02540 | 0.112276135 | 2.225844396 | Ten DAP | 5  | 29.5.11.01  | protein               |
| cassava4.1_010800m PA | AT4G29120 | 0.113438562 | 1.093053604 | Ten DAP | 7  | 7.1.03      | OPP                   |
| cassava4.1_000908m PA | AT2G05710 | 0.113489976 | 1.26897535  | Ten DAP | 34 | 8.1.03      | TCA / org             |
| cassava4.1_008657m PA | AT3G47520 | 0.114050678 | 1.285920773 | Ten DAP | 15 | 8.2.09      | TCA / org             |
| cassava4.1_015974m PA | AT5G06720 | 0.114366918 | 1.860333256 | Ten DAP | 3  | 26.12       | misc                  |
| cassava4.1_009445m PA | AT5G22300 | 0.115069058 | 1.278215514 | Ten DAP | 2  | 26.08       | misc                  |
| cassava4.1_015100m PA | AT5G02790 | 0.115136676 | 4.120762135 | Ten DAP | 3  | 35.1        | not assigned          |
| cassava4.1_003086m PA | AT4G39690 | 0.115305126 | 1.112800648 | Ten DAP | 15 | 35.2        | not assigned          |
| cassava4.1_014642m PA | AT1G07890 | 0.115515397 | 2.004814059 | Ten DAP | 6  | 21.2.1      | redox                 |
| cassava4.1_008437m PA | AT5G19990 | 0.116050842 | 1.229496628 | Ten DAP | 22 | 29.5.11.20  | protein               |
| cassava4.1_011300m PA | AT4G08900 | 0.116376462 | 1.674871193 | Ten DAP | 10 | 13.2.2.3    | amino acid metabolism |
| cassava4.1_013984m PA | AT1G64520 | 0.117593767 | 2.131915673 | Ten DAP | 7  | 29.5.11.20  | protein               |
| cassava4.1_005217m PA | AT2G22480 | 0.117662228 | 1.670368678 | Ten DAP | 10 | 4.04        | glycolysis            |
| cassava4.1_006241m PA | AT5G41670 | 0.117793077 | 1.609821025 | Ten DAP | 20 | 7.1.03      | OPP                   |
| cassava4.1_009310m PA | AT2G05990 | 0.117967302 | 1.449345644 | Ten DAP | 15 | 11.1.06     | lipid metabolism      |
| cassava4.1_012793m PA | AT5G19760 | 0.118178435 | 1.395746889 | Ten DAP | 2  | 34.9        | transport             |
| cassava4.1_009796m PA | AT5G03290 | 0.118338472 | 1.328447738 | Ten DAP | 11 | 8.2.04      | TCA / org             |
| cassava4.1_003850m PA | AT2G42520 | 0.118514027 | 1.290346032 | Ten DAP | 9  | 27.1.2      | RNA                   |
| cassava4.1_024362m PA | -         | 0.11957709  | 2.585239527 | Ten DAP | 51 | -           | -                     |
| cassava4.1_012014m PA | AT3G59890 | 0.119599332 | 1.897646739 | Ten DAP | 2  | 13.1.3.5.02 | amino acid metabolism |
| cassava4.1_012581m PA | AT3G03800 | 0.120542801 | 1.446378885 | Ten DAP | 4  | 31.4        | cell                  |
| cassava4.1_015623m PA | AT2G02390 | 0.120568896 | 1.916276017 | Ten DAP | 6  | 26.09       | misc                  |
| cassava4.1_016751m PA | AT5G23750 | 0.12143601  | 1.65994725  | Ten DAP | 5  | 27.3.99     | RNA                   |
| cassava4.1_022523m PA | AT2G46520 | 0.122538113 | 1.338119305 | Ten DAP | 15 | 20.1        | stress                |
| cassava4.1_022523m PA | AT2G46520 | 0.122538113 | 1.338119305 | Ten DAP | 15 | 29.3.1      | protein               |
| cassava4.1_015597m PA | AT1G67360 | 0.122667975 | 1.842562216 | Ten DAP | 3  | 35.1        | not assigned          |
| cassava4.1_011776m PA | AT1G52760 | 0.122703254 | 1.550010546 | Ten DAP | 5  | 11.9.2      | lipid metabolism      |
| cassava4.1_017118m PA | AT4G23690 | 0.123358814 | 1.18419055  | Ten DAP | 2  | 20.1        | stress                |
| cassava4.1_003828m PA | AT3G19420 | 0.124048356 | 1.579814812 | Ten DAP | 3  | 29.4        | protein               |
| cassava4.1_029023m PA | AT1G33590 | 0.124148931 | 1.214383251 | Ten DAP | 5  | 20.1        | stress                |

|                        |           |             |             |         |    |             |                       |
|------------------------|-----------|-------------|-------------|---------|----|-------------|-----------------------|
| cassava4.1_012448m PA  | AT4G39230 | 0.124182302 | 1.291397582 | Ten DAP | 19 | 16.8.5      | secondary metabolism  |
| cassava4.1_014503m PA  | AT3G55440 | 0.124504536 | 1.722312487 | Ten DAP | 4  | 4.08        | glycolysis            |
| cassava4.1_012111m PA  | AT4G02340 | 0.124679634 | 2.293402251 | Ten DAP | 3  | 26.01       | misc                  |
| cassava4.1_016095m PA  | AT3G26420 | 0.124689652 | 1.200801559 | Ten DAP | 3  | 27.4        | RNA                   |
| cassava4.1_031433m PA  | AT4G13010 | 0.124984694 | 1.168757775 | Ten DAP | 24 | 26.07       | misc                  |
| cassava4.1_007005m PA  | AT5G64430 | 0.125313955 | 1.692603144 | Ten DAP | 7  | 35.1        | not assigned          |
| cassava4.1_001858m PA  | AT3G15730 | 0.125808286 | 1.256587923 | Ten DAP | 44 | 11.9.3.01   | lipid metabolism      |
| cassava4.1_016102m PA  | AT1G75270 | 0.125935919 | 1.856076082 | Ten DAP | 6  | 21.2.1      | redox                 |
| cassava4.1_015875m PA  | AT1G20440 | 0.125958623 | 1.892394426 | Ten DAP | 11 | 20.2.99     | stress                |
| cassava4.1_004740m PA  | AT2G23420 | 0.125998287 | 1.587311852 | Ten DAP | 3  | 35.1        | not assigned          |
| cassava4.1_008132m PA  | AT5G07030 | 0.128229688 | 1.415961373 | Ten DAP | 4  | 27.3.67     | RNA                   |
| cassava4.1_014065m PA  | AT3G02520 | 0.128409936 | 1.164910071 | Ten DAP | 6  | 30.7        | signalling            |
| cassava4.1_010344m PA  | AT4G37990 | 0.12924818  | 1.315275224 | Ten DAP | 11 | 16.2.1.010  | secondary metabolism  |
| cassava4.1_011215m PA  | AT4G38460 | 0.129432822 | 1.366033569 | Ten DAP | 7  | 16.1.1.010  | secondary metabolism  |
| cassava4.1_012687m PA  | AT2G42130 | 0.129456199 | 2.113531173 | Ten DAP | 4  | 35.2        | not assigned          |
| cassava4.1_022023m PA  | AT5G63220 | 0.129538139 | 1.278214383 | Ten DAP | 4  | 35.2        | not assigned          |
| cassava4.1_015573m PA  | AT3G01850 | 0.129652966 | 2.324416414 | Ten DAP | 2  | 7.2.03      | OPP                   |
| cassava4.1_007736m PA  | AT1G44820 | 0.129727155 | 1.292639102 | Ten DAP | 4  | 26.08       | misc                  |
| gi 169794081 ref YP_OC | -         | 0.130938682 | 1.49258646  | Ten DAP | 25 | -           | -                     |
| cassava4.1_018015m PA  | AT1G24020 | 0.131542999 | 2.79451859  | Ten DAP | 2  | 20.2.99     | stress                |
| cassava4.1_014787m PA  | AT2G01250 | 0.132060716 | 1.715439587 | Ten DAP | 3  | 29.2.2      | protein               |
| cassava4.1_003997m PA  | AT5G13110 | 0.13211543  | 1.270766833 | Ten DAP | 2  | 7.1.01      | OPP                   |
| cassava4.1_015119m PA  | AT3G12390 | 0.132246489 | 1.37088616  | Ten DAP | 7  | 29.2.4      | protein               |
| cassava4.1_005750m PA  | AT3G24200 | 0.132306972 | 1.481000472 | Ten DAP | 3  | 26.07       | misc                  |
| cassava4.1_034435m PA  | AT2G15490 | 0.133359806 | 1.329493123 | Ten DAP | 2  | 26.02       | misc                  |
| cassava4.1_009013m PA  | AT1G62640 | 0.13473601  | 1.260540024 | Ten DAP | 2  | 11.1.03     | lipid metabolism      |
| cassava4.1_013772m PA  | AT5G06060 | 0.134868707 | 1.779255439 | Ten DAP | 2  | 26.08       | misc                  |
| cassava4.1_013119m PA  | AT5G45390 | 0.134940556 | 1.948054619 | Ten DAP | 5  | 29.5.05     | protein               |
| cassava4.1_009799m PA  | AT4G16260 | 0.135189841 | 1.359937941 | Ten DAP | 3  | 26.04       | misc                  |
| cassava4.1_009901m PA  | AT5G08170 | 0.135221193 | 1.266541616 | Ten DAP | 2  | 22.1.04     | polyamine metabolism  |
| cassava4.1_009735m PA  | AT3G52180 | 0.135771612 | 2.142486305 | Ten DAP | 7  | 35.1        | not assigned          |
| cassava4.1_010221m PA  | AT3G02540 | 0.136497744 | 1.177026973 | Ten DAP | 7  | 29.5.11.01  | protein               |
| cassava4.1_008948m PA  | AT5G57850 | 0.136673603 | 1.475229168 | Ten DAP | 11 | 26.26.1     | misc                  |
| cassava4.1_013237m PA  | AT1G59960 | 0.136691543 | 1.742945345 | Ten DAP | 2  | 16.8.2      | secondary metabolism  |
| cassava4.1_009456m PA  | AT3G20330 | 0.136779974 | 1.152139989 | Ten DAP | 7  | 23.1.1.02   | nucleotide metabolism |
| cassava4.1_014303m PA  | AT2G45300 | 0.137496082 | 1.306516982 | Ten DAP | 3  | 13.1.6.1.06 | amino acid metabolism |
| cassava4.1_002579m PA  | AT3G01680 | 0.137631582 | 1.112520095 | Ten DAP | 20 | 35.2        | not assigned          |
| cassava4.1_010989m PA  | AT3G56460 | 0.138451284 | 2.70541493  | Ten DAP | 2  | 26.01       | misc                  |
| cassava4.1_008997m PA  | AT3G17880 | 0.138970581 | 1.577286504 | Ten DAP | 13 | 21.01       | redox                 |
| cassava4.1_009187m PA  | AT3G09350 | 0.139100531 | 1.782427134 | Ten DAP | 6  | 35.1.3      | not assigned          |
| cassava4.1_012919m PA  | AT1G18640 | 0.13955574  | 1.775868678 | Ten DAP | 3  | 13.1.5.1.03 | amino acid metabolism |
| cassava4.1_006694m PA  | AT3G09940 | 0.139889848 | 1.379383744 | Ten DAP | 9  | 21.2.1      | redox                 |
| cassava4.1_000751m PA  | AT3G45850 | 0.140830767 | 2.894303682 | Ten DAP | 2  | 28.1        | DNA                   |
| cassava4.1_017458m PA  | AT3G23790 | 0.140921749 | 1.768589971 | Ten DAP | 3  | 11.1.08     | lipid metabolism      |

|                       |           |             |             |         |    |             |                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|----------------------------------|
| cassava4.1_006628m PA | AT5G58090 | 0.14094893  | 1.476696688 | Ten DAP | 7  | 35.1        | not assigned                     |
| cassava4.1_006439m PA | AT4G36195 | 0.141408492 | 1.216205254 | Ten DAP | 7  | 29.5.05     | protein                          |
| cassava4.1_004822m PA | AT5G16150 | 0.143239995 | 1.647159078 | Ten DAP | 2  | 2.2.2.06    | major CHO metabolism             |
| cassava4.1_004822m PA | AT5G16150 | 0.143239995 | 1.647159078 | Ten DAP | 2  | 34.2        | transporter                      |
| cassava4.1_006843m PA | AT3G54470 | 0.143534558 | 1.279785675 | Ten DAP | 15 | 23.1.1.05   | nucleotide metabolism            |
| cassava4.1_010959m PA | AT5G08300 | 0.143699243 | 1.388010412 | Ten DAP | 14 | 8.1.06      | TCA / org                        |
| cassava4.1_011972m PA | AT1G24360 | 0.143736145 | 1.886584677 | Ten DAP | 4  | 11.1.04     | lipid metabolism                 |
| cassava4.1_002380m PA | AT1G03090 | 0.143806965 | 1.677205102 | Ten DAP | 4  | 13.2.4.4    | amino acid metabolism            |
| cassava4.1_016660m PA | AT3G06390 | 0.145027917 | 1.920382692 | Ten DAP | 2  | 35.1        | not assigned                     |
| cassava4.1_005282m PA | AT1G09830 | 0.14532559  | 1.206211827 | Ten DAP | 4  | 23.1.2.02   | nucleotide metabolism            |
| cassava4.1_015338m PA | AT2G18110 | 0.145538293 | 1.215746511 | Ten DAP | 4  | 29.2.4      | protein                          |
| cassava4.1_011761m PA | AT1G09130 | 0.14559431  | 2.45156516  | Ten DAP | 3  | 29.5.05     | protein                          |
| cassava4.1_002470m PA | AT5G19620 | 0.1456893   | 1.34840287  | Ten DAP | 8  | 35.1        | not assigned                     |
| cassava4.1_004309m PA | AT3G15410 | 0.145803989 | 1.408860436 | Ten DAP | 5  | 35.1        | not assigned                     |
| cassava4.1_005155m PA | AT2G14170 | 0.146659443 | 1.344217415 | Ten DAP | 11 | 13.2.4.3    | amino acid metabolism            |
| cassava4.1_002466m PA | AT3G46970 | 0.14731939  | 1.304477076 | Ten DAP | 17 | 2.2.2.02    | major CHO metabolism             |
| cassava4.1_013399m PA | AT4G28400 | 0.149369472 | 1.500677506 | Ten DAP | 9  | 29.4        | protein                          |
| cassava4.1_015380m PA | AT4G11150 | 0.149638012 | 1.876903114 | Ten DAP | 12 | 34.1.01     | transport                        |
| cassava4.1_010597m PA | AT5G37600 | 0.150226394 | 1.385437199 | Ten DAP | 5  | 12.2.02     | N-metabolism                     |
| cassava4.1_016943m PA | AT1G10200 | 0.150818868 | 1.553702378 | Ten DAP | 2  | 27.3.67     | RNA                              |
| cassava4.1_033064m PA | AT1G65590 | 0.151549948 | 1.203318337 | Ten DAP | 2  | 35.1        | not assigned                     |
| cassava4.1_011783m PA | AT2G42910 | 0.151866716 | 1.45798603  | Ten DAP | 3  | 13.1.7.011  | amino acid metabolism            |
| cassava4.1_011783m PA | AT2G42910 | 0.151866716 | 1.45798603  | Ten DAP | 3  | 23.1.03     | nucleotide metabolism            |
| cassava4.1_014284m PA | AT4G13720 | 0.152079354 | 1.157927141 | Ten DAP | 7  | 35.1        | not assigned                     |
| cassava4.1_018158m PA | AT5G12020 | 0.152181229 | 2.297352002 | Ten DAP | 7  | 20.2.1      | stress                           |
| cassava4.1_011142m PA | AT5G06720 | 0.152240907 | 1.740625242 | Ten DAP | 5  | 26.12       | misc                             |
| cassava4.1_006877m PA | AT5G04990 | 0.153157786 | 1.389127443 | Ten DAP | 6  | 35.1        | not assigned                     |
| cassava4.1_014432m PA | AT3G55440 | 0.153717454 | 1.550469005 | Ten DAP | 9  | 4.08        | glycolysis                       |
| cassava4.1_005825m PA | AT3G58750 | 0.153986539 | 1.726053013 | Ten DAP | 6  | 6.01        | gluconeogenese/ glyoxylate cycle |
| cassava4.1_012430m PA | AT3G58840 | 0.155223324 | 1.256174144 | Ten DAP | 4  | 35.2        | not assigned                     |
| cassava4.1_001724m PA | AT3G06810 | 0.155239958 | 1.3728602   | Ten DAP | 15 | 11.9.4.02   | lipid metabolism                 |
| cassava4.1_021137m PA | AT2G31810 | 0.156128679 | 1.297916733 | Ten DAP | 10 | 13.1.4.1    | amino acid metabolism            |
| cassava4.1_027575m PA | AT3G10690 | 0.156154049 | 1.164292797 | Ten DAP | 5  | 28.1        | DNA                              |
| cassava4.1_008220m PA | AT1G16300 | 0.156368217 | 1.245150077 | Ten DAP | 13 | 4.09        | glycolysis                       |
| cassava4.1_013756m PA | AT4G02610 | 0.156698671 | 2.904417771 | Ten DAP | 3  | 13.1.6.5.05 | amino acid metabolism            |
| cassava4.1_010553m PA | AT3G08640 | 0.156840179 | 1.24827519  | Ten DAP | 7  | 35.1        | not assigned                     |
| cassava4.1_014093m PA | AT2G45060 | 0.157485941 | 1.463446796 | Ten DAP | 4  | 35.2        | not assigned                     |
| cassava4.1_010870m PA | AT5G10010 | 0.157631391 | 1.606725317 | Ten DAP | 4  | 35.2        | not assigned                     |
| cassava4.1_019672m PA | AT5G47550 | 0.157982784 | 1.581793652 | Ten DAP | 3  | 20.1        | stress                           |
| cassava4.1_019672m PA | AT5G47550 | 0.157982784 | 1.581793652 | Ten DAP | 3  | 29.5.03     | protein                          |
| cassava4.1_000097m PA | AT3G22790 | 0.158757509 | 1.17223175  | Ten DAP | 2  | 29.4        | protein                          |
| cassava4.1_016475m PA | AT1G14900 | 0.158828042 | 1.596609554 | Ten DAP | 3  | 28.1        | DNA                              |
| cassava4.1_002614m PA | AT3G29320 | 0.159453092 | 1.403512667 | Ten DAP | 16 | 2.2.2.02    | major CHO metabolism             |
| cassava4.1_009927m PA | AT4G11260 | 0.159669678 | 1.199940349 | Ten DAP | 6  | 29.4        | protein                          |

|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_013908m PA | AT3G17440 | 0.160116025 | 1.434000825 | Ten DAP | 4  | 31.4        | cell                                             |
| cassava4.1_012558m PA | AT3G54110 | 0.160695162 | 2.245330522 | Ten DAP | 3  | 9.8         | mitochondrial electron transport / ATP synthesis |
| cassava4.1_009781m PA | AT5G50550 | 0.160815515 | 1.397641197 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_000255m PA | AT1G15520 | 0.161043991 | 1.356373812 | Ten DAP | 23 | 34.16       | transport                                        |
| cassava4.1_006624m PA | AT1G72160 | 0.161692272 | 1.327880464 | Ten DAP | 22 | 28.99       | DNA                                              |
| cassava4.1_006624m PA | AT1G72160 | 0.161692272 | 1.327880464 | Ten DAP | 22 | 34.99       | transport                                        |
| cassava4.1_012070m PA | AT4G00560 | 0.162198166 | 1.602976423 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_012416m PA | AT1G76150 | 0.162648597 | 1.4718774   | Ten DAP | 2  | 11.8        | lipid metabolism                                 |
| cassava4.1_005004m PA | AT3G26310 | 0.163684417 | 1.365154447 | Ten DAP | 2  | 26.10       | misc                                             |
| cassava4.1_010419m PA | AT1G67280 | 0.164035207 | 1.222203298 | Ten DAP | 13 | 13.2.3.2    | amino acid metabolism                            |
| cassava4.1_010419m PA | AT1G67280 | 0.164035207 | 1.222203298 | Ten DAP | 13 | 24.02       | Biodegradation of Xenobiotics                    |
| cassava4.1_013151m PA | AT3G56190 | 0.164532623 | 1.220861496 | Ten DAP | 6  | 35.1        | not assigned                                     |
| cassava4.1_006991m PA | AT5G46290 | 0.164659675 | 1.508417849 | Ten DAP | 11 | 11.1.03     | lipid metabolism                                 |
| cassava4.1_027961m PA | AT3G04790 | 0.165238087 | 1.378612947 | Ten DAP | 4  | 1.3.010     | PS                                               |
| cassava4.1_027961m PA | AT3G04790 | 0.165238087 | 1.378612947 | Ten DAP | 4  | 7.2.04      | OPP                                              |
| cassava4.1_001014m PA | AT1G09010 | 0.165647616 | 1.613332796 | Ten DAP | 3  | 10.6.2      | cell wall                                        |
| cassava4.1_001014m PA | AT1G09010 | 0.165647616 | 1.613332796 | Ten DAP | 3  | 26.03       | misc                                             |
| cassava4.1_008399m PA | AT5G67290 | 0.165862705 | 1.287798858 | Ten DAP | 2  | 26.07       | misc                                             |
| cassava4.1_007302m PA | AT4G34480 | 0.166136738 | 1.81995484  | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_009729m PA | AT5G43940 | 0.16630625  | 1.314192012 | Ten DAP | 11 | 25.11       | C1-metabolism                                    |
| cassava4.1_009729m PA | AT5G43940 | 0.16630625  | 1.314192012 | Ten DAP | 11 | 26.11       | misc                                             |
| cassava4.1_022796m PA | AT1G12230 | 0.16667314  | 1.474429065 | Ten DAP | 13 | 7.2.02      | OPP                                              |
| cassava4.1_004441m PA | AT4G12400 | 0.166744092 | 1.393226809 | Ten DAP | 37 | 20          | stress                                           |
| cassava4.1_006278m PA | AT1G79440 | 0.167929112 | 1.552981411 | Ten DAP | 10 | 8.2.99      | TCA / org                                        |
| cassava4.1_006278m PA | AT1G79440 | 0.167929112 | 1.552981411 | Ten DAP | 10 | 13.1.1.1.03 | amino acid metabolism                            |
| cassava4.1_013704m PA | AT4G03140 | 0.169735767 | 2.484905239 | Ten DAP | 6  | 26.22       | misc                                             |
| cassava4.1_006438m PA | AT3G57610 | 0.16992243  | 1.480939935 | Ten DAP | 9  | 23.1.2.20   | nucleotide metabolism                            |
| cassava4.1_015011m PA | AT2G21870 | 0.170082557 | 1.961272465 | Ten DAP | 22 | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_007900m PA | AT1G09750 | 0.170206359 | 1.109267201 | Ten DAP | 7  | 27.3.99     | RNA                                              |
| cassava4.1_019697m PA | AT3G01390 | 0.17071522  | 1.646760569 | Ten DAP | 3  | 34.1        | transport                                        |
| cassava4.1_015738m PA | AT5G55190 | 0.171999406 | 1.562508637 | Ten DAP | 4  | 30.5        | signalling                                       |
| cassava4.1_005882m PA | AT1G54100 | 0.172134009 | 1.26532535  | Ten DAP | 11 | 5.10        | fermentation                                     |
| cassava4.1_006078m PA | AT3G60130 | 0.172325711 | 5.424339969 | Ten DAP | 3  | 26.03       | misc                                             |
| cassava4.1_027816m PA | AT2G34680 | 0.173277258 | 1.828798307 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_008386m PA | AT5G55530 | 0.173686893 | 1.431849839 | Ten DAP | 3  | 20.2.2      | stress                                           |
| cassava4.1_008738m PA | AT3G19450 | 0.174318087 | 1.163159793 | Ten DAP | 9  | 16.2.1.010  | secondary metabolism                             |
| cassava4.1_014677m PA | AT5G66140 | 0.174435359 | 1.579507455 | Ten DAP | 8  | 29.5.11.20  | protein                                          |
| cassava4.1_006730m PA | AT3G48730 | 0.174894594 | 1.198048467 | Ten DAP | 4  | 19.03       | tetrapyrrole synthesis                           |
| cassava4.1_034034m PA | AT5G55860 | 0.175455423 | 1.362649273 | Ten DAP | 9  | 35.2        | not assigned                                     |
| cassava4.1_011852m PA | AT5G20080 | 0.175507767 | 1.827815582 | Ten DAP | 11 | 21.99       | redox                                            |
| cassava4.1_007957m PA | AT1G77120 | 0.176082112 | 2.022238374 | Ten DAP | 4  | 5.03        | fermentation                                     |
| cassava4.1_014837m PA | AT2G01250 | 0.176672888 | 1.633752667 | Ten DAP | 13 | 29.2.2      | protein                                          |
| cassava4.1_008063m PA | AT5G48930 | 0.176749688 | 1.841378332 | Ten DAP | 9  | 16.2.1.04   | secondary metabolism                             |
| cassava4.1_011810m PA | AT4G21580 | 0.177270989 | 1.447487266 | Ten DAP | 12 | 26.01       | misc                                             |

|                       |           |             |             |         |    |              |                        |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|------------------------|
| cassava4.1_002801m PA | AT1G61010 | 0.177421905 | 1.127597816 | Ten DAP | 3  | 27.1         | RNA                    |
| cassava4.1_029718m PA | AT4G23850 | 0.179128844 | 1.333396037 | Ten DAP | 11 | 11.1.09      | lipid metabolism       |
| cassava4.1_004490m PA | AT1G33320 | 0.179719114 | 1.819017997 | Ten DAP | 3  | 13.1.3.4.01  | amino acid metabolism  |
| cassava4.1_014627m PA | AT3G22110 | 0.180551261 | 1.867885562 | Ten DAP | 7  | 29.5.11.20   | protein                |
| cassava4.1_002044m PA | AT5G65620 | 0.180626404 | 1.167258642 | Ten DAP | 28 | 29.5         | protein                |
| cassava4.1_007037m PA | AT5G67630 | 0.181135868 | 1.128821798 | Ten DAP | 15 | 28.1         | DNA                    |
| cassava4.1_010948m PA | AT3G46440 | 0.181535215 | 1.559198475 | Ten DAP | 5  | 10.1.05      | cell wall              |
| cassava4.1_005009m PA | AT5G17530 | 0.181725429 | 1.372335882 | Ten DAP | 13 | 4.02         | glycolysis             |
| cassava4.1_029521m PA | AT5G56000 | 0.182113053 | 1.191222766 | Ten DAP | 10 | 20.2.1       | stress                 |
| cassava4.1_001802m PA | AT5G17920 | 0.182927258 | 1.083683862 | Ten DAP | 39 | 13.1.3.4     | amino acid metabolism  |
| cassava4.1_009000m PA | AT1G03475 | 0.183185178 | 1.339301334 | Ten DAP | 6  | 19.08        | tetrapyrrole synthesis |
| cassava4.1_029062m PA | AT1G80360 | 0.183471573 | 1.262502537 | Ten DAP | 4  | 13.1.6.2     | amino acid metabolism  |
| cassava4.1_001084m PA | AT5G62670 | 0.183499081 | 1.315417428 | Ten DAP | 6  | 34.1         | transport              |
| cassava4.1_012284m PA | AT5G23540 | 0.183766266 | 1.243936082 | Ten DAP | 10 | 29.5.11.20   | protein                |
| cassava4.1_015183m PA | AT1G79210 | 0.184321418 | 1.461992832 | Ten DAP | 2  | 29.5.11.20   | protein                |
| cassava4.1_008376m PA | AT1G08450 | 0.185227248 | 1.748001198 | Ten DAP | 6  | 30.3         | signalling             |
| cassava4.1_009477m PA | AT1G68010 | 0.18533929  | 1.730487959 | Ten DAP | 2  | 1.2.06       | PS                     |
| cassava4.1_009477m PA | AT1G68010 | 0.18533929  | 1.730487959 | Ten DAP | 2  | 13.2.5.1     | amino acid metabolism  |
| cassava4.1_007405m PA | AT5G14590 | 0.185710917 | 1.376514432 | Ten DAP | 5  | 8.1.04       | TCA / org              |
| cassava4.1_002712m PA | AT5G63190 | 0.186660482 | 1.406280973 | Ten DAP | 20 | 28.99        | DNA                    |
| cassava4.1_008337m PA | AT1G57720 | 0.186992953 | 1.205671768 | Ten DAP | 27 | 29.2.4       | protein                |
| cassava4.1_008143m PA | AT5G11170 | 0.187767386 | 1.274099146 | Ten DAP | 16 | 28.1         | DNA                    |
| cassava4.1_009887m PA | AT1G08490 | 0.187971264 | 1.597828424 | Ten DAP | 5  | 30.1.01      | signalling             |
| cassava4.1_014637m PA | AT5G14240 | 0.188590911 | 1.423461116 | Ten DAP | 2  | 35.2         | not assigned           |
| cassava4.1_007702m PA | AT1G48620 | 0.189116256 | 1.22919659  | Ten DAP | 5  | 28.1.3       | DNA                    |
| cassava4.1_012745m PA | AT5G09650 | 0.189129233 | 1.223212637 | Ten DAP | 10 | 23.4.99      | nucleotide metabolism  |
| cassava4.1_008036m PA | AT1G69740 | 0.189966942 | 1.392324151 | Ten DAP | 12 | 19.04        | tetrapyrrole synthesis |
| cassava4.1_034056m PA | AT1G47480 | 0.190316801 | 1.175662707 | Ten DAP | 6  | 35.1         | not assigned           |
| cassava4.1_010424m PA | AT1G01910 | 0.190338386 | 1.441716109 | Ten DAP | 3  | 34.18.01     | transport              |
| cassava4.1_003897m PA | AT1G06820 | 0.190499398 | 1.177493373 | Ten DAP | 8  | 16.1.4       | secondary metabolism   |
| cassava4.1_008567m PA | AT3G54250 | 0.191774513 | 1.279815931 | Ten DAP | 3  | 16.1.2.06    | secondary metabolism   |
| cassava4.1_006384m PA | AT1G09020 | 0.193170958 | 1.66202018  | Ten DAP | 2  | 29.4         | protein                |
| cassava4.1_014967m PA | AT3G10220 | 0.193483419 | 1.173217952 | Ten DAP | 4  | 31.1         | cell                   |
| cassava4.1_031695m PA | AT2G28470 | 0.194104834 | 1.404811068 | Ten DAP | 9  | 10.6.2       | cell wall              |
| cassava4.1_031695m PA | AT2G28470 | 0.194104834 | 1.404811068 | Ten DAP | 9  | 26.03        | misc                   |
| cassava4.1_013202m PA | AT2G37170 | 0.194784697 | 1.259616676 | Ten DAP | 5  | 34.19.1      | transport              |
| cassava4.1_009020m PA | AT1G79550 | 0.195049664 | 1.619909834 | Ten DAP | 23 | 4.010        | glycolysis             |
| cassava4.1_020020m PA | AT2G04400 | 0.195521885 | 1.199452984 | Ten DAP | 3  | 13.1.6.5.04  | amino acid metabolism  |
| cassava4.1_009268m PA | AT5G54800 | 0.196091071 | 1.598831466 | Ten DAP | 4  | 2.1.2.05     | major CHO metabolism   |
| cassava4.1_009268m PA | AT5G54800 | 0.196091071 | 1.598831466 | Ten DAP | 4  | 34.8         | transport              |
| cassava4.1_011635m PA | AT2G38550 | 0.196161485 | 1.566361646 | Ten DAP | 5  | 35.2         | not assigned           |
| cassava4.1_016386m PA | AT5G10860 | 0.19621274  | 1.877067847 | Ten DAP | 6  | 35.1         | not assigned           |
| cassava4.1_005207m PA | AT5G10920 | 0.196440908 | 1.281885226 | Ten DAP | 4  | 13.1.2.3.023 | amino acid metabolism  |
| cassava4.1_013362m PA | AT5G40650 | 0.196617706 | 2.337190674 | Ten DAP | 4  | 8.1.07       | TCA / org              |

|                       |           |             |             |         |    |              |                       |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|-----------------------|
| cassava4.1_005766m PA | AT2G44450 | 0.197073759 | 1.430884362 | Ten DAP | 7  | 26.03        | misc                  |
| cassava4.1_021900m PA | AT5G06860 | 0.197094283 | 1.420545274 | Ten DAP | 3  | 10.6.3       | cell wall             |
| cassava4.1_011347m PA | AT3G04120 | 0.197749641 | 1.219588669 | Ten DAP | 35 | 4.09         | glycolysis            |
| cassava4.1_007552m PA | AT4G26000 | 0.197839353 | 1.310694227 | Ten DAP | 4  | 27.3.99      | RNA                   |
| cassava4.1_012465m PA | AT3G61220 | 0.197943978 | 2.571041827 | Ten DAP | 2  | 26.22        | misc                  |
| cassava4.1_016056m PA | AT2G18230 | 0.197959383 | 1.414196793 | Ten DAP | 4  | 23.4.99      | nucleotide metabolism |
| cassava4.1_011176m PA | AT3G04120 | 0.198445418 | 1.364651348 | Ten DAP | 14 | 4.09         | glycolysis            |
| cassava4.1_008978m PA | AT5G64380 | 0.198494275 | 1.397492877 | Ten DAP | 2  | 1.3.07       | PS                    |
| cassava4.1_008674m PA | AT5G15610 | 0.201459286 | 1.633795735 | Ten DAP | 3  | 29.5.11.20   | protein               |
| cassava4.1_005098m PA | AT5G19320 | 0.201910767 | 1.182109785 | Ten DAP | 9  | 30.5         | signalling            |
| cassava4.1_007022m PA | AT2G33150 | 0.202881357 | 1.339763806 | Ten DAP | 4  | 11.9.4.05    | lipid metabolism      |
| cassava4.1_007022m PA | AT2G33150 | 0.202881357 | 1.339763806 | Ten DAP | 4  | 13.2.4.1     | amino acid metabolism |
| cassava4.1_004139m PA | AT5G51480 | 0.203010811 | 1.249173685 | Ten DAP | 10 | 26.07        | misc                  |
| cassava4.1_009956m PA | AT4G17520 | 0.203234177 | 1.35060261  | Ten DAP | 15 | 27.4         | RNA                   |
| cassava4.1_010369m PA | AT2G39770 | 0.203337967 | 1.399898286 | Ten DAP | 4  | 10.1.1.01    | cell wall             |
| cassava4.1_006506m PA | AT3G61140 | 0.203646963 | 1.388688858 | Ten DAP | 5  | 30.11.1      | signalling            |
| cassava4.1_015424m PA | AT5G41210 | 0.203899002 | 1.575802874 | Ten DAP | 5  | 26.09        | misc                  |
| cassava4.1_008319m PA | AT3G27740 | 0.204066305 | 1.373745314 | Ten DAP | 5  | 13.1.2.3.011 | amino acid metabolism |
| cassava4.1_008319m PA | AT3G27740 | 0.204066305 | 1.373745314 | Ten DAP | 5  | 23.1.1.01    | nucleotide metabolism |
| cassava4.1_003505m PA | AT1G49670 | 0.204499677 | 1.534919466 | Ten DAP | 11 | 26.07        | misc                  |
| cassava4.1_007624m PA | AT1G52380 | 0.204523388 | 1.213707106 | Ten DAP | 3  | 30.5         | signalling            |
| cassava4.1_013356m PA | AT5G04170 | 0.204726227 | 1.596097212 | Ten DAP | 3  | 30.3         | signalling            |
| cassava4.1_007681m PA | AT1G09850 | 0.204852553 | 1.281273884 | Ten DAP | 3  | 29.5.03      | protein               |
| cassava4.1_009001m PA | AT5G62390 | 0.205642308 | 1.770945057 | Ten DAP | 5  | 30.3         | signalling            |
| cassava4.1_002427m PA | AT1G52320 | 0.205689252 | 9.558877778 | Ten DAP | 2  | 35.2         | not assigned          |
| cassava4.1_005293m PA | AT4G32520 | 0.20661457  | 1.308309878 | Ten DAP | 16 | 1.2.05       | PS                    |
| cassava4.1_005293m PA | AT4G32520 | 0.20661457  | 1.308309878 | Ten DAP | 16 | 13.1.5.2.01  | amino acid metabolism |
| cassava4.1_005293m PA | AT4G32520 | 0.20661457  | 1.308309878 | Ten DAP | 16 | 25.01        | C1-metabolism         |
| cassava4.1_012016m PA | AT2G21170 | 0.206730912 | 1.375325681 | Ten DAP | 5  | 1.3.05       | PS                    |
| cassava4.1_010194m PA | AT4G17520 | 0.207083219 | 1.492993885 | Ten DAP | 3  | 27.4         | RNA                   |
| cassava4.1_011091m PA | AT3G26340 | 0.207405692 | 1.277059355 | Ten DAP | 11 | 29.5.11.20   | protein               |
| cassava4.1_006445m PA | AT1G20200 | 0.20816789  | 1.144333442 | Ten DAP | 4  | 29.5.11.20   | protein               |
| cassava4.1_007750m PA | AT5G09900 | 0.209207856 | 1.375988838 | Ten DAP | 10 | 29.5.11.20   | protein               |
| cassava4.1_007394m PA | AT4G18950 | 0.209314163 | 1.775092794 | Ten DAP | 5  | 29.4         | protein               |
| cassava4.1_007394m PA | AT4G18950 | 0.209314163 | 1.775092794 | Ten DAP | 5  | 31.1         | cell                  |
| cassava4.1_027307m PA | AT1G80460 | 0.210752582 | 1.209969748 | Ten DAP | 3  | 11.5.01      | lipid metabolism      |
| cassava4.1_002829m PA | AT1G29880 | 0.211694385 | 1.173245649 | Ten DAP | 24 | 29.1.014     | protein               |
| cassava4.1_002307m PA | AT2G45290 | 0.211754741 | 1.343756161 | Ten DAP | 36 | 1.3.08       | PS                    |
| cassava4.1_002307m PA | AT2G45290 | 0.211754741 | 1.343756161 | Ten DAP | 36 | 7.2.01       | OPP                   |
| cassava4.1_009613m PA | AT5G65760 | 0.211765259 | 1.394151175 | Ten DAP | 2  | 29.5         | protein               |
| cassava4.1_002124m PA | AT1G15690 | 0.212300603 | 1.243447943 | Ten DAP | 14 | 34.30        | transport             |
| cassava4.1_012289m PA | AT3G14750 | 0.212515649 | 1.29305773  | Ten DAP | 2  | 35.1         | not assigned          |
| cassava4.1_006975m PA | AT1G80600 | 0.213609758 | 1.743242821 | Ten DAP | 2  | 13.1.2.3.04  | amino acid metabolism |
| cassava4.1_002761m PA | AT5G27600 | 0.213671198 | 1.581802835 | Ten DAP | 4  | 11.1.08      | lipid metabolism      |



|                       |           |             |             |         |    |                |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|----------------|--------------------------------------------------|
| cassava4.1_006909m PA | AT4G26910 | 0.214072247 | 1.510417328 | Ten DAP | 15 | 35.1           | not assigned                                     |
| cassava4.1_020495m PA | AT2G13560 | 0.214297831 | 1.460325103 | Ten DAP | 2  | 8.2.10         | TCA / org                                        |
| cassava4.1_009293m PA | AT1G47710 | 0.214914351 | 1.244709985 | Ten DAP | 14 | 20.1           | stress                                           |
| cassava4.1_009293m PA | AT1G47710 | 0.214914351 | 1.244709985 | Ten DAP | 14 | 29.5.05        | protein                                          |
| cassava4.1_009185m PA | AT1G65930 | 0.215458892 | 1.358067264 | Ten DAP | 8  | 8.1.04         | TCA / org                                        |
| cassava4.1_003704m PA | AT5G64570 | 0.215842054 | 1.324212017 | Ten DAP | 9  | 35.1           | not assigned                                     |
| cassava4.1_004043m PA | AT2G39940 | 0.216143781 | 1.366521723 | Ten DAP | 7  | 29.5.11.4.3.02 | protein                                          |
| cassava4.1_014131m PA | AT5G58420 | 0.2171081   | 1.648918987 | Ten DAP | 2  | 29.2.2         | protein                                          |
| cassava4.1_012067m PA | AT1G19140 | 0.217322652 | 1.090820027 | Ten DAP | 4  | 35.2           | not assigned                                     |
| cassava4.1_014799m PA | AT2G05840 | 0.217682487 | 1.593129998 | Ten DAP | 12 | 29.5.11.20     | protein                                          |
| cassava4.1_009845m PA | AT1G79230 | 0.217993551 | 1.583438478 | Ten DAP | 5  | 13.2.5.3       | amino acid metabolism                            |
| cassava4.1_006906m PA | AT1G34430 | 0.218824284 | 1.430551967 | Ten DAP | 12 | 8.1.01.02      | TCA / org                                        |
| cassava4.1_026109m PA | AT4G19880 | 0.220125146 | 1.395969431 | Ten DAP | 6  | 26.09          | misc                                             |
| cassava4.1_009294m PA | AT3G02720 | 0.220753064 | 1.936735153 | Ten DAP | 5  | 35.1           | not assigned                                     |
| cassava4.1_006476m PA | AT5G08530 | 0.221707488 | 1.10958104  | Ten DAP | 14 | 9.1.2          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_017342m PA | AT2G21620 | 0.222162403 | 1.596158839 | Ten DAP | 5  | 20.2.99        | stress                                           |
| cassava4.1_010509m PA | AT2G36460 | 0.222164189 | 1.152940825 | Ten DAP | 33 | 4.07           | glycolysis                                       |
| cassava4.1_007011m PA | AT5G43060 | 0.222566275 | 1.154674258 | Ten DAP | 4  | 29.5.03        | protein                                          |
| cassava4.1_011513m PA | AT4G24340 | 0.222793534 | 1.874873862 | Ten DAP | 2  | 35.1           | not assigned                                     |
| cassava4.1_012257m PA | AT2G42500 | 0.222937315 | 1.633778016 | Ten DAP | 3  | 29.4           | protein                                          |
| cassava4.1_016417m PA | AT3G22630 | 0.223888583 | 1.392010564 | Ten DAP | 2  | 29.5.11.20     | protein                                          |
| cassava4.1_033369m PA | AT2G22590 | 0.223901915 | 1.672644369 | Ten DAP | 2  | 16.8.1         | secondary metabolism                             |
| cassava4.1_010547m PA | AT3G19320 | 0.223926953 | 1.318353165 | Ten DAP | 5  | 35.1           | not assigned                                     |
| cassava4.1_010165m PA | AT4G17520 | 0.224224593 | 1.136952973 | Ten DAP | 4  | 27.4           | RNA                                              |
| cassava4.1_010037m PA | AT2G41810 | 0.224367785 | 1.666953139 | Ten DAP | 3  | 35.2           | not assigned                                     |
| cassava4.1_015618m PA | AT5G12110 | 0.224594498 | 1.310168788 | Ten DAP | 10 | 29.2.4         | protein                                          |
| cassava4.1_029886m PA | AT5G42650 | 0.226513182 | 1.267680859 | Ten DAP | 12 | 17.7.1.03      | hormone metabolism                               |
| cassava4.1_012107m PA | AT5G57330 | 0.229434561 | 1.132547405 | Ten DAP | 4  | 3.5            | minor CHO metabolism                             |
| cassava4.1_012458m PA | AT1G02560 | 0.229472115 | 2.204088314 | Ten DAP | 2  | 29.5.05        | protein                                          |
| cassava4.1_007573m PA | AT5G10770 | 0.229869941 | 1.376230551 | Ten DAP | 6  | 27.3.99        | RNA                                              |
| cassava4.1_003640m PA | AT4G32250 | 0.230584339 | 1.677092483 | Ten DAP | 3  | 29.4           | protein                                          |
| cassava4.1_005392m PA | AT3G13860 | 0.230637077 | 1.300432067 | Ten DAP | 13 | 29.6           | protein                                          |
| cassava4.1_008936m PA | AT5G02500 | 0.23071684  | 1.178106471 | Ten DAP | 2  | 29.6           | protein                                          |
| cassava4.1_011191m PA | AT3G02360 | 0.231086905 | 1.194851295 | Ten DAP | 11 | 7.1.03         | OPP                                              |
| cassava4.1_013160m PA | AT4G16060 | 0.232165143 | 1.305060748 | Ten DAP | 2  | 35.2           | not assigned                                     |
| cassava4.1_002278m PA | AT3G01180 | 0.235049795 | 1.373885415 | Ten DAP | 12 | 2.1.2.02       | major CHO metabolism                             |
| cassava4.1_011883m PA | AT2G33040 | 0.235992394 | 1.430886555 | Ten DAP | 15 | 9.09           | mitochondrial electron transport / ATP synthesis |
| cassava4.1_005468m PA | AT5G36210 | 0.236001118 | 1.290750016 | Ten DAP | 11 | 29.5           | protein                                          |
| cassava4.1_000869m PA | AT5G13980 | 0.236516771 | 1.249670989 | Ten DAP | 2  | 26.03          | misc                                             |
| cassava4.1_029798m PA | AT5G01260 | 0.236819804 | 1.646134072 | Ten DAP | 2  | 2.2.2.04       | major CHO metabolism                             |
| cassava4.1_002502m PA | AT5G67090 | 0.237153725 | 1.254353545 | Ten DAP | 5  | 29.5.01        | protein                                          |
| cassava4.1_014499m PA | AT3G58600 | 0.237309731 | 1.117304593 | Ten DAP | 4  | 35.2           | not assigned                                     |
| cassava4.1_009300m PA | AT3G25700 | 0.237917388 | 1.580893201 | Ten DAP | 4  | 27.3.99        | RNA                                              |
| cassava4.1_004453m PA | AT4G12400 | 0.238546377 | 1.25319202  | Ten DAP | 26 | 20             | stress                                           |

|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_034169m PA | AT1G05150 | 0.240072307 | 1.898305225 | Ten DAP | 4  | 30.3        | signalling                                       |
| cassava4.1_003265m PA | AT2G26730 | 0.24044058  | 1.476831735 | Ten DAP | 6  | 30.2.3      | signalling                                       |
| cassava4.1_011782m PA | AT1G16700 | 0.240807272 | 1.767069801 | Ten DAP | 5  | 9.1.2       | mitochondrial electron transport / ATP synthesis |
| cassava4.1_026755m PA | AT4G11290 | 0.240868909 | 2.679319815 | Ten DAP | 2  | 26.12       | misc                                             |
| cassava4.1_008727m PA | AT1G74910 | 0.241650561 | 1.299649707 | Ten DAP | 6  | 2.1.2.01    | major CHO metabolism                             |
| cassava4.1_001007m PA | AT2G47390 | 0.242277642 | 1.423503769 | Ten DAP | 11 | 35.2        | not assigned                                     |
| cassava4.1_031960m PA | AT4G33680 | 0.242447407 | 2.045445187 | Ten DAP | 4  | 13.1.3.5.03 | amino acid metabolism                            |
| cassava4.1_015312m PA | AT2G45790 | 0.242553961 | 1.238395427 | Ten DAP | 3  | 10.1.021    | cell wall                                        |
| cassava4.1_012516m PA | AT2G33800 | 0.242581264 | 1.356065274 | Ten DAP | 3  | 29.2.1.99   | protein                                          |
| cassava4.1_010502m PA | AT2G36460 | 0.243091463 | 1.275518191 | Ten DAP | 10 | 4.07        | glycolysis                                       |
| cassava4.1_006785m PA | AT3G13920 | 0.24339004  | 1.149106658 | Ten DAP | 29 | 29.2.3      | protein                                          |
| cassava4.1_013524m PA | AT5G40770 | 0.243426689 | 1.247327147 | Ten DAP | 11 | 35.1        | not assigned                                     |
| cassava4.1_014819m PA | AT5G63400 | 0.245238626 | 1.1110552   | Ten DAP | 12 | 23.4.01     | nucleotide metabolism                            |
| cassava4.1_026399m PA | AT1G78860 | 0.24524261  | 1.974348304 | Ten DAP | 2  | 26.16       | misc                                             |
| cassava4.1_012407m PA | AT2G21250 | 0.245775157 | 1.361977986 | Ten DAP | 4  | 3.5         | minor CHO metabolism                             |
| cassava4.1_032931m PA | AT1G15520 | 0.246022369 | 1.363656604 | Ten DAP | 2  | 34.16       | transport                                        |
| cassava4.1_011356m PA | AT5G23050 | 0.247008937 | 2.301824116 | Ten DAP | 5  | 11.1.08     | lipid metabolism                                 |
| cassava4.1_004170m PA | AT5G25880 | 0.248158705 | 1.508189973 | Ten DAP | 2  | 8.2.10      | TCA / org                                        |
| cassava4.1_009412m PA | AT2G26930 | 0.24860403  | 1.144938073 | Ten DAP | 2  | 16.1.1.04   | secondary metabolism                             |
| cassava4.1_013784m PA | AT5G51100 | 0.249382311 | 1.672711555 | Ten DAP | 2  | 21.6        | redox                                            |
| cassava4.1_034167m PA | AT4G10750 | 0.249725888 | 1.285887132 | Ten DAP | 5  | 35.1        | not assigned                                     |
| cassava4.1_013881m PA | AT1G30890 | 0.249898389 | 1.141854828 | Ten DAP | 3  | 35.1        | not assigned                                     |
| cassava4.1_003861m PA | AT4G01660 | 0.250052072 | 1.55412865  | Ten DAP | 4  | 35.1.1      | not assigned                                     |
| cassava4.1_004677m PA | AT5G27380 | 0.250732346 | 1.870919195 | Ten DAP | 4  | 21.2.2      | redox                                            |
| cassava4.1_017439m PA | -         | 0.251100416 | 1.204464045 | Ten DAP | 14 | -           | -                                                |
| cassava4.1_007366m PA | AT1G80480 | 0.252858067 | 1.105706958 | Ten DAP | 5  | 31.1        | cell                                             |
| cassava4.1_001414m PA | AT1G03310 | 0.253063848 | 1.361153605 | Ten DAP | 6  | 2.1.2.04    | major CHO metabolism                             |
| cassava4.1_011947m PA | AT4G15960 | 0.254566346 | 1.322124094 | Ten DAP | 8  | 26.01       | misc                                             |
| cassava4.1_012794m PA | AT5G19760 | 0.255229562 | 1.334041053 | Ten DAP | 10 | 34.9        | transport                                        |
| cassava4.1_008701m PA | AT5G07440 | 0.25523341  | 1.661348081 | Ten DAP | 3  | 12.3.01     | N-metabolism                                     |
| cassava4.1_015571m PA | AT1G67360 | 0.255396173 | 1.645971266 | Ten DAP | 11 | 35.1        | not assigned                                     |
| cassava4.1_006009m PA | AT1G51980 | 0.255404062 | 1.38137865  | Ten DAP | 14 | 29.3.2      | protein                                          |
| cassava4.1_013761m PA | AT5G42790 | 0.255798808 | 1.227475355 | Ten DAP | 9  | 29.5.11.20  | protein                                          |
| cassava4.1_008604m PA | AT1G65930 | 0.257333496 | 1.266361963 | Ten DAP | 23 | 8.1.04      | TCA / org                                        |
| cassava4.1_009710m PA | AT3G19320 | 0.258894533 | 1.145406289 | Ten DAP | 8  | 35.1        | not assigned                                     |
| cassava4.1_007545m PA | AT1G07930 | 0.258958446 | 1.294882477 | Ten DAP | 33 | 29.2.4      | protein                                          |
| cassava4.1_013904m PA | AT3G47590 | 0.260090476 | 1.655467618 | Ten DAP | 3  | 35.1        | not assigned                                     |
| cassava4.1_009172m PA | AT2G20360 | 0.260475842 | 1.32830518  | Ten DAP | 10 | 9.1.2       | mitochondrial electron transport / ATP synthesis |
| cassava4.1_010950m PA | AT3G03080 | 0.261184917 | 1.338568932 | Ten DAP | 19 | 26.07       | misc                                             |
| cassava4.1_022780m PA | AT3G57880 | 0.262775559 | 1.193627045 | Ten DAP | 14 | 35.1.19     | not assigned                                     |
| cassava4.1_003708m PA | AT1G48480 | 0.263110776 | 1.489697948 | Ten DAP | 3  | 30.2.3      | signalling                                       |
| cassava4.1_001086m PA | AT2G40840 | 0.264003241 | 1.250485455 | Ten DAP | 7  | 2.2.2.04    | major CHO metabolism                             |
| cassava4.1_014881m PA | AT4G17830 | 0.264228098 | 1.321297262 | Ten DAP | 3  | 29.5        | protein                                          |
| cassava4.1_011707m PA | AT4G04020 | 0.26526688  | 1.220851886 | Ten DAP | 6  | 31.1        | cell                                             |

|                       |           |             |             |         |    |                |                       |
|-----------------------|-----------|-------------|-------------|---------|----|----------------|-----------------------|
| cassava4.1_016997m PA | AT2G37790 | 0.265289513 | 1.245156765 | Ten DAP | 7  | 3.5            | minor CHO metabolism  |
| cassava4.1_003756m PA | AT5G13520 | 0.26546009  | 1.166953804 | Ten DAP | 11 | 29.5           | protein               |
| cassava4.1_013855m PA | AT5G10860 | 0.265473742 | 1.317348682 | Ten DAP | 12 | 35.1           | not assigned          |
| cassava4.1_008363m PA | AT1G29150 | 0.265545566 | 1.174032878 | Ten DAP | 20 | 29.5.11.20     | protein               |
| cassava4.1_006543m PA | AT4G33030 | 0.265634698 | 1.25688037  | Ten DAP | 9  | 11.10.03       | lipid metabolism      |
| cassava4.1_013833m PA | AT2G40060 | 0.265974309 | 1.185620443 | Ten DAP | 3  | 35.2           | not assigned          |
| cassava4.1_007509m PA | AT4G34480 | 0.267100739 | 1.136566537 | Ten DAP | 6  | 35.1           | not assigned          |
| cassava4.1_007157m PA | AT4G34480 | 0.267610463 | 3.949257967 | Ten DAP | 2  | 35.1           | not assigned          |
| cassava4.1_006024m PA | AT1G74920 | 0.267674974 | 1.195445435 | Ten DAP | 12 | 16.4.2.01      | secondary metabolism  |
| cassava4.1_009803m PA | AT1G14810 | 0.268924838 | 1.420865544 | Ten DAP | 5  | 13.1.3.6.1.02  | amino acid metabolism |
| cassava4.1_009971m PA | AT1G06690 | 0.269696027 | 1.32719413  | Ten DAP | 2  | 3.5            | minor CHO metabolism  |
| cassava4.1_001595m PA | AT2G36390 | 0.273468576 | 1.319348939 | Ten DAP | 51 | 2.1.2.03       | major CHO metabolism  |
| cassava4.1_009321m PA | AT3G15010 | 0.273549556 | 1.184176417 | Ten DAP | 7  | 27.4           | RNA                   |
| cassava4.1_011778m PA | AT5G05340 | 0.273922495 | 1.437085572 | Ten DAP | 6  | 26.12          | misc                  |
| cassava4.1_003888m PA | AT2G29690 | 0.274495295 | 1.158538147 | Ten DAP | 12 | 13.1.6.5.01    | amino acid metabolism |
| cassava4.1_010113m PA | AT4G19810 | 0.274709203 | 1.198971424 | Ten DAP | 2  | 20.1           | stress                |
| cassava4.1_000828m PA | AT5G65750 | 0.275259972 | 1.248060804 | Ten DAP | 6  | 8.1.05         | TCA / org             |
| cassava4.1_001635m PA | AT2G32810 | 0.27564693  | 1.507490154 | Ten DAP | 2  | 10.6.2         | cell wall             |
| cassava4.1_001635m PA | AT2G32810 | 0.27564693  | 1.507490154 | Ten DAP | 2  | 26.03          | misc                  |
| cassava4.1_004717m PA | AT3G29320 | 0.275694189 | 1.420309134 | Ten DAP | 41 | 2.2.2.02       | major CHO metabolism  |
| cassava4.1_014529m PA | AT1G17880 | 0.275944085 | 2.318617916 | Ten DAP | 2  | 27.3.50        | RNA                   |
| cassava4.1_021032m PA | AT4G19710 | 0.276692123 | 1.137422637 | Ten DAP | 14 | 13.1.3.6.1.010 | amino acid metabolism |
| cassava4.1_025269m PA | AT2G27920 | 0.276693341 | 1.37804778  | Ten DAP | 2  | 29.5           | protein               |
| cassava4.1_012862m PA | AT3G12800 | 0.276930751 | 1.333385401 | Ten DAP | 3  | 26.22          | misc                  |
| cassava4.1_001478m PA | AT1G15130 | 0.278160208 | 1.150563746 | Ten DAP | 13 | 35.1.41        | not assigned          |
| cassava4.1_014325m PA | AT3G62870 | 0.278162586 | 1.251860406 | Ten DAP | 17 | 29.2.2         | protein               |
| cassava4.1_014123m PA | AT5G07090 | 0.278233423 | 1.311007803 | Ten DAP | 13 | 29.2.2         | protein               |
| cassava4.1_008634m PA | AT5G22790 | 0.278471246 | 1.429984079 | Ten DAP | 3  | 35.2           | not assigned          |
| cassava4.1_014160m PA | AT2G40300 | 0.279067793 | 1.373033508 | Ten DAP | 2  | 15.2           | metal handling        |
| cassava4.1_014710m PA | AT3G16240 | 0.279461013 | 1.227574304 | Ten DAP | 2  | 34.19.2        | transport             |
| cassava4.1_011025m PA | AT2G43950 | 0.279841329 | 1.41996038  | Ten DAP | 8  | 35.2           | not assigned          |
| cassava4.1_014480m PA | AT2G28790 | 0.28243327  | 1.33201853  | Ten DAP | 3  | 20.2           | stress                |
| cassava4.1_009354m PA | AT5G13490 | 0.283166033 | 1.972139563 | Ten DAP | 4  | 34.14          | transport             |
| cassava4.1_005411m PA | AT5G20890 | 0.283428878 | 1.287333867 | Ten DAP | 14 | 29.6           | protein               |
| cassava4.1_015362m PA | AT1G16210 | 0.284364536 | 1.222239749 | Ten DAP | 6  | 35.2           | not assigned          |
| cassava4.1_004825m PA | AT5G16370 | 0.287532813 | 1.408612804 | Ten DAP | 3  | 11.1.08        | lipid metabolism      |
| cassava4.1_004277m PA | AT1G53210 | 0.288365841 | 1.77598753  | Ten DAP | 3  | 30.3           | signalling            |
| cassava4.1_004544m PA | AT1G16900 | 0.288908346 | 10.70472557 | Ten DAP | 2  | 29.4           | protein               |
| cassava4.1_010071m PA | AT3G59350 | 0.289454379 | 1.140529309 | Ten DAP | 7  | 29.4.1.58      | protein               |
| cassava4.1_015004m PA | AT5G02790 | 0.289602269 | 1.73939417  | Ten DAP | 9  | 35.1           | not assigned          |
| cassava4.1_007558m PA | AT5G19180 | 0.289741479 | 1.79721153  | Ten DAP | 2  | 29.5.11.02     | protein               |
| cassava4.1_013596m PA | AT2G34470 | 0.289924582 | 1.136816896 | Ten DAP | 6  | 13.2.2.3       | amino acid metabolism |
| cassava4.1_014729m PA | AT2G45790 | 0.289930114 | 1.802359789 | Ten DAP | 3  | 10.1.021       | cell wall             |
| cassava4.1_004083m PA | AT4G34200 | 0.291387344 | 1.122572134 | Ten DAP | 34 | 13.1.5.1.01    | amino acid metabolism |

|                       |           |             |             |         |    |               |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|---------------|--------------------------------------------------|
| cassava4.1_001005m PA | AT2G25140 | 0.291398063 | 1.114160938 | Ten DAP | 15 | 20.2.1        | stress                                           |
| cassava4.1_034211m PA | AT3G19950 | 0.292492413 | 1.139416306 | Ten DAP | 4  | 29.5.11.04.02 | protein                                          |
| cassava4.1_025020m PA | AT5G40990 | 0.292958686 | 3.522533517 | Ten DAP | 2  | 26.28         | misc                                             |
| cassava4.1_013042m PA | AT1G03210 | 0.293722756 | 1.327520312 | Ten DAP | 5  | 35.1          | not assigned                                     |
| cassava4.1_019717m PA | AT3G17210 | 0.296258178 | 1.380432172 | Ten DAP | 2  | 35.1          | not assigned                                     |
| cassava4.1_017974m PA | AT1G53540 | 0.296343926 | 2.077299902 | Ten DAP | 2  | 20.2.1        | stress                                           |
| cassava4.1_013889m PA | AT3G57490 | 0.29704583  | 1.744164102 | Ten DAP | 12 | 29.2.2        | protein                                          |
| cassava4.1_001589m PA | AT5G23890 | 0.297130636 | 1.098638586 | Ten DAP | 7  | 35.1          | not assigned                                     |
| cassava4.1_016465m PA | AT1G21720 | 0.297288507 | 1.849489944 | Ten DAP | 3  | 29.5.11.20    | protein                                          |
| cassava4.1_024545m PA | AT4G39080 | 0.297808552 | 1.134090951 | Ten DAP | 13 | 34.1          | transport                                        |
| cassava4.1_015854m PA | AT1G78380 | 0.300165074 | 1.577772431 | Ten DAP | 2  | 26.09         | misc                                             |
| cassava4.1_009191m PA | AT3G55010 | 0.300362407 | 1.453932258 | Ten DAP | 3  | 23.1.2.05     | nucleotide metabolism                            |
| cassava4.1_032232m PA | AT5G48220 | 0.300732801 | 1.164862846 | Ten DAP | 5  | 13.1.6.5.04   | amino acid metabolism                            |
| cassava4.1_020466m PA | AT1G01170 | 0.300882019 | 1.258313736 | Ten DAP | 2  | 20.2.99       | stress                                           |
| cassava4.1_005146m PA | AT4G38350 | 0.301370716 | 1.756119579 | Ten DAP | 5  | 35.1          | not assigned                                     |
| cassava4.1_015495m PA | AT4G38400 | 0.302165955 | 1.372378836 | Ten DAP | 3  | 10.7          | cell wall                                        |
| cassava4.1_014720m PA | AT5G63510 | 0.302478244 | 2.112446556 | Ten DAP | 3  | 35.1          | not assigned                                     |
| cassava4.1_015762m PA | AT2G44050 | 0.30274562  | 1.485300788 | Ten DAP | 4  | 18.3.02       | Co-factor and vitamine metabolism                |
| cassava4.1_017151m PA | AT1G70670 | 0.303151086 | 1.584720306 | Ten DAP | 4  | 33.99         | development                                      |
| cassava4.1_003768m PA | AT5G50400 | 0.303175764 | 1.246916852 | Ten DAP | 9  | 26.13         | misc                                             |
| cassava4.1_018909m PA | AT3G03590 | 0.304804219 | 1.401795783 | Ten DAP | 2  | 35.1          | not assigned                                     |
| cassava4.1_004141m PA | AT3G08510 | 0.304938692 | 1.39926466  | Ten DAP | 18 | 11.9.3.05     | lipid metabolism                                 |
| cassava4.1_004141m PA | AT3G08510 | 0.304938692 | 1.39926466  | Ten DAP | 18 | 30.4.04       | signalling                                       |
| cassava4.1_014454m PA | AT3G55440 | 0.305726618 | 1.180865848 | Ten DAP | 2  | 4.08          | glycolysis                                       |
| cassava4.1_026354m PA | AT3G52570 | 0.306925844 | 1.09936401  | Ten DAP | 3  | 35.2          | not assigned                                     |
| cassava4.1_022943m PA | AT3G63520 | 0.307431289 | 1.167261114 | Ten DAP | 11 | 16.1.4.10     | secondary metabolism                             |
| cassava4.1_007013m PA | AT5G63910 | 0.307752397 | 1.210196981 | Ten DAP | 2  | 35.2          | not assigned                                     |
| cassava4.1_010097m PA | AT5G48020 | 0.308535859 | 1.5786211   | Ten DAP | 2  | 35.2          | not assigned                                     |
| cassava4.1_014495m PA | AT3G15260 | 0.309322707 | 1.127316526 | Ten DAP | 7  | 29.4          | protein                                          |
| cassava4.1_014930m PA | AT3G51780 | 0.309636695 | 1.228321144 | Ten DAP | 3  | 35.1          | not assigned                                     |
| cassava4.1_014442m PA | AT4G29260 | 0.310118017 | 1.925704228 | Ten DAP | 2  | 26.13         | misc                                             |
| cassava4.1_002356m PA | AT5G37510 | 0.310647517 | 1.119518848 | Ten DAP | 31 | 9.1.2         | mitochondrial electron transport / ATP synthesis |
| cassava4.1_011672m PA | AT1G24360 | 0.310671389 | 1.352236436 | Ten DAP | 9  | 11.1.04       | lipid metabolism                                 |
| cassava4.1_004991m PA | AT2G24820 | 0.312439527 | 2.015954215 | Ten DAP | 2  | 29.3.3        | protein                                          |
| cassava4.1_010116m PA | AT5G50850 | 0.313050014 | 1.122741376 | Ten DAP | 11 | 8.1.01.01     | TCA / org                                        |
| cassava4.1_002528m PA | AT3G01680 | 0.313529432 | 1.26677368  | Ten DAP | 16 | 35.2          | not assigned                                     |
| cassava4.1_009117m PA | AT5G42970 | 0.31366166  | 1.399699608 | Ten DAP | 3  | 30.11.1       | signalling                                       |
| cassava4.1_007234m PA | AT4G33680 | 0.314994227 | 1.406954901 | Ten DAP | 10 | 13.1.3.5.03   | amino acid metabolism                            |
| cassava4.1_033530m PA | AT2G07698 | 0.315294196 | 1.072453001 | Ten DAP | 2  | 9.09          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_025245m PA | AT3G16640 | 0.316373319 | 1.199945461 | Ten DAP | 3  | 35.1          | not assigned                                     |
| cassava4.1_031819m PA | AT4G17190 | 0.318830879 | 1.678003967 | Ten DAP | 2  | 16.1.2.09     | secondary metabolism                             |
| cassava4.1_009084m PA | AT3G04600 | 0.319447398 | 1.269305928 | Ten DAP | 6  | 29.1          | protein                                          |
| cassava4.1_010153m PA | AT4G37970 | 0.319621263 | 1.310919671 | Ten DAP | 10 | 16.2.1.010    | secondary metabolism                             |
| cassava4.1_002509m PA | AT1G30360 | 0.319754479 | 1.29512792  | Ten DAP | 3  | 20.2.3        | stress                                           |

|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_003454m PA | AT5G38640 | 0.321144404 | 1.34009831  | Ten DAP | 4  | 29.2.3      | protein                                          |
| cassava4.1_002128m PA | AT1G15690 | 0.321430183 | 1.151475361 | Ten DAP | 22 | 34.30       | transport                                        |
| cassava4.1_009566m PA | AT2G27680 | 0.321460791 | 1.238517696 | Ten DAP | 4  | 3.5         | minor CHO metabolism                             |
| cassava4.1_003885m PA | AT5G64440 | 0.321920209 | 1.313432185 | Ten DAP | 3  | 26.08       | misc                                             |
| cassava4.1_007608m PA | AT3G16480 | 0.323103027 | 1.303480878 | Ten DAP | 4  | 29.3.2      | protein                                          |
| cassava4.1_001605m PA | AT1G23180 | 0.323187153 | 1.204668776 | Ten DAP | 3  | 35.1.3      | not assigned                                     |
| cassava4.1_015358m PA | AT1G18540 | 0.323233589 | 2.049679238 | Ten DAP | 4  | 29.2.2      | protein                                          |
| cassava4.1_011826m PA | AT4G14880 | 0.323405622 | 1.150869717 | Ten DAP | 22 | 13.1.5.3.01 | amino acid metabolism                            |
| cassava4.1_009003m PA | AT1G79550 | 0.323730242 | 1.046669029 | Ten DAP | 44 | 4.010       | glycolysis                                       |
| cassava4.1_014643m PA | AT1G07890 | 0.324429292 | 1.26813682  | Ten DAP | 22 | 21.2.1      | redox                                            |
| cassava4.1_004303m PA | AT5G13030 | 0.325379006 | 1.085522064 | Ten DAP | 11 | 35.2        | not assigned                                     |
| cassava4.1_006577m PA | AT1G72330 | 0.327432841 | 1.260349922 | Ten DAP | 9  | 13.1.1.3.01 | amino acid metabolism                            |
| cassava4.1_016521m PA | AT5G47030 | 0.327494787 | 1.61273057  | Ten DAP | 3  | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_000329m PA | AT3G13290 | 0.327783369 | 2.630167856 | Ten DAP | 2  | 33.99       | development                                      |
| cassava4.1_006456m PA | AT5G26030 | 0.331490128 | 1.826811146 | Ten DAP | 4  | 19.020      | tetrapyrrole synthesis                           |
| cassava4.1_016307m PA | AT3G49010 | 0.332609031 | 1.698476697 | Ten DAP | 7  | 29.2.2      | protein                                          |
| cassava4.1_001915m PA | AT5G57870 | 0.333412087 | 1.101384847 | Ten DAP | 8  | 29.2.3      | protein                                          |
| cassava4.1_017016m PA | AT2G27030 | 0.33605523  | 1.208267749 | Ten DAP | 5  | 30.3        | signalling                                       |
| cassava4.1_017537m PA | AT3G56070 | 0.33706801  | 1.247159295 | Ten DAP | 3  | 31.3.01     | cell                                             |
| cassava4.1_009715m PA | AT4G28390 | 0.338253209 | 1.447312727 | Ten DAP | 10 | 34.9        | transport                                        |
| cassava4.1_000646m PA | AT5G49030 | 0.338700394 | 1.290886133 | Ten DAP | 2  | 29.1.05     | protein                                          |
| cassava4.1_004671m PA | AT3G11710 | 0.339216471 | 1.066682824 | Ten DAP | 21 | 29.1.06     | protein                                          |
| cassava4.1_003615m PA | AT2G13560 | 0.339703277 | 1.041972912 | Ten DAP | 14 | 8.2.10      | TCA / org                                        |
| cassava4.1_015127m PA | AT3G14290 | 0.339766448 | 1.555464468 | Ten DAP | 8  | 29.5.11.20  | protein                                          |
| cassava4.1_011958m PA | AT1G05620 | 0.341288571 | 1.451006338 | Ten DAP | 3  | 23.2        | nucleotide metabolism                            |
| cassava4.1_004228m PA | AT3G17970 | 0.341361591 | 1.202938313 | Ten DAP | 4  | 29.3.3      | protein                                          |
| cassava4.1_015434m PA | AT4G26220 | 0.342743029 | 1.20574566  | Ten DAP | 4  | 16.2.1.06   | secondary metabolism                             |
| cassava4.1_004630m PA | AT5G08680 | 0.343302159 | 1.165361737 | Ten DAP | 27 | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_013921m PA | AT5G06290 | 0.344037686 | 1.545225211 | Ten DAP | 8  | 21.05       | redox                                            |
| cassava4.1_012057m PA | AT2G21170 | 0.344269378 | 1.237065937 | Ten DAP | 3  | 1.3.05      | PS                                               |
| cassava4.1_010182m PA | AT2G45440 | 0.344885219 | 1.232827248 | Ten DAP | 3  | 13.1.3.5.01 | amino acid metabolism                            |
| cassava4.1_033827m PA | AT3G55260 | 0.346162586 | 1.286145115 | Ten DAP | 7  | 35.1        | not assigned                                     |
| cassava4.1_030462m PA | AT5G42180 | 0.346920268 | 1.237565298 | Ten DAP | 2  | 26.12       | misc                                             |
| cassava4.1_012630m PA | AT2G42130 | 0.347381349 | 1.202836008 | Ten DAP | 13 | 35.2        | not assigned                                     |
| cassava4.1_018010m PA | AT3G53990 | 0.347470385 | 1.335264101 | Ten DAP | 8  | 20.2.2      | stress                                           |
| cassava4.1_032915m PA | AT2G35490 | 0.348894389 | 1.348188973 | Ten DAP | 2  | 31.1        | cell                                             |
| cassava4.1_006126m PA | AT3G17760 | 0.349429876 | 1.238726536 | Ten DAP | 7  | 13.1.1.1.01 | amino acid metabolism                            |
| cassava4.1_003064m PA | AT3G05900 | 0.350529124 | 1.251771814 | Ten DAP | 30 | 35.1        | not assigned                                     |
| cassava4.1_003245m PA | AT1G45150 | 0.351239492 | 1.32982647  | Ten DAP | 7  | 35.2        | not assigned                                     |
| cassava4.1_015325m PA | AT1G20225 | 0.352438263 | 1.45497267  | Ten DAP | 4  | 35.2        | not assigned                                     |
| cassava4.1_016640m PA | AT5G63620 | 0.354306567 | 1.545974534 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_014032m PA | AT3G09740 | 0.354921952 | 1.070693756 | Ten DAP | 8  | 31.4        | cell                                             |
| cassava4.1_010908m PA | AT1G12780 | 0.355039838 | 1.21550372  | Ten DAP | 17 | 10.1.02     | cell wall                                        |
| cassava4.1_014339m PA | AT5G13120 | 0.355273486 | 1.327502033 | Ten DAP | 6  | 31.3.01     | cell                                             |

|                       |           |             |             |         |    |              |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_004513m PA | AT3G16950 | 0.355327854 | 1.255723499 | Ten DAP | 13 | 8.1.01.03    | TCA / org                                        |
| cassava4.1_019312m PA | AT5G25450 | 0.356567728 | 1.336257434 | Ten DAP | 5  | 9.05         | mitochondrial electron transport / ATP synthesis |
| cassava4.1_027790m PA | AT1G73370 | 0.356569843 | 1.132328345 | Ten DAP | 2  | 2.2.1.05     | major CHO metabolism                             |
| cassava4.1_007986m PA | AT1G04980 | 0.35688277  | 1.221255692 | Ten DAP | 9  | 21.01        | redox                                            |
| cassava4.1_006431m PA | AT3G02360 | 0.357872224 | 1.171183241 | Ten DAP | 10 | 7.1.03       | OPP                                              |
| cassava4.1_013545m PA | AT4G35220 | 0.359315648 | 1.478018155 | Ten DAP | 6  | 28.99        | DNA                                              |
| cassava4.1_025681m PA | AT1G10510 | 0.359547901 | 1.284454675 | Ten DAP | 3  | 33.99        | development                                      |
| cassava4.1_016825m PA | AT5G16400 | 0.360655545 | 1.400409713 | Ten DAP | 2  | 21.01        | redox                                            |
| cassava4.1_003136m PA | AT4G16760 | 0.363012308 | 1.339363523 | Ten DAP | 6  | 11.9.4.02    | lipid metabolism                                 |
| cassava4.1_001205m PA | AT1G68560 | 0.363669101 | 1.290821362 | Ten DAP | 10 | 2.2.2.1      | major CHO metabolism                             |
| cassava4.1_001205m PA | AT1G68560 | 0.363669101 | 1.290821362 | Ten DAP | 10 | 26.03        | misc                                             |
| cassava4.1_017451m PA | AT2G22170 | 0.363851642 | 1.282441248 | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_010847m PA | AT1G52730 | 0.364026576 | 1.325306314 | Ten DAP | 2  | 27.3.99      | RNA                                              |
| cassava4.1_010847m PA | AT1G52730 | 0.364026576 | 1.325306314 | Ten DAP | 2  | 33.99        | development                                      |
| cassava4.1_013244m PA | AT3G53900 | 0.366267989 | 1.212582056 | Ten DAP | 4  | 23.3.1.03    | nucleotide metabolism                            |
| cassava4.1_013511m PA | AT2G20690 | 0.366304429 | 1.502393845 | Ten DAP | 3  | 18.3.02      | Co-factor and vitamine metabolism                |
| cassava4.1_007347m PA | AT5G59420 | 0.366463544 | 1.141026237 | Ten DAP | 7  | 31.4         | cell                                             |
| cassava4.1_014421m PA | AT5G20720 | 0.367273711 | 1.211841298 | Ten DAP | 5  | 29.6         | protein                                          |
| cassava4.1_009196m PA | AT5G16880 | 0.368599383 | 1.122314718 | Ten DAP | 5  | 31.4         | cell                                             |
| cassava4.1_013801m PA | AT3G02520 | 0.368770217 | 1.082408846 | Ten DAP | 14 | 30.7         | signalling                                       |
| cassava4.1_003951m PA | AT3G49220 | 0.369153168 | 1.107975296 | Ten DAP | 3  | 10.8.01      | cell wall                                        |
| cassava4.1_012376m PA | AT4G36800 | 0.369829034 | 1.335426368 | Ten DAP | 2  | 29.5.11.03   | protein                                          |
| cassava4.1_000003m PA | AT1G55860 | 0.372904527 | 1.194876019 | Ten DAP | 3  | 29.5.11.4.01 | protein                                          |
| cassava4.1_006417m PA | AT4G38510 | 0.373164713 | 1.142162958 | Ten DAP | 3  | 34.1.01      | transport                                        |
| cassava4.1_023292m PA | AT3G10690 | 0.373245796 | 1.236149296 | Ten DAP | 3  | 28.1         | DNA                                              |
| cassava4.1_003675m PA | AT3G49240 | 0.374293473 | 1.057417397 | Ten DAP | 5  | 35.1.5       | not assigned                                     |
| cassava4.1_006966m PA | AT4G34490 | 0.374323627 | 1.161413236 | Ten DAP | 19 | 28.99        | DNA                                              |
| cassava4.1_004791m PA | AT1G77590 | 0.378392434 | 1.172509373 | Ten DAP | 11 | 11.1.09      | lipid metabolism                                 |
| cassava4.1_018483m PA | AT3G45980 | 0.378634731 | 1.245698376 | Ten DAP | 10 | 28.1.3       | DNA                                              |
| cassava4.1_002716m PA | AT5G49910 | 0.378902009 | 1.272858531 | Ten DAP | 35 | 20.2.1       | stress                                           |
| cassava4.1_005096m PA | AT4G13700 | 0.379491299 | 1.583264074 | Ten DAP | 2  | 26.13        | misc                                             |
| cassava4.1_009351m PA | AT5G13490 | 0.37988375  | 1.601326537 | Ten DAP | 25 | 34.14        | transport                                        |
| cassava4.1_013120m PA | AT4G28510 | 0.380275112 | 1.178080654 | Ten DAP | 7  | 35.1         | not assigned                                     |
| cassava4.1_007795m PA | AT1G18450 | 0.380809964 | 1.203678846 | Ten DAP | 2  | 31.1         | cell                                             |
| cassava4.1_015911m PA | AT1G30070 | 0.381325198 | 1.389215731 | Ten DAP | 2  | 35.1         | not assigned                                     |
| cassava4.1_011832m PA | AT4G34050 | 0.381826562 | 1.594142971 | Ten DAP | 5  | 16.2.1.06    | secondary metabolism                             |
| cassava4.1_009944m PA | AT5G53400 | 0.38533401  | 1.159532821 | Ten DAP | 5  | 31.1         | cell                                             |
| cassava4.1_003376m PA | AT2G23350 | 0.386520742 | 1.489169829 | Ten DAP | 6  | 27.1         | RNA                                              |
| cassava4.1_016116m PA | AT3G48890 | 0.389211545 | 1.503679734 | Ten DAP | 6  | 21.2         | redox                                            |
| cassava4.1_001937m PA | AT2G17790 | 0.38953689  | 1.093856805 | Ten DAP | 7  | 29.3.4.3     | protein                                          |
| cassava4.1_003199m PA | AT5G04885 | 0.389918028 | 1.252656188 | Ten DAP | 5  | 26.03        | misc                                             |
| cassava4.1_015848m PA | AT4G36750 | 0.390846052 | 1.212722003 | Ten DAP | 2  | 11.8         | lipid metabolism                                 |
| cassava4.1_017425m PA | AT1G67430 | 0.391841855 | 1.180793071 | Ten DAP | 5  | 29.2.2       | protein                                          |
| cassava4.1_014582m PA | AT3G25780 | 0.392678075 | 1.28304514  | Ten DAP | 11 | 17.7.1.04    | hormone metabolism                               |

|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_018351m PA | AT1G70830 | 0.395858808 | 1.238853255 | Ten DAP | 8  | 20.2.99     | stress                                           |
| cassava4.1_017764m PA | AT1G80230 | 0.396012665 | 1.174891178 | Ten DAP | 2  | 9.07        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_013442m PA | AT4G23900 | 0.396735515 | 1.259858351 | Ten DAP | 5  | 23.4.010    | nucleotide metabolism                            |
| cassava4.1_016933m PA | AT1G48830 | 0.397394291 | 1.30731631  | Ten DAP | 10 | 29.2.2      | protein                                          |
| cassava4.1_014684m PA | AT2G27020 | 0.397487659 | 1.379711075 | Ten DAP | 9  | 29.5.11.20  | protein                                          |
| cassava4.1_018385m PA | AT5G02560 | 0.398060046 | 1.465837786 | Ten DAP | 4  | 28.1.3      | DNA                                              |
| cassava4.1_031031m PA | AT1G13750 | 0.398279194 | 1.267897145 | Ten DAP | 6  | 26.13       | misc                                             |
| cassava4.1_011691m PA | AT4G02340 | 0.398400564 | 1.207667745 | Ten DAP | 7  | 26.01       | misc                                             |
| cassava4.1_018616m PA | AT2G44310 | 0.399493152 | 1.216766819 | Ten DAP | 2  | 30.3        | signalling                                       |
| cassava4.1_016073m PA | AT3G25580 | 0.401394376 | 1.193346108 | Ten DAP | 2  | 21.01       | redox                                            |
| cassava4.1_013978m PA | AT2G40300 | 0.402828259 | 1.1564929   | Ten DAP | 9  | 15.2        | metal handling                                   |
| cassava4.1_016023m PA | AT3G17020 | 0.402924694 | 1.180845866 | Ten DAP | 3  | 20.2.2      | stress                                           |
| cassava4.1_005314m PA | AT3G58610 | 0.404093702 | 1.404617236 | Ten DAP | 8  | 13.1.4.1    | amino acid metabolism                            |
| cassava4.1_032669m PA | AT2G44450 | 0.4059898   | 2.207208249 | Ten DAP | 2  | 26.03       | misc                                             |
| cassava4.1_016576m PA | AT4G17830 | 0.40690464  | 1.188317736 | Ten DAP | 4  | 29.5        | protein                                          |
| cassava4.1_002785m PA | AT1G77590 | 0.407199249 | 1.279226235 | Ten DAP | 3  | 11.1.09     | lipid metabolism                                 |
| cassava4.1_011323m PA | AT4G08900 | 0.407884397 | 1.526062466 | Ten DAP | 2  | 13.2.2.3    | amino acid metabolism                            |
| cassava4.1_017647m PA | AT5G38410 | 0.409052039 | 1.401832487 | Ten DAP | 4  | 1.3.02      | PS                                               |
| cassava4.1_004702m PA | AT5G18070 | 0.409161171 | 1.160892843 | Ten DAP | 5  | 10.1        | cell wall                                        |
| cassava4.1_004164m PA | AT5G25880 | 0.409932835 | 1.370762959 | Ten DAP | 59 | 8.2.10      | TCA / org                                        |
| cassava4.1_012783m PA | AT3G12290 | 0.411045463 | 1.074067198 | Ten DAP | 14 | 25.05       | C1-metabolism                                    |
| cassava4.1_006391m PA | AT5G63890 | 0.411285133 | 1.080965417 | Ten DAP | 8  | 13.1.7.08   | amino acid metabolism                            |
| cassava4.1_008631m PA | AT3G13920 | 0.411533546 | 1.549947232 | Ten DAP | 5  | 29.2.3      | protein                                          |
| cassava4.1_007023m PA | AT2G01720 | 0.412231265 | 1.177729717 | Ten DAP | 8  | 29.7        | protein                                          |
| cassava4.1_016823m PA | AT4G10450 | 0.412312993 | 1.202999724 | Ten DAP | 14 | 29.2.2      | protein                                          |
| cassava4.1_011977m PA | AT3G48890 | 0.413938316 | 1.362453552 | Ten DAP | 2  | 21.2        | redox                                            |
| cassava4.1_011932m PA | AT4G29830 | 0.41415835  | 1.386194604 | Ten DAP | 3  | 30.5        | signalling                                       |
| cassava4.1_009598m PA | AT3G45770 | 0.41723009  | 1.179147563 | Ten DAP | 8  | 29.3.1      | protein                                          |
| cassava4.1_001640m PA | AT1G56070 | 0.417597042 | 1.133145981 | Ten DAP | 16 | 29.2.4      | protein                                          |
| cassava4.1_002005m PA | AT4G33090 | 0.417775598 | 1.064657549 | Ten DAP | 15 | 29.5        | protein                                          |
| cassava4.1_007404m PA | AT1G59900 | 0.417952736 | 1.093499112 | Ten DAP | 25 | 8.1.01.01   | TCA / org                                        |
| cassava4.1_000501m PA | AT1G79280 | 0.419078515 | 1.717262941 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_014465m PA | AT3G62030 | 0.419321569 | 1.180687438 | Ten DAP | 3  | 31.3.01     | cell                                             |
| cassava4.1_001448m PA | AT2G16950 | 0.419647717 | 1.10117892  | Ten DAP | 5  | 29.3.1      | protein                                          |
| cassava4.1_014220m PA | AT5G24650 | 0.419787893 | 1.168333441 | Ten DAP | 5  | 29.3.2      | protein                                          |
| cassava4.1_002627m PA | AT3G01680 | 0.420338202 | 1.161859738 | Ten DAP | 7  | 35.2        | not assigned                                     |
| cassava4.1_005164m PA | AT1G20080 | 0.420506364 | 1.164548596 | Ten DAP | 12 | 35.1.19     | not assigned                                     |
| cassava4.1_005539m PA | AT2G45300 | 0.420760728 | 1.289188782 | Ten DAP | 9  | 13.1.6.1.06 | amino acid metabolism                            |
| cassava4.1_018936m PA | AT1G51060 | 0.422502589 | 1.242431203 | Ten DAP | 3  | 28.1.3      | DNA                                              |
| cassava4.1_002624m PA | AT5G26830 | 0.422540469 | 1.273159959 | Ten DAP | 22 | 29.1.03     | protein                                          |
| cassava4.1_004493m PA | AT4G20980 | 0.422913326 | 1.119615305 | Ten DAP | 13 | 29.2.3      | protein                                          |
| cassava4.1_005257m PA | AT5G62890 | 0.423904165 | 1.245464817 | Ten DAP | 5  | 34.99       | transport                                        |
| cassava4.1_011177m PA | AT5G03300 | 0.424276732 | 1.103520743 | Ten DAP | 18 | 23.3.2.01   | nucleotide metabolism                            |
| cassava4.1_005898m PA | AT1G51980 | 0.425336972 | 1.106670583 | Ten DAP | 12 | 29.3.2      | protein                                          |

|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_001787m PA | AT4G39080 | 0.428373657 | 1.158062044 | Ten DAP | 5  | 34.1        | transport                                        |
| cassava4.1_015272m PA | AT3G10920 | 0.429182382 | 1.164547288 | Ten DAP | 7  | 21.6        | redox                                            |
| cassava4.1_009490m PA | AT4G19006 | 0.429668087 | 1.069940883 | Ten DAP | 12 | 29.5.11.20  | protein                                          |
| cassava4.1_010835m PA | AT1G64970 | 0.429689483 | 1.057518331 | Ten DAP | 5  | 16.1.3.05   | secondary metabolism                             |
| cassava4.1_015090m PA | AT2G25080 | 0.429941879 | 1.228329064 | Ten DAP | 2  | 21.2        | redox                                            |
| cassava4.1_010349m PA | AT4G11120 | 0.429967535 | 1.167298445 | Ten DAP | 6  | 29.2.4      | protein                                          |
| cassava4.1_015238m PA | AT5G35530 | 0.431583123 | 1.107434929 | Ten DAP | 6  | 29.2.2      | protein                                          |
| cassava4.1_000214m PA | AT5G04140 | 0.432801218 | 1.262220889 | Ten DAP | 14 | 12.2.1.01   | N-metabolism                                     |
| cassava4.1_018711m PA | AT1G04480 | 0.433485509 | 1.249610425 | Ten DAP | 2  | 29.2.2      | protein                                          |
| cassava4.1_000342m PA | AT1G05570 | 0.433730254 | 1.288008997 | Ten DAP | 3  | 3.6         | minor CHO metabolism                             |
| cassava4.1_018079m PA | AT1G68300 | 0.434046687 | 1.215338876 | Ten DAP | 2  | 20.2.99     | stress                                           |
| cassava4.1_022949m PA | AT1G65980 | 0.434532054 | 1.565352241 | Ten DAP | 2  | 21.05       | redox                                            |
| cassava4.1_020346m PA | AT3G61110 | 0.435091532 | 1.089561345 | Ten DAP | 3  | 29.2.2      | protein                                          |
| cassava4.1_012361m PA | AT5G13440 | 0.435347938 | 1.174871035 | Ten DAP | 6  | 9.05        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_018170m PA | -         | 0.437661803 | 1.409145641 | Ten DAP | 11 | -           | -                                                |
| cassava4.1_009305m PA | AT1G08470 | 0.437828391 | 1.181760482 | Ten DAP | 7  | 16.4.1      | secondary metabolism                             |
| cassava4.1_030498m PA | AT3G15090 | 0.438785512 | 1.20482267  | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_003340m PA | AT3G12580 | 0.438874913 | 1.325632284 | Ten DAP | 11 | 20.2.1      | stress                                           |
| cassava4.1_023204m PA | AT3G08960 | 0.439375157 | 1.754886117 | Ten DAP | 2  | 27.3.99     | RNA                                              |
| cassava4.1_011839m PA | AT5G39740 | 0.439926969 | 1.368973583 | Ten DAP | 15 | 29.2.2      | protein                                          |
| cassava4.1_005323m PA | AT3G20410 | 0.440441151 | 1.179084533 | Ten DAP | 3  | 30.3        | signalling                                       |
| cassava4.1_014302m PA | AT4G23630 | 0.440622405 | 1.269110862 | Ten DAP | 5  | 35.1        | not assigned                                     |
| cassava4.1_013498m PA | AT2G16850 | 0.4406411   | 1.360692223 | Ten DAP | 5  | 34.19.1     | transport                                        |
| cassava4.1_012868m PA | AT5G46800 | 0.442380152 | 2.128587443 | Ten DAP | 7  | 34.9        | transport                                        |
| cassava4.1_000431m PA | AT4G00630 | 0.444440864 | 1.150462649 | Ten DAP | 10 | 34.15       | transport                                        |
| cassava4.1_008086m PA | AT5G37600 | 0.444580128 | 1.168559628 | Ten DAP | 9  | 12.2.02     | N-metabolism                                     |
| cassava4.1_013786m PA | AT2G26800 | 0.444860532 | 1.504312361 | Ten DAP | 2  | 13.2.4.4    | amino acid metabolism                            |
| cassava4.1_003079m PA | AT2G32850 | 0.44560197  | 3.475238053 | Ten DAP | 2  | 29.4        | protein                                          |
| cassava4.1_003274m PA | AT3G13060 | 0.44642218  | 1.391352466 | Ten DAP | 2  | 35.2        | not assigned                                     |
| cassava4.1_017832m PA | AT5G16450 | 0.446613452 | 1.16616913  | Ten DAP | 3  | 25          | C1-metabolism                                    |
| cassava4.1_002254m PA | AT2G05920 | 0.446920464 | 1.307875719 | Ten DAP | 14 | 29.5.01     | protein                                          |
| cassava4.1_027677m PA | AT1G15520 | 0.44712201  | 1.163753634 | Ten DAP | 7  | 34.16       | transport                                        |
| cassava4.1_004214m PA | AT5G01320 | 0.447266017 | 1.063657916 | Ten DAP | 10 | 5.02        | fermentation                                     |
| cassava4.1_004085m PA | AT4G34200 | 0.447469639 | 1.189443723 | Ten DAP | 17 | 13.1.5.1.01 | amino acid metabolism                            |
| cassava4.1_009602m PA | AT5G14780 | 0.447757326 | 6.33041327  | Ten DAP | 2  | 25.10       | C1-metabolism                                    |
| cassava4.1_011439m PA | AT2G03390 | 0.450092527 | 1.268424358 | Ten DAP | 2  | 28.1        | DNA                                              |
| cassava4.1_014943m PA | AT4G36910 | 0.451562959 | 1.193283134 | Ten DAP | 5  | 35.1        | not assigned                                     |
| cassava4.1_016781m PA | AT4G01900 | 0.452073156 | 1.13626153  | Ten DAP | 2  | 30.1        | signalling                                       |
| cassava4.1_013351m PA | AT2G41530 | 0.452581152 | 1.180958023 | Ten DAP | 8  | 35.1        | not assigned                                     |
| cassava4.1_017973m PA | AT1G65980 | 0.453077239 | 1.198801445 | Ten DAP | 5  | 21.05       | redox                                            |
| cassava4.1_008270m PA | AT5G10330 | 0.453680529 | 1.648173646 | Ten DAP | 6  | 13.1.7.06   | amino acid metabolism                            |
| cassava4.1_004407m PA | AT5G61900 | 0.455045428 | 1.267516425 | Ten DAP | 3  | 35.1        | not assigned                                     |
| cassava4.1_016543m PA | AT3G22630 | 0.456336581 | 1.190354573 | Ten DAP | 8  | 29.5.11.20  | protein                                          |
| cassava4.1_001983m PA | AT2G42490 | 0.4571225   | 1.36387091  | Ten DAP | 2  | 26.07       | misc                                             |



|                       |           |             |             |         |    |             |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|--------------------------------------------------|
| cassava4.1_019826m PA | AT4G14320 | 0.457263504 | 1.129065532 | Ten DAP | 3  | 29.2.2      | protein                                          |
| cassava4.1_011964m PA | AT5G66720 | 0.458016724 | 1.104723364 | Ten DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_031166m PA | AT1G05500 | 0.458032785 | 1.404886067 | Ten DAP | 8  | 35.1.19     | not assigned                                     |
| cassava4.1_013407m PA | AT1G14410 | 0.459263658 | 1.112970173 | Ten DAP | 3  | 27.3.67     | RNA                                              |
| cassava4.1_004947m PA | AT1G76160 | 0.459716576 | 1.299910913 | Ten DAP | 6  | 35.1        | not assigned                                     |
| cassava4.1_016175m PA | AT2G30860 | 0.460680241 | 1.319406552 | Ten DAP | 17 | 26.09       | misc                                             |
| cassava4.1_018308m PA | AT1G34030 | 0.463163671 | 1.161911941 | Ten DAP | 14 | 29.2.2      | protein                                          |
| cassava4.1_019559m PA | AT5G01650 | 0.463438013 | 1.365749217 | Ten DAP | 4  | 35.1        | not assigned                                     |
| cassava4.1_013579m PA | AT5G19140 | 0.465137153 | 1.158552249 | Ten DAP | 6  | 15          | metal handling                                   |
| cassava4.1_020696m PA | AT3G52730 | 0.46742504  | 1.093321488 | Ten DAP | 2  | 9.05        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_015644m PA | AT5G37475 | 0.467985123 | 1.102287032 | Ten DAP | 8  | 27.3.99     | RNA                                              |
| cassava4.1_015644m PA | AT5G37475 | 0.467985123 | 1.102287032 | Ten DAP | 8  | 29.2.3      | protein                                          |
| cassava4.1_005910m PA | AT2G40890 | 0.468923502 | 1.2488587   | Ten DAP | 10 | 16.2.1.05   | secondary metabolism                             |
| cassava4.1_005910m PA | AT2G40890 | 0.468923502 | 1.2488587   | Ten DAP | 10 | 26.10       | misc                                             |
| cassava4.1_011885m PA | AT5G16990 | 0.469781369 | 1.097758394 | Ten DAP | 3  | 26.07       | misc                                             |
| cassava4.1_006349m PA | AT5G40010 | 0.470136297 | 1.452699255 | Ten DAP | 2  | 29.5.09     | protein                                          |
| cassava4.1_018436m PA | AT4G09320 | 0.470340414 | 1.206059127 | Ten DAP | 11 | 23.4.010    | nucleotide metabolism                            |
| cassava4.1_010514m PA | AT1G31190 | 0.471295777 | 1.174773426 | Ten DAP | 4  | 3.4.05      | minor CHO metabolism                             |
| cassava4.1_009757m PA | AT5G40490 | 0.472703102 | 1.215554011 | Ten DAP | 6  | 27.4        | RNA                                              |
| cassava4.1_015586m PA | AT3G17770 | 0.472742777 | 1.292479338 | Ten DAP | 6  | 3.5         | minor CHO metabolism                             |
| cassava4.1_007661m PA | AT1G77670 | 0.473681122 | 1.261297456 | Ten DAP | 4  | 16.2        | secondary metabolism                             |
| cassava4.1_019974m PA | AT2G24940 | 0.473794589 | 1.125225641 | Ten DAP | 3  | 21.2        | redox                                            |
| cassava4.1_006281m PA | AT1G22380 | 0.474336281 | 1.352626614 | Ten DAP | 3  | 26.02       | misc                                             |
| cassava4.1_010643m PA | AT3G57560 | 0.474712044 | 1.326808871 | Ten DAP | 6  | 13.1.2.3.02 | amino acid metabolism                            |
| cassava4.1_010643m PA | AT3G57560 | 0.474712044 | 1.326808871 | Ten DAP | 6  | 23.4.99     | nucleotide metabolism                            |
| cassava4.1_006452m PA | AT3G19340 | 0.474789914 | 1.156896019 | Ten DAP | 8  | 35.2        | not assigned                                     |
| cassava4.1_020331m PA | AT3G24540 | 0.474805414 | 1.373361087 | Ten DAP | 3  | 30.2.22     | signalling                                       |
| cassava4.1_013615m PA | AT5G67500 | 0.475234311 | 1.154176    | Ten DAP | 2  | 34.20       | transport                                        |
| cassava4.1_009800m PA | AT4G05390 | 0.476262611 | 1.249294151 | Ten DAP | 4  | 7.3         | OPP                                              |
| cassava4.1_008923m PA | AT4G19410 | 0.478500386 | 1.049459551 | Ten DAP | 18 | 10.8.02     | cell wall                                        |
| cassava4.1_017771m PA | AT3G52300 | 0.479634494 | 1.120817371 | Ten DAP | 14 | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_027417m PA | AT5G64580 | 0.483798873 | 1.407005454 | Ten DAP | 2  | 29.5.09     | protein                                          |
| cassava4.1_012158m PA | AT2G41790 | 0.484270518 | 1.136207712 | Ten DAP | 3  | 29.5.07     | protein                                          |
| cassava4.1_027390m PA | AT5G12370 | 0.486683863 | 1.429311257 | Ten DAP | 4  | 35.1        | not assigned                                     |
| cassava4.1_013892m PA | AT3G57490 | 0.487375307 | 1.126417479 | Ten DAP | 12 | 29.2.2      | protein                                          |
| cassava4.1_013922m PA | AT5G12370 | 0.487752671 | 1.197000612 | Ten DAP | 3  | 35.1        | not assigned                                     |
| cassava4.1_003563m PA | AT5G48960 | 0.489232508 | 1.308540642 | Ten DAP | 12 | 35.1        | not assigned                                     |
| cassava4.1_012429m PA | AT3G48330 | 0.489349417 | 1.115834343 | Ten DAP | 3  | 29.4        | protein                                          |
| cassava4.1_024143m PA | AT5G16510 | 0.49208473  | 1.412911061 | Ten DAP | 3  | 10.5.5      | cell wall                                        |
| cassava4.1_014693m PA | AT5G13450 | 0.493108104 | 1.204089184 | Ten DAP | 16 | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_015992m PA | AT5G01750 | 0.494548266 | 1.149353908 | Ten DAP | 7  | 35.2        | not assigned                                     |
| cassava4.1_005272m PA | AT2G16070 | 0.494559326 | 1.17935106  | Ten DAP | 7  | 35.2        | not assigned                                     |
| cassava4.1_011901m PA | AT5G58220 | 0.494589238 | 1.206010136 | Ten DAP | 4  | 35.2        | not assigned                                     |
| cassava4.1_016698m PA | AT5G39850 | 0.494763628 | 1.101999974 | Ten DAP | 13 | 29.2.2      | protein                                          |

|                       |           |             |             |         |    |            |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|------------|--------------------------------------------------|
| cassava4.1_018628m PA | AT2G33220 | 0.494916715 | 1.16100412  | Ten DAP | 3  | 35.2       | not assigned                                     |
| cassava4.1_015393m PA | AT5G57950 | 0.495109137 | 1.050188337 | Ten DAP | 2  | 29.5.11.20 | protein                                          |
| cassava4.1_006660m PA | AT4G30810 | 0.49572561  | 1.63248472  | Ten DAP | 3  | 29.5.05    | protein                                          |
| cassava4.1_002629m PA | AT3G01680 | 0.49725085  | 1.132259788 | Ten DAP | 19 | 35.2       | not assigned                                     |
| cassava4.1_016079m PA | AT5G42960 | 0.497843324 | 1.123098932 | Ten DAP | 3  | 35.2       | not assigned                                     |
| cassava4.1_018546m PA | AT3G56490 | 0.498320938 | 1.088543873 | Ten DAP | 5  | 29.4       | protein                                          |
| cassava4.1_029911m PA | AT5G57850 | 0.499282873 | 1.351879309 | Ten DAP | 6  | 26.26.1    | misc                                             |
| cassava4.1_024456m PA | AT5G19680 | 0.500390892 | 1.120726049 | Ten DAP | 2  | 29.4       | protein                                          |
| cassava4.1_007236m PA | AT5G58100 | 0.500654269 | 1.181506536 | Ten DAP | 3  | 35.2       | not assigned                                     |
| cassava4.1_015256m PA | AT4G27670 | 0.500658758 | 1.170432359 | Ten DAP | 17 | 20.2.1     | stress                                           |
| cassava4.1_019078m PA | AT1G07770 | 0.502929679 | 1.113574077 | Ten DAP | 5  | 29.2.2     | protein                                          |
| cassava4.1_018061m PA | AT3G03100 | 0.505095154 | 1.06059607  | Ten DAP | 4  | 9.1.2      | mitochondrial electron transport / ATP synthesis |
| cassava4.1_006562m PA | AT2G36070 | 0.505518313 | 1.146187141 | Ten DAP | 2  | 29.3.2     | protein                                          |
| cassava4.1_031909m PA | AT4G29680 | 0.505994384 | 1.430257047 | Ten DAP | 4  | 23.4.99    | nucleotide metabolism                            |
| cassava4.1_015850m PA | AT5G59240 | 0.507199783 | 1.298474887 | Ten DAP | 4  | 29.2.2     | protein                                          |
| cassava4.1_003366m PA | AT2G01190 | 0.507513896 | 1.163377566 | Ten DAP | 9  | 35.1       | not assigned                                     |
| cassava4.1_014674m PA | AT2G43090 | 0.508014116 | 1.167276749 | Ten DAP | 7  | 35.1.23    | not assigned                                     |
| cassava4.1_012143m PA | AT3G18140 | 0.508459282 | 1.118758176 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_016348m PA | AT3G49010 | 0.509441779 | 1.263391859 | Ten DAP | 5  | 29.2.2     | protein                                          |
| cassava4.1_004263m PA | AT2G28000 | 0.513291853 | 1.110031131 | Ten DAP | 35 | 1.3.013    | PS                                               |
| cassava4.1_004458m PA | AT3G23990 | 0.514433757 | 1.127685188 | Ten DAP | 39 | 20.2.1     | stress                                           |
| cassava4.1_004458m PA | AT3G23990 | 0.514433757 | 1.127685188 | Ten DAP | 39 | 29.6       | protein                                          |
| cassava4.1_018405m PA | AT3G52580 | 0.517862537 | 1.058747651 | Ten DAP | 4  | 29.2.2     | protein                                          |
| cassava4.1_030272m PA | AT3G52990 | 0.518210808 | 1.09196647  | Ten DAP | 9  | 4.013      | glycolysis                                       |
| cassava4.1_002794m PA | AT4G24490 | 0.518452523 | 1.11251776  | Ten DAP | 9  | 16.1.1     | secondary metabolism                             |
| cassava4.1_009589m PA | AT1G66430 | 0.51925422  | 1.059469231 | Ten DAP | 9  | 2.2.1.01   | major CHO metabolism                             |
| cassava4.1_011604m PA | AT2G37130 | 0.519766372 | 1.335961159 | Ten DAP | 2  | 26.12      | misc                                             |
| cassava4.1_006449m PA | AT1G71380 | 0.520970573 | 1.436725749 | Ten DAP | 5  | 26.03      | misc                                             |
| cassava4.1_006696m PA | AT3G25860 | 0.521457238 | 1.120844204 | Ten DAP | 10 | 11.1.031   | lipid metabolism                                 |
| cassava4.1_018343m PA | AT5G09500 | 0.522253993 | 1.187948698 | Ten DAP | 8  | 29.2.2     | protein                                          |
| cassava4.1_013318m PA | AT1G28290 | 0.522266663 | 1.291810548 | Ten DAP | 12 | 20.2.99    | stress                                           |
| cassava4.1_017005m PA | AT3G20390 | 0.522376374 | 1.119842778 | Ten DAP | 5  | 35.1       | not assigned                                     |
| cassava4.1_001348m PA | AT4G35830 | 0.523119751 | 1.026568624 | Ten DAP | 45 | 8.1.03     | TCA / org                                        |
| cassava4.1_003683m PA | AT1G80300 | 0.523492883 | 1.116025358 | Ten DAP | 15 | 34.99      | transport                                        |
| cassava4.1_016194m PA | AT5G14670 | 0.524466306 | 1.101509102 | Ten DAP | 13 | 29.3.4.99  | protein                                          |
| cassava4.1_023099m PA | AT1G65590 | 0.52534827  | 1.696367081 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_006289m PA | AT2G19520 | 0.527328259 | 1.095997435 | Ten DAP | 6  | 27.3.62    | RNA                                              |
| cassava4.1_006289m PA | AT2G19520 | 0.527328259 | 1.095997435 | Ten DAP | 6  | 33.99      | development                                      |
| cassava4.1_011869m PA | AT3G04880 | 0.527899594 | 1.114181408 | Ten DAP | 14 | 28.2       | DNA                                              |
| cassava4.1_018867m PA | AT3G54560 | 0.528031009 | 1.073188726 | Ten DAP | 2  | 28.1.3     | DNA                                              |
| cassava4.1_008283m PA | AT1G57720 | 0.529383663 | 1.150272781 | Ten DAP | 11 | 29.2.4     | protein                                          |
| cassava4.1_014694m PA | AT3G10260 | 0.5294189   | 1.099415488 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_004565m PA | AT1G60420 | 0.529510857 | 1.684251928 | Ten DAP | 11 | 35.1       | not assigned                                     |
| cassava4.1_014355m PA | AT1G04040 | 0.531292696 | 1.072624505 | Ten DAP | 13 | 26.13      | misc                                             |

|                        |           |             |             |         |    |           |                       |
|------------------------|-----------|-------------|-------------|---------|----|-----------|-----------------------|
| cassava4.1_016255m PA  | AT3G22845 | 0.531808878 | 1.170957721 | Ten DAP | 2  | 35.1      | not assigned          |
| cassava4.1_004007m PA  | AT2G37690 | 0.532207848 | 1.176638626 | Ten DAP | 6  | 23.1.2.06 | nucleotide metabolism |
| cassava4.1_015737m PA  | AT3G49470 | 0.532743982 | 1.155933557 | Ten DAP | 5  | 29.2.4    | protein               |
| cassava4.1_027096m PA  | AT4G13550 | 0.532751646 | 1.271682179 | Ten DAP | 2  | 11.9.2.01 | lipid metabolism      |
| cassava4.1_017636m PA  | AT4G39520 | 0.533581226 | 1.205737838 | Ten DAP | 2  | 30.5      | signalling            |
| cassava4.1_014293m PA  | AT1G78300 | 0.534086194 | 1.026279164 | Ten DAP | 7  | 30.7      | signalling            |
| cassava4.1_010360m PA  | AT3G62220 | 0.534788363 | 1.188410613 | Ten DAP | 10 | 29.4.1.58 | protein               |
| cassava4.1_006757m PA  | AT2G34250 | 0.535209745 | 1.057123938 | Ten DAP | 10 | 29.3.4.99 | protein               |
| cassava4.1_001124m PA  | AT1G50180 | 0.535469775 | 1.267189874 | Ten DAP | 3  | 20.1      | stress                |
| cassava4.1_018207m PA  | AT2G35120 | 0.537647262 | 1.054354253 | Ten DAP | 2  | 1.2.4     | PS                    |
| cassava4.1_009436m PA  | AT5G63140 | 0.537927411 | 1.28103021  | Ten DAP | 5  | 26.13     | misc                  |
| cassava4.1_000732m PA  | AT5G20280 | 0.538192248 | 1.146477123 | Ten DAP | 19 | 2.1.1.01  | major CHO metabolism  |
| cassava4.1_033375m PA  | AT4G10960 | 0.539648025 | 1.052751287 | Ten DAP | 4  | 10.1.02   | cell wall             |
| cassava4.1_002342m PA  | AT3G05900 | 0.539691525 | 1.065590285 | Ten DAP | 17 | 35.1      | not assigned          |
| gi 169794058 ref YP_OC | -         | 0.539983166 | 1.09196     | Ten DAP | 10 | -         | -                     |
| cassava4.1_007416m PA  | AT1G11580 | 0.544083584 | 1.078682374 | Ten DAP | 3  | 10.8.01   | cell wall             |
| cassava4.1_015116m PA  | AT1G47420 | 0.544853489 | 1.075271566 | Ten DAP | 6  | 35.2      | not assigned          |
| cassava4.1_012401m PA  | AT2G26230 | 0.545974996 | 1.211378306 | Ten DAP | 6  | 23.2      | nucleotide metabolism |
| cassava4.1_008840m PA  | AT3G59350 | 0.546106429 | 1.377057627 | Ten DAP | 2  | 29.4.1.58 | protein               |
| cassava4.1_016588m PA  | AT3G06050 | 0.54615609  | 1.098968316 | Ten DAP | 5  | 20.2.99   | stress                |
| cassava4.1_016588m PA  | AT3G06050 | 0.54615609  | 1.098968316 | Ten DAP | 5  | 21.05     | redox                 |
| cassava4.1_017910m PA  | AT1G09590 | 0.546600603 | 1.067518609 | Ten DAP | 6  | 29.2.2    | protein               |
| cassava4.1_002471m PA  | AT2G01690 | 0.547941233 | 1.194204238 | Ten DAP | 5  | 35.2      | not assigned          |
| cassava4.1_019568m PA  | AT3G44590 | 0.548114542 | 1.04398566  | Ten DAP | 4  | 29.2.2    | protein               |
| cassava4.1_015802m PA  | AT1G26910 | 0.54842191  | 1.086947303 | Ten DAP | 2  | 29.2.2    | protein               |
| cassava4.1_011801m PA  | AT5G55610 | 0.548734198 | 1.040017799 | Ten DAP | 6  | 35.2      | not assigned          |
| cassava4.1_011305m PA  | AT2G38380 | 0.548824354 | 1.564145403 | Ten DAP | 2  | 26.12     | misc                  |
| cassava4.1_016783m PA  | AT4G25740 | 0.549250852 | 1.039251793 | Ten DAP | 10 | 29.2.2    | protein               |
| cassava4.1_007920m PA  | AT5G02130 | 0.550414182 | 1.181701843 | Ten DAP | 4  | 31.1      | cell                  |
| cassava4.1_016516m PA  | AT2G29960 | 0.552446353 | 1.184173413 | Ten DAP | 7  | 31.3.01   | cell                  |
| cassava4.1_014688m PA  | AT5G10360 | 0.555233033 | 1.173377264 | Ten DAP | 8  | 29.2.2    | protein               |
| cassava4.1_006921m PA  | AT5G16620 | 0.556158835 | 1.255006252 | Ten DAP | 7  | 29.3.3    | protein               |
| cassava4.1_010684m PA  | AT1G10940 | 0.556203693 | 1.086250248 | Ten DAP | 14 | 29.4      | protein               |
| cassava4.1_010205m PA  | AT1G55510 | 0.556506397 | 1.373946586 | Ten DAP | 2  | 13.2.4.1  | amino acid metabolism |
| cassava4.1_010863m PA  | AT5G16990 | 0.556897972 | 1.202270878 | Ten DAP | 23 | 26.07     | misc                  |
| cassava4.1_013131m PA  | AT2G18730 | 0.557142138 | 1.089146477 | Ten DAP | 3  | 11.3.05   | lipid metabolism      |
| cassava4.1_013627m PA  | AT1G19190 | 0.557344039 | 1.081027977 | Ten DAP | 6  | 35.1      | not assigned          |
| cassava4.1_005709m PA  | AT4G34880 | 0.559039137 | 1.031182026 | Ten DAP | 2  | 26.08     | misc                  |
| cassava4.1_003883m PA  | AT3G13470 | 0.559587481 | 1.14786853  | Ten DAP | 11 | 29.6      | protein               |
| cassava4.1_008940m PA  | AT4G38630 | 0.560004554 | 1.130672749 | Ten DAP | 7  | 29.5.11   | protein               |
| cassava4.1_003479m PA  | AT1G50480 | 0.561101657 | 1.323934539 | Ten DAP | 18 | 25.02     | C1-metabolism         |
| cassava4.1_007933m PA  | AT1G09750 | 0.561305029 | 1.060069699 | Ten DAP | 9  | 27.3.99   | RNA                   |
| cassava4.1_018932m PA  | AT5G53560 | 0.56196856  | 1.060967965 | Ten DAP | 2  | 35.1      | not assigned          |
| cassava4.1_010581m PA  | AT5G37600 | 0.56272964  | 1.679825302 | Ten DAP | 2  | 12.2.02   | N-metabolism          |

|                       |           |              |             |         |    |              |                                                  |
|-----------------------|-----------|--------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_004187m PA | AT1G31800 | 0.562957666  | 1.311831506 | Ten DAP | 3  | 26.10        | misc                                             |
| cassava4.1_018294m PA | AT1G08830 | 0.563472541  | 1.062460578 | Ten DAP | 3  | 21.6         | redox                                            |
| cassava4.1_003582m PA | AT5G20950 | 0.563616773  | 1.171537803 | Ten DAP | 27 | 10.6.1       | cell wall                                        |
| cassava4.1_024093m PA | AT5G67400 | 0.563971862  | 1.274088986 | Ten DAP | 9  | 26.12        | misc                                             |
| cassava4.1_014978m PA | AT2G37020 | 0.564207573  | 1.507103585 | Ten DAP | 2  | 35.1         | not assigned                                     |
| cassava4.1_015349m PA | AT4G31300 | 0.564475473  | 1.054623614 | Ten DAP | 6  | 29.5.11.20   | protein                                          |
| cassava4.1_019588m PA | AT3G44590 | 0.56531707   | 1.038957218 | Ten DAP | 2  | 29.2.2       | protein                                          |
| cassava4.1_025763m PA | AT5G57190 | 0.565468245  | 1.232607547 | Ten DAP | 4  | 11.3.08      | lipid metabolism                                 |
| cassava4.1_027481m PA | AT4G37070 | 0.565817152  | 2.716364616 | Ten DAP | 2  | 33.1         | development                                      |
| cassava4.1_015271m PA | AT3G18580 | 0.567734826  | 1.123673018 | Ten DAP | 2  | 27.3.99      | RNA                                              |
| cassava4.1_019411m PA | AT2G32060 | 0.568378304  | 1.062211946 | Ten DAP | 3  | 29.2.2       | protein                                          |
| cassava4.1_018852m PA | AT5G10390 | 0.569833561  | 1.083380437 | Ten DAP | 6  | 28.1.3       | DNA                                              |
| cassava4.1_009188m PA | AT1G77420 | 0.570507807  | 1.106828687 | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_010438m PA | AT1G02305 | 0.571203439  | 1.07690079  | Ten DAP | 2  | 29.5.03      | protein                                          |
| cassava4.1_020258m PA | AT4G30010 | 0.571291679  | 1.15218838  | Ten DAP | 2  | 35.2         | not assigned                                     |
| cassava4.1_012602m PA | AT1G56450 | 0.571364928  | 1.110599928 | Ten DAP | 4  | 29.5.11.20   | protein                                          |
| cassava4.1_006924m PA | AT4G13930 | 0.571584774  | 1.059521237 | Ten DAP | 24 | 1.2.05       | PS                                               |
| cassava4.1_006924m PA | AT4G13930 | 0.571584774  | 1.059521237 | Ten DAP | 24 | 13.1.5.2.01  | amino acid metabolism                            |
| cassava4.1_020027m PA | AT5G47890 | 0.571780791  | 1.073611558 | Ten DAP | 2  | 25.01        | C1-metabolism                                    |
| cassava4.1_002358m PA | AT5G60600 | 0.571879673  | 1.100088181 | Ten DAP | 17 | 9.1.2        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_002667m PA | AT1G65540 | 0.573164601  | 1.107513997 | Ten DAP | 8  | 16.1.1.06    | secondary metabolism                             |
| cassava4.1_007932m PA | AT1G48850 | 0.573311733  | 1.282174963 | Ten DAP | 6  | 30.3         | signalling                                       |
| cassava4.1_018900m PA | AT4G15000 | 0.57348261   | 1.07746629  | Ten DAP | 5  | 13.1.6.1.07  | amino acid metabolism                            |
| cassava4.1_011662m PA | AT2G18980 | 0.574596435  | 1.285041988 | Ten DAP | 8  | 29.2.2       | protein                                          |
| cassava4.1_027198m PA | AT4G35630 | 0.574664541  | 1.120835261 | Ten DAP | 8  | 26.12        | misc                                             |
| cassava4.1_018529m PA | AT1G08480 | 0.5745427155 | 1.064054573 | Ten DAP | 2  | 13.1.5.1.02  | amino acid metabolism                            |
| cassava4.1_016492m PA | AT1G08480 | 0.575427155  | 1.064054573 | Ten DAP | 3  | 35.2         | not assigned                                     |
| cassava4.1_016492m PA | AT4G27270 | 0.575536365  | 1.075283543 | Ten DAP | 9  | 11.8         | lipid metabolism                                 |
| cassava4.1_013216m PA | AT5G10730 | 0.575827882  | 1.225152215 | Ten DAP | 3  | 35.2         | not assigned                                     |
| cassava4.1_000010m PA | AT2G45540 | 0.576367307  | 1.496321716 | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_000080m PA | AT4G38600 | 0.576762684  | 1.10038342  | Ten DAP | 4  | 29.5.11.4.01 | protein                                          |
| cassava4.1_008552m PA | AT5G64860 | 0.576997277  | 1.064269048 | Ten DAP | 8  | 2.2.2.04     | major CHO metabolism                             |
| cassava4.1_015411m PA | AT5G10160 | 0.577437775  | 1.056171151 | Ten DAP | 5  | 11.1.05      | lipid metabolism                                 |
| cassava4.1_026808m PA | AT2G16950 | 0.578046806  | 1.142165959 | Ten DAP | 3  | 29.3.1       | protein                                          |
| cassava4.1_019309m PA | AT1G27970 | 0.580342759  | 1.057862777 | Ten DAP | 3  | 29.3.1       | protein                                          |
| cassava4.1_014419m PA | AT5G58270 | 0.580875052  | 1.572590149 | Ten DAP | 2  | 34.16        | transport                                        |
| cassava4.1_018106m PA | AT1G24020 | 0.581038844  | 1.029769467 | Ten DAP | 6  | 20.2.99      | stress                                           |
| cassava4.1_008465m PA | AT5G17990 | 0.581630658  | 1.165360493 | Ten DAP | 7  | 13.1.6.5.02  | amino acid metabolism                            |
| cassava4.1_008164m PA | AT3G16850 | 0.583155636  | 1.523084953 | Ten DAP | 4  | 10.6.3       | cell wall                                        |
| cassava4.1_015382m PA | AT1G08110 | 0.584282967  | 1.042051748 | Ten DAP | 3  | 13.2.3.2     | amino acid metabolism                            |
| cassava4.1_015382m PA | AT1G08110 | 0.584282967  | 1.042051748 | Ten DAP | 3  | 24.02        | Biodegradation of Xenobiotics                    |
| cassava4.1_015768m PA | AT1G26910 | 0.584284251  | 1.047135728 | Ten DAP | 7  | 29.2.2       | protein                                          |
| cassava4.1_016920m PA | AT1G48830 | 0.584445408  | 1.001783869 | Ten DAP | 2  | 29.2.2       | protein                                          |
| cassava4.1_019062m PA | AT5G08060 | 0.584770845  | 1.027616892 | Ten DAP | 4  | 35.1         | not assigned                                     |

|                       |           |             |             |         |    |                |                       |
|-----------------------|-----------|-------------|-------------|---------|----|----------------|-----------------------|
| cassava4.1_008511m PA | AT5G26670 | 0.585349932 | 1.270047801 | Ten DAP | 2  | 10.8.02        | cell wall             |
| cassava4.1_018671m PA | AT5G02960 | 0.586046196 | 1.076056284 | Ten DAP | 4  | 29.2.2         | protein               |
| cassava4.1_006370m PA | AT1G60770 | 0.587881083 | 1.112535174 | Ten DAP | 3  | 27.3.67        | RNA                   |
| cassava4.1_005106m PA | AT3G63130 | 0.588628072 | 1.108226575 | Ten DAP | 6  | 30.5           | signalling            |
| cassava4.1_016159m PA | AT4G17170 | 0.589386993 | 1.007124654 | Ten DAP | 8  | 30.5           | signalling            |
| cassava4.1_028407m PA | AT5G19440 | 0.589748288 | 1.458511638 | Ten DAP | 3  | 26.11          | misc                  |
| cassava4.1_002839m PA | AT1G29880 | 0.59009623  | 1.122019069 | Ten DAP | 9  | 29.1.014       | protein               |
| cassava4.1_029035m PA | AT3G60140 | 0.590129566 | 1.139165316 | Ten DAP | 3  | 26.03          | misc                  |
| cassava4.1_003805m PA | AT3G23940 | 0.590182853 | 1.124613494 | Ten DAP | 18 | 35.1           | not assigned          |
| cassava4.1_015351m PA | AT1G35780 | 0.591006582 | 1.202983448 | Ten DAP | 4  | 35.2           | not assigned          |
| cassava4.1_004040m PA | AT4G18810 | 0.593293724 | 1.246732298 | Ten DAP | 4  | 20.2.99        | stress                |
| cassava4.1_004040m PA | AT4G18810 | 0.593293724 | 1.246732298 | Ten DAP | 4  | 30.11          | signalling            |
| cassava4.1_010214m PA | AT1G08370 | 0.593935467 | 1.288688154 | Ten DAP | 2  | 27.3.67        | RNA                   |
| cassava4.1_011275m PA | AT3G17940 | 0.59410198  | 1.219360615 | Ten DAP | 3  | 3.5            | minor CHO metabolism  |
| cassava4.1_016772m PA | AT5G11900 | 0.595225705 | 1.165327236 | Ten DAP | 2  | 29.2.3         | protein               |
| cassava4.1_014206m PA | AT4G36130 | 0.595888819 | 1.052623838 | Ten DAP | 11 | 29.2.2         | protein               |
| cassava4.1_015085m PA | AT4G11600 | 0.596162159 | 1.056729731 | Ten DAP | 2  | 21.2.2         | redox                 |
| cassava4.1_004703m PA | AT2G01600 | 0.597183512 | 1.002074675 | Ten DAP | 4  | 35.1.21        | not assigned          |
| cassava4.1_005690m PA | AT5G40760 | 0.598006009 | 1.06405769  | Ten DAP | 23 | 7.1.01         | OPP                   |
| cassava4.1_002157m PA | AT1G20160 | 0.598207597 | 1.056274832 | Ten DAP | 8  | 29.5.01        | protein               |
| cassava4.1_014112m PA | AT4G34670 | 0.598449025 | 1.078970949 | Ten DAP | 11 | 29.2.2         | protein               |
| cassava4.1_007709m PA | AT5G11520 | 0.598795759 | 1.281891986 | Ten DAP | 4  | 13.1.1.2.01    | amino acid metabolism |
| cassava4.1_003628m PA | AT1G26110 | 0.598862672 | 1.188537748 | Ten DAP | 2  | 35.2           | not assigned          |
| cassava4.1_002461m PA | AT5G49570 | 0.601004412 | 1.105524388 | Ten DAP | 5  | 35.1           | not assigned          |
| cassava4.1_007212m PA | AT5G19485 | 0.604486282 | 1.091080344 | Ten DAP | 2  |                |                       |
| cassava4.1_015872m PA | AT1G78380 | 0.605788204 | 1.04706008  | Ten DAP | 8  | 26.09          | misc                  |
| cassava4.1_019888m PA | AT1G07660 | 0.607008927 | 1.022377948 | Ten DAP | 11 | 28.1.3         | DNA                   |
| cassava4.1_019888m PA | AT1G07660 | 0.607008927 | 1.022377948 | Ten DAP | 11 | 31.1           | cell                  |
| cassava4.1_012374m PA | AT4G14930 | 0.607057098 | 1.0332793   | Ten DAP | 2  | 26.13          | misc                  |
| cassava4.1_027765m PA | AT5G23400 | 0.60758125  | 1.069685075 | Ten DAP | 17 | 20.1           | stress                |
| cassava4.1_008996m PA | AT2G19940 | 0.608752056 | 1.261053989 | Ten DAP | 9  | 35.1           | not assigned          |
| cassava4.1_034378m PA | AT5G17380 | 0.609688827 | 1.1944795   | Ten DAP | 13 | 5.02           | fermentation          |
| cassava4.1_000217m PA | AT5G41790 | 0.610125936 | 1.105367267 | Ten DAP | 11 | 30.11.1        | signalling            |
| cassava4.1_000217m PA | AT5G41790 | 0.610125936 | 1.105367267 | Ten DAP | 11 | 33.99          | development           |
| cassava4.1_000938m PA | AT4G16130 | 0.610966457 | 1.244906174 | Ten DAP | 7  | 3.8.01         | minor CHO metabolism  |
| cassava4.1_005091m PA | AT5G28900 | 0.611087647 | 1.159782591 | Ten DAP | 4  | 29.4           | protein               |
| cassava4.1_015998m PA | AT1G09630 | 0.613891179 | 1.017328005 | Ten DAP | 3  | 30.5           | signalling            |
| cassava4.1_018051m PA | AT2G42210 | 0.616600768 | 1.024928559 | Ten DAP | 3  | 29.3.2         | protein               |
| cassava4.1_011276m PA | AT1G74030 | 0.616694214 | 1.055071282 | Ten DAP | 6  | 4.012          | glycolysis            |
| cassava4.1_010379m PA | AT4G31860 | 0.617460458 | 1.381685734 | Ten DAP | 2  | 29.4           | protein               |
| cassava4.1_001254m PA | AT4G19710 | 0.617841482 | 1.056085498 | Ten DAP | 34 | 13.1.3.6.1.010 | amino acid metabolism |
| cassava4.1_002669m PA | AT5G19740 | 0.617970946 | 1.049690324 | Ten DAP | 3  | 29.5           | protein               |
| cassava4.1_015045m PA | AT4G12060 | 0.619574179 | 1.048190392 | Ten DAP | 2  | 29.3.99        | protein               |
| cassava4.1_011026m PA | AT1G53240 | 0.619622671 | 1.176113478 | Ten DAP | 8  | 8.1.09         | TCA / org             |

|                       |           |             |             |         |    |             |                               |
|-----------------------|-----------|-------------|-------------|---------|----|-------------|-------------------------------|
| cassava4.1_004987m PA | AT4G01690 | 0.621702214 | 1.100534904 | Ten DAP | 4  | 19.09       | tetrapyrrole synthesis        |
| cassava4.1_012198m PA | AT4G02340 | 0.622191278 | 1.17939896  | Ten DAP | 4  | 26.01       | misc                          |
| cassava4.1_002897m PA | AT2G38040 | 0.622240148 | 1.100220755 | Ten DAP | 15 | 16.99       | secondary metabolism          |
| cassava4.1_025071m PA | AT1G72250 | 0.622585743 | 1.131509457 | Ten DAP | 3  | 31.1        | cell                          |
| cassava4.1_003967m PA | AT4G00570 | 0.622612636 | 1.104674933 | Ten DAP | 24 | 8.2.10      | TCA / org                     |
| cassava4.1_004839m PA | AT3G13930 | 0.622759215 | 1.067178615 | Ten DAP | 13 | 8.1.01.02   | TCA / org                     |
| cassava4.1_015599m PA | AT5G65270 | 0.622792875 | 1.082843716 | Ten DAP | 4  | 30.5        | signalling                    |
| cassava4.1_017409m PA | AT2G34480 | 0.624379554 | 1.038157144 | Ten DAP | 7  | 29.2.2      | protein                       |
| cassava4.1_008930m PA | AT2G45240 | 0.62481308  | 1.170799017 | Ten DAP | 2  | 29.5.07     | protein                       |
| cassava4.1_019152m PA | AT1G29850 | 0.625318346 | 1.013673248 | Ten DAP | 2  | 35.1        | not assigned                  |
| cassava4.1_011417m PA | AT2G45630 | 0.629936881 | 1.009777145 | Ten DAP | 2  | 1.2.06      | PS                            |
| cassava4.1_011417m PA | AT2G45630 | 0.629936881 | 1.009777145 | Ten DAP | 2  | 13.2.5.1    | amino acid metabolism         |
| cassava4.1_011417m PA | AT2G45630 | 0.629936881 | 1.009777145 | Ten DAP | 2  | 26.01       | misc                          |
| cassava4.1_016323m PA | AT2G25950 | 0.631820171 | 1.127040409 | Ten DAP | 3  | 35.2        | not assigned                  |
| cassava4.1_014208m PA | AT1G78300 | 0.632481482 | 1.132571816 | Ten DAP | 3  | 30.7        | signalling                    |
| cassava4.1_004070m PA | AT4G12420 | 0.633176832 | 1.38681867  | Ten DAP | 12 | 35.1        | not assigned                  |
| cassava4.1_013074m PA | AT1G53580 | 0.634915586 | 1.145006654 | Ten DAP | 10 | 13.2.3.2    | amino acid metabolism         |
| cassava4.1_013074m PA | AT1G53580 | 0.634915586 | 1.145006654 | Ten DAP | 10 | 24.01       | Biodegradation of Xenobiotics |
| cassava4.1_004610m PA | AT4G11740 | 0.63566873  | 1.05176332  | Ten DAP | 2  | 31.4        | cell                          |
| cassava4.1_027071m PA | AT3G06580 | 0.63653083  | 1.046894678 | Ten DAP | 7  | 3.8.01      | minor CHO metabolism          |
| cassava4.1_001874m PA | AT1G73370 | 0.639252054 | 1.103336919 | Ten DAP | 7  | 2.2.1.05    | major CHO metabolism          |
| cassava4.1_011180m PA | AT5G47540 | 0.640474524 | 1.243683804 | Ten DAP | 2  | 35.1        | not assigned                  |
| cassava4.1_031676m PA | AT3G23790 | 0.642536998 | 1.396436246 | Ten DAP | 2  | 11.1.08     | lipid metabolism              |
| cassava4.1_011868m PA | AT5G24400 | 0.643063887 | 1.161632061 | Ten DAP | 3  | 7.1.02      | OPP                           |
| cassava4.1_003892m PA | AT2G35040 | 0.64341346  | 1.218546227 | Ten DAP | 9  | 23.1.2.09   | nucleotide metabolism         |
| cassava4.1_026278m PA | AT4G33865 | 0.645330482 | 1.237314951 | Ten DAP | 2  | 29.2.2      | protein                       |
| cassava4.1_009840m PA | AT1G12840 | 0.648186722 | 1.060618846 | Ten DAP | 16 | 34.1        | transport                     |
| cassava4.1_009997m PA | AT3G53580 | 0.649059036 | 1.063680667 | Ten DAP | 4  | 13.1.3.5.04 | amino acid metabolism         |
| cassava4.1_008286m PA | AT3G18270 | 0.652232231 | 1.094959889 | Ten DAP | 4  | 26.01       | misc                          |
| cassava4.1_016433m PA | AT2G33470 | 0.652272858 | 1.020290791 | Ten DAP | 5  | 35.1        | not assigned                  |
| cassava4.1_016843m PA | AT4G02080 | 0.653685041 | 1.017726857 | Ten DAP | 9  | 30.5        | signalling                    |
| cassava4.1_013773m PA | AT5G16710 | 0.655846774 | 1.979349927 | Ten DAP | 2  | 21.2.1      | redox                         |
| cassava4.1_004706m PA | AT5G48570 | 0.656207588 | 1.07177473  | Ten DAP | 17 | 31.3.01     | cell                          |
| cassava4.1_003677m PA | AT1G78900 | 0.657889152 | 1.152417359 | Ten DAP | 41 | 34.1        | transport                     |
| cassava4.1_026035m PA | AT1G77120 | 0.660050818 | 1.034810387 | Ten DAP | 24 | 5.03        | fermentation                  |
| cassava4.1_013706m PA | AT5G37720 | 0.660484374 | 1.231686917 | Ten DAP | 2  | 27.4        | RNA                           |
| cassava4.1_017662m PA | AT4G38740 | 0.660927824 | 1.055041869 | Ten DAP | 10 | 31.3.01     | cell                          |
| cassava4.1_019244m PA | AT1G11530 | 0.661276429 | 1.013187652 | Ten DAP | 2  | 21.01       | redox                         |
| cassava4.1_009951m PA | AT4G35260 | 0.661935377 | 1.020030079 | Ten DAP | 7  | 8.1.04      | TCA / org                     |
| cassava4.1_033800m PA | AT5G55200 | 0.661952208 | 1.01471254  | Ten DAP | 3  | 29.6        | protein                       |
| cassava4.1_029910m PA | AT5G54140 | 0.662041406 | 1.536798143 | Ten DAP | 2  | 17.2.1      | hormone metabolism            |
| cassava4.1_000857m PA | AT4G37640 | 0.664040619 | 1.059518029 | Ten DAP | 2  | 30.3        | signalling                    |
| cassava4.1_014326m PA | AT2G42590 | 0.66500669  | 1.157075617 | Ten DAP | 3  | 30.7        | signalling                    |
| cassava4.1_008935m PA | AT4G17890 | 0.665044616 | 1.170525072 | Ten DAP | 6  | 29.5.11.05  | protein                       |

|                       |           |             |             |         |    |            |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|------------|--------------------------------------------------|
| cassava4.1_006479m PA | AT2G42810 | 0.666430779 | 1.048477206 | Ten DAP | 5  | 29.4       | protein                                          |
| cassava4.1_006851m PA | AT4G28300 | 0.666949426 | 1.034092869 | Ten DAP | 3  | 10.5.4     | cell wall                                        |
| cassava4.1_014135m PA | AT5G27470 | 0.667049298 | 1.145221742 | Ten DAP | 8  | 29.1.011   | protein                                          |
| cassava4.1_010846m PA | AT2G17390 | 0.667141871 | 1.268329945 | Ten DAP | 4  | 27.3.39    | RNA                                              |
| cassava4.1_021211m PA | AT5G58090 | 0.667241901 | 1.369876376 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_013465m PA | AT4G17720 | 0.668246942 | 1.092446793 | Ten DAP | 17 | 27.4       | RNA                                              |
| cassava4.1_004945m PA | AT5G15270 | 0.669871718 | 1.039080292 | Ten DAP | 7  | 35.1       | not assigned                                     |
| cassava4.1_005721m PA | AT5G40760 | 0.670259761 | 1.080528375 | Ten DAP | 2  | 7.1.01     | OPP                                              |
| cassava4.1_012522m PA | AT3G60100 | 0.671786518 | 1.371513252 | Ten DAP | 6  | 8.1.02     | TCA / org                                        |
| cassava4.1_009035m PA | AT3G02540 | 0.673321617 | 1.114769183 | Ten DAP | 4  | 29.5.11.01 | protein                                          |
| cassava4.1_021385m PA | AT5G19460 | 0.673348388 | 1.168931077 | Ten DAP | 3  | 35.1       | not assigned                                     |
| cassava4.1_003622m PA | AT3G11710 | 0.675096237 | 1.033820519 | Ten DAP | 4  | 29.1.06    | protein                                          |
| cassava4.1_014251m PA | AT1G78300 | 0.675476391 | 1.031398558 | Ten DAP | 25 | 30.7       | signalling                                       |
| cassava4.1_008885m PA | AT4G21800 | 0.677741544 | 1.292223765 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_001934m PA | AT5G57870 | 0.678729731 | 1.044626938 | Ten DAP | 20 | 29.2.3     | protein                                          |
| cassava4.1_003276m PA | AT5G27540 | 0.679037421 | 1.124138565 | Ten DAP | 4  | 30.5       | signalling                                       |
| cassava4.1_018514m PA | AT1G17160 | 0.680104111 | 1.003602549 | Ten DAP | 5  | 3.5        | minor CHO metabolism                             |
| cassava4.1_001527m PA | AT4G35790 | 0.681162773 | 1.106076759 | Ten DAP | 13 | 11.9.3.01  | lipid metabolism                                 |
| cassava4.1_016808m PA | AT1G33140 | 0.683545813 | 1.063991834 | Ten DAP | 4  | 29.2.2     | protein                                          |
| cassava4.1_005052m PA | AT4G05160 | 0.683569249 | 1.029351558 | Ten DAP | 2  | 16.2       | secondary metabolism                             |
| cassava4.1_021316m PA | AT2G28100 | 0.683688942 | 1.11319261  | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_001960m PA | AT5G10560 | 0.684154846 | 1.050722269 | Ten DAP | 6  | 10.6.2     | cell wall                                        |
| cassava4.1_004114m PA | AT3G58610 | 0.686477132 | 1.039618343 | Ten DAP | 8  | 13.1.4.1   | amino acid metabolism                            |
| cassava4.1_011830m PA | AT3G03890 | 0.688944169 | 1.101065817 | Ten DAP | 5  | 35.2       | not assigned                                     |
| cassava4.1_005830m PA | AT3G63130 | 0.689146628 | 1.274707635 | Ten DAP | 11 | 30.5       | signalling                                       |
| cassava4.1_010437m PA | AT4G09670 | 0.689503617 | 1.041242731 | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_014473m PA | AT4G02580 | 0.689783825 | 1.05618186  | Ten DAP | 6  | 9.1.2      | mitochondrial electron transport / ATP synthesis |
| cassava4.1_003096m PA | AT4G26300 | 0.690013085 | 1.052826245 | Ten DAP | 15 | 29.1.019   | protein                                          |
| cassava4.1_003396m PA | AT1G49760 | 0.691409967 | 1.125804578 | Ten DAP | 16 | 27.1       | RNA                                              |
| cassava4.1_019698m PA | AT5G02450 | 0.692625627 | 1.035769806 | Ten DAP | 2  | 29.2.2     | protein                                          |
| cassava4.1_019638m PA | AT1G74270 | 0.693271275 | 1.41033239  | Ten DAP | 2  | 29.2.2     | protein                                          |
| cassava4.1_025886m PA | AT3G01510 | 0.694236214 | 1.206461149 | Ten DAP | 2  | 26.13      | misc                                             |
| cassava4.1_008473m PA | AT2G42710 | 0.696230589 | 1.452579598 | Ten DAP | 3  | 29.2.2     | protein                                          |
| cassava4.1_008987m PA | AT4G21800 | 0.699841424 | 1.08735633  | Ten DAP | 2  | 35.1       | not assigned                                     |
| cassava4.1_011190m PA | AT1G23820 | 0.700481635 | 1.030214518 | Ten DAP | 4  | 22.1.06    | polyamine metabolism                             |
| cassava4.1_011157m PA | AT3G63410 | 0.701037404 | 1.150436199 | Ten DAP | 6  | 16.1.3.03  | secondary metabolism                             |
| cassava4.1_003773m PA | AT5G03650 | 0.702243606 | 1.111449633 | Ten DAP | 23 | 2.1.2.03   | major CHO metabolism                             |
| cassava4.1_009441m PA | AT3G53110 | 0.704311716 | 1.137201185 | Ten DAP | 9  | 28.1       | DNA                                              |
| cassava4.1_012454m PA | AT2G32080 | 0.70931135  | 1.055623605 | Ten DAP | 5  | 27.3.99    | RNA                                              |
| cassava4.1_013184m PA | AT4G35000 | 0.709731245 | 1.449770746 | Ten DAP | 2  | 21.2.1     | redox                                            |
| cassava4.1_004237m PA | AT4G36690 | 0.710485928 | 1.070142892 | Ten DAP | 4  | 27.1.1     | RNA                                              |
| cassava4.1_013254m PA | AT5G04740 | 0.710783603 | 1.07949141  | Ten DAP | 12 | 13         | amino acid metabolism                            |
| cassava4.1_009220m PA | AT2G05990 | 0.710899885 | 1.088983058 | Ten DAP | 6  | 11.1.06    | lipid metabolism                                 |
| cassava4.1_010187m PA | AT5G54160 | 0.71374806  | 1.04535163  | Ten DAP | 17 | 16.2.1.09  | secondary metabolism                             |

|                       |           |             |             |         |    |            |                        |
|-----------------------|-----------|-------------|-------------|---------|----|------------|------------------------|
| cassava4.1_009378m PA | AT2G40490 | 0.714663656 | 1.223966684 | Ten DAP | 7  | 19.07      | tetrapyrrole synthesis |
| cassava4.1_016942m PA | AT1G54860 | 0.715009406 | 1.349561399 | Ten DAP | 2  | 35.2       | not assigned           |
| cassava4.1_004233m PA | AT2G15620 | 0.715678328 | 1.047246806 | Ten DAP | 6  | 12.1.02    | N-metabolism           |
| cassava4.1_008307m PA | AT1G09430 | 0.71629274  | 1.032393284 | Ten DAP | 7  | 8.2.011    | TCA / org              |
| cassava4.1_003343m PA | AT3G12580 | 0.718780712 | 1.23062273  | Ten DAP | 7  | 20.2.1     | stress                 |
| cassava4.1_029022m PA | AT5G40480 | 0.719286128 | 1.06018545  | Ten DAP | 3  | 29.3.1     | protein                |
| cassava4.1_010020m PA | AT4G36810 | 0.719964229 | 1.319289776 | Ten DAP | 4  | 16.1.1.010 | secondary metabolism   |
| cassava4.1_003924m PA | AT3G10050 | 0.720126798 | 1.034605981 | Ten DAP | 4  | 13.2.5.1   | amino acid metabolism  |
| cassava4.1_009911m PA | AT2G30200 | 0.720256216 | 1.073731739 | Ten DAP | 7  | 11.1.02    | lipid metabolism       |
| cassava4.1_006414m PA | AT4G24620 | 0.721798105 | 1.052986579 | Ten DAP | 12 | 4.03       | glycolysis             |
| cassava4.1_012601m PA | AT2G43180 | 0.722988186 | 1.123192152 | Ten DAP | 11 | 35.2       | not assigned           |
| cassava4.1_011791m PA | AT4G08790 | 0.725003905 | 1.891511104 | Ten DAP | 2  | 26.08      | misc                   |
| cassava4.1_017700m PA | AT4G40030 | 0.725261993 | 1.008604129 | Ten DAP | 2  | 28.1.3     | DNA                    |
| cassava4.1_006309m PA | AT3G60140 | 0.725795856 | 1.189548767 | Ten DAP | 8  | 26.03      | misc                   |
| cassava4.1_006586m PA | AT4G14040 | 0.726970276 | 1.056119743 | Ten DAP | 10 | 15         | metal handling         |
| cassava4.1_028848m PA | AT3G04710 | 0.728199727 | 1.302642038 | Ten DAP | 2  | 31.1       | cell                   |
| cassava4.1_006494m PA | AT1G54730 | 0.728331172 | 1.053543706 | Ten DAP | 2  | 34.2       | transporter            |
| cassava4.1_004384m PA | AT5G21105 | 0.728643033 | 1.080768401 | Ten DAP | 2  | 21.2.1     | redox                  |
| cassava4.1_003907m PA | AT3G13470 | 0.729131131 | 1.035227991 | Ten DAP | 41 | 29.6       | protein                |
| cassava4.1_003519m PA | AT5G66760 | 0.731786795 | 1.20097796  | Ten DAP | 14 | 8.1.07     | TCA / org              |
| cassava4.1_007587m PA | AT3G11330 | 0.732179747 | 1.052466036 | Ten DAP | 2  | 35.1       | not assigned           |
| cassava4.1_026522m PA | AT1G32780 | 0.735203468 | 1.00997587  | Ten DAP | 3  | 26.11.01   | misc                   |
| cassava4.1_007669m PA | AT4G35850 | 0.735924118 | 1.049605743 | Ten DAP | 6  | 35.1.5     | not assigned           |
| cassava4.1_003757m PA | AT1G20950 | 0.737585428 | 1.070408139 | Ten DAP | 23 | 4.05       | glycolysis             |
| cassava4.1_010708m PA | AT4G35160 | 0.73950961  | 1.119199147 | Ten DAP | 3  | 16.2       | secondary metabolism   |
| cassava4.1_012894m PA | AT5G65940 | 0.740187789 | 1.006619062 | Ten DAP | 2  | 11.9.4.05  | lipid metabolism       |
| cassava4.1_012894m PA | AT5G65940 | 0.740187789 | 1.006619062 | Ten DAP | 2  | 13.2.3.5   | amino acid metabolism  |
| cassava4.1_006988m PA | AT2G44640 | 0.740348701 | 1.130252778 | Ten DAP | 2  | 35.2       | not assigned           |
| cassava4.1_011781m PA | AT3G02280 | 0.741603652 | 1.065295716 | Ten DAP | 2  | 19.99      | tetrapyrrole synthesis |
| cassava4.1_014448m PA | AT3G18165 | 0.744336861 | 1.168315852 | Ten DAP | 2  | 35.1       | not assigned           |
| cassava4.1_021498m PA | AT1G60500 | 0.745810998 | 1.001030325 | Ten DAP | 3  | 26.17      | misc                   |
| cassava4.1_011472m PA | AT4G24770 | 0.747382867 | 1.145137628 | Ten DAP | 4  | 27.3.99    | RNA                    |
| cassava4.1_013717m PA | AT3G01280 | 0.755270655 | 1.161847292 | Ten DAP | 15 | 34.20      | transport              |
| cassava4.1_010208m PA | AT3G08030 | 0.756672953 | 1.150945156 | Ten DAP | 16 | 35.2       | not assigned           |
| cassava4.1_021342m PA | AT3G45140 | 0.758675264 | 1.283327948 | Ten DAP | 7  | 17.7.1.02  | hormone metabolism     |
| cassava4.1_000822m PA | AT5G65750 | 0.759404502 | 1.038313945 | Ten DAP | 45 | 8.1.05     | TCA / org              |
| cassava4.1_002448m PA | AT1G76140 | 0.759855998 | 1.016477165 | Ten DAP | 5  | 29.5       | protein                |
| cassava4.1_003150m PA | AT1G67680 | 0.760610733 | 1.061629036 | Ten DAP | 8  | 29.3.4.99  | protein                |
| cassava4.1_009703m PA | AT5G61510 | 0.76126965  | 1.247551492 | Ten DAP | 8  | 26.07      | misc                   |
| cassava4.1_008058m PA | AT2G27600 | 0.761969821 | 1.127459236 | Ten DAP | 10 | 29.3.99    | protein                |
| cassava4.1_004981m PA | AT1G76160 | 0.762168161 | 1.174124621 | Ten DAP | 15 | 35.1       | not assigned           |
| cassava4.1_004773m PA | AT2G17290 | 0.762359189 | 1.046979102 | Ten DAP | 2  | 30.3       | signalling             |
| cassava4.1_004332m PA | AT1G70730 | 0.762414575 | 1.124431419 | Ten DAP | 29 | 4.02       | glycolysis             |
| cassava4.1_024672m PA | AT5G04360 | 0.763370856 | 1.345789754 | Ten DAP | 2  | 2.1.2.04   | major CHO metabolism   |



|                       |           |             |             |         |    |               |                                                  |
|-----------------------|-----------|-------------|-------------|---------|----|---------------|--------------------------------------------------|
| cassava4.1_004491m PA | AT4G24040 | 0.764361068 | 1.020367089 | Ten DAP | 8  | 3.2.04        | minor CHO metabolism                             |
| cassava4.1_031207m PA | AT5G57590 | 0.76596927  | 1.067409429 | Ten DAP | 3  | 16.99         | secondary metabolism                             |
| cassava4.1_003145m PA | AT4G16760 | 0.768968092 | 1.026901389 | Ten DAP | 6  | 11.9.4.02     | lipid metabolism                                 |
| cassava4.1_005231m PA | AT4G31180 | 0.770425815 | 1.085526277 | Ten DAP | 22 | 29.1.012      | protein                                          |
| cassava4.1_001932m PA | AT2G39930 | 0.772568075 | 1.157479924 | Ten DAP | 5  | 2.1.2.04      | major CHO metabolism                             |
| cassava4.1_012219m PA | AT5G58270 | 0.772754281 | 1.012848187 | Ten DAP | 2  | 34.16         | transport                                        |
| cassava4.1_003971m PA | AT3G47930 | 0.773845251 | 1.049720694 | Ten DAP | 16 | 21.2.1.02     | redox                                            |
| cassava4.1_010572m PA | AT5G03610 | 0.775687077 | 1.106844388 | Ten DAP | 2  | 26.28         | misc                                             |
| cassava4.1_000437m PA | AT2G02560 | 0.776438978 | 1.026963099 | Ten DAP | 15 | 35.1          | not assigned                                     |
| cassava4.1_002419m PA | AT4G35300 | 0.784652062 | 1.166354201 | Ten DAP | 5  | 34.2          | transporter                                      |
| cassava4.1_007565m PA | AT1G07930 | 0.785594101 | 1.220154584 | Ten DAP | 2  | 29.2.4        | protein                                          |
| cassava4.1_013290m PA | AT5G62740 | 0.78645352  | 1.008623722 | Ten DAP | 12 | 35.1          | not assigned                                     |
| cassava4.1_013998m PA | AT2G29570 | 0.786732869 | 1.080619622 | Ten DAP | 16 | 27.3.67       | RNA                                              |
| cassava4.1_005265m PA | AT1G72330 | 0.790084699 | 1.037164715 | Ten DAP | 10 | 13.1.1.3.01   | amino acid metabolism                            |
| cassava4.1_015314m PA | AT2G39020 | 0.792724826 | 1.165209435 | Ten DAP | 3  | 26.24         | misc                                             |
| cassava4.1_021650m PA | AT1G60710 | 0.794606886 | 1.107537218 | Ten DAP | 5  | 17.2.3        | hormone metabolism                               |
| cassava4.1_007909m PA | AT5G66680 | 0.797104263 | 1.149915617 | Ten DAP | 12 | 26.01         | misc                                             |
| cassava4.1_004726m PA | AT5G08680 | 0.798271833 | 1.013961356 | Ten DAP | 36 | 9.09          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_002837m PA | AT1G06950 | 0.798585257 | 1.050838527 | Ten DAP | 12 | 29.3.3        | protein                                          |
| cassava4.1_022495m PA | AT5G20990 | 0.79915816  | 1.067243431 | Ten DAP | 6  | 18.1.01       | Co-factor and vitamine metabolism                |
| cassava4.1_000833m PA | AT4G11610 | 0.799790169 | 1.079107573 | Ten DAP | 4  | 13.1.6.5      | amino acid metabolism                            |
| cassava4.1_000233m PA | AT3G13300 | 0.799960479 | 1.254465012 | Ten DAP | 6  | 35.1          | not assigned                                     |
| cassava4.1_033533m PA | AT3G07160 | 0.800135142 | 1.064935529 | Ten DAP | 3  | 3.6           | minor CHO metabolism                             |
| cassava4.1_002079m PA | AT3G23980 | 0.800613098 | 1.45454257  | Ten DAP | 2  | 35.1          | not assigned                                     |
| cassava4.1_011200m PA | AT1G10840 | 0.80114766  | 1.00980798  | Ten DAP | 8  | 29.2.3        | protein                                          |
| cassava4.1_013194m PA | AT3G10690 | 0.802027084 | 1.063615915 | Ten DAP | 4  | 28.1          | DNA                                              |
| cassava4.1_001582m PA | AT2G26570 | 0.803155838 | 1.198245535 | Ten DAP | 2  | 35.2          | not assigned                                     |
| cassava4.1_017195m PA | AT5G37850 | 0.805457444 | 1.15066742  | Ten DAP | 3  | 3.5           | minor CHO metabolism                             |
| cassava4.1_009358m PA | AT1G08200 | 0.808485133 | 1.104546988 | Ten DAP | 3  | 10.1.03       | cell wall                                        |
| cassava4.1_008254m PA | AT2G35840 | 0.811210375 | 1.085711675 | Ten DAP | 8  | 2.1.1.02      | major CHO metabolism                             |
| cassava4.1_007396m PA | AT3G55120 | 0.812011909 | 1.108277601 | Ten DAP | 16 | 16.8.2        | secondary metabolism                             |
| cassava4.1_005233m PA | AT3G22520 | 0.812472324 | 1.010710403 | Ten DAP | 3  | 35.2          | not assigned                                     |
| cassava4.1_005366m PA | AT3G06720 | 0.81351216  | 1.007063505 | Ten DAP | 6  | 29.3.1        | protein                                          |
| cassava4.1_009740m PA | AT3G26700 | 0.813666609 | 1.074450896 | Ten DAP | 2  | 29.4.1.59     | protein                                          |
| cassava4.1_012113m PA | AT3G59760 | 0.814356045 | 1.027357882 | Ten DAP | 11 | 13.1.5.3.01   | amino acid metabolism                            |
| cassava4.1_010018m PA | AT3G44190 | 0.815914494 | 1.098967774 | Ten DAP | 2  | 35.1          | not assigned                                     |
| cassava4.1_028456m PA | AT2G01410 | 0.816610415 | 1.224352073 | Ten DAP | 2  | 35.2          | not assigned                                     |
| cassava4.1_012151m PA | AT1G31230 | 0.818659735 | 1.040621927 | Ten DAP | 9  | 13.1.3.6.1.01 | amino acid metabolism                            |
| cassava4.1_012360m PA | AT5G05780 | 0.819870947 | 1.041926318 | Ten DAP | 3  | 29.5.11.20    | protein                                          |
| cassava4.1_002361m PA | AT4G04910 | 0.820486475 | 1.04645568  | Ten DAP | 23 | 29.5.09       | protein                                          |
| cassava4.1_033167m PA | AT2G25050 | 0.822180114 | 1.048746925 | Ten DAP | 2  | 35.1.20       | not assigned                                     |
| cassava4.1_004718m PA | AT5G05520 | 0.82309976  | 1.082478264 | Ten DAP | 10 | 35.1          | not assigned                                     |
| cassava4.1_011872m PA | -         | 0.824350759 | 1.166000176 | Ten DAP | 3  | -             | -                                                |
| cassava4.1_001472m PA | AT1G15130 | 0.826133153 | 1.115299673 | Ten DAP | 5  | 35.1.41       | not assigned                                     |

|                        |           |             |             |         |    |                |                                                  |
|------------------------|-----------|-------------|-------------|---------|----|----------------|--------------------------------------------------|
| cassava4.1_002741m PA  | AT5G36880 | 0.826415524 | 1.065465671 | Ten DAP | 6  | 11.1.08        | lipid metabolism                                 |
| cassava4.1_009501m PA  | AT4G24820 | 0.826443788 | 1.065593612 | Ten DAP | 11 | 29.5.11.20     | protein                                          |
| cassava4.1_002622m PA  | AT3G01680 | 0.826532459 | 1.136684746 | Ten DAP | 8  | 35.2           | not assigned                                     |
| cassava4.1_003452m PA  | AT5G51820 | 0.831527628 | 1.033803977 | Ten DAP | 26 | 4.02           | glycolysis                                       |
| cassava4.1_011619m PA  | AT5G42150 | 0.832642234 | 1.658476459 | Ten DAP | 2  | 35.2           | not assigned                                     |
| cassava4.1_002075m PA  | AT4G14570 | 0.833362994 | 1.053213917 | Ten DAP | 4  | 29.5           | protein                                          |
| cassava4.1_026977m PA  | AT5G16390 | 0.835208492 | 1.001575169 | Ten DAP | 2  | 11.1.01        | lipid metabolism                                 |
| cassava4.1_006965m PA  | AT5G17310 | 0.836268835 | 1.029815509 | Ten DAP | 30 | 4.01           | glycolysis                                       |
| cassava4.1_013577m PA  | AT3G51680 | 0.837677915 | 1.02240832  | Ten DAP | 5  | 26.22          | misc                                             |
| cassava4.1_009678m PA  | AT5G08280 | 0.837688317 | 1.089944465 | Ten DAP | 3  | 19.05          | tetrapyrrole synthesis                           |
| cassava4.1_013110m PA  | AT1G79500 | 0.838668818 | 1.155439132 | Ten DAP | 5  | 3.5            | minor CHO metabolism                             |
| cassava4.1_002326m PA  | AT4G02570 | 0.840746233 | 1.098632883 | Ten DAP | 4  | 29.5.11.4.3.03 | protein                                          |
| cassava4.1_010051m PA  | AT2G25710 | 0.841811317 | 1.471720262 | Ten DAP | 2  | 18             | Co-factor and vitamins metabolism                |
| cassava4.1_012009m PA  | AT1G48550 | 0.842378415 | 1.038457804 | Ten DAP | 2  | 29.3.4.3       | protein                                          |
| cassava4.1_009397m PA  | AT1G61580 | 0.844253162 | 1.034094961 | Ten DAP | 25 | 29.2.2         | protein                                          |
| cassava4.1_026681m PA  | AT5G67500 | 0.844614731 | 1.071182591 | Ten DAP | 4  | 34.20          | transport                                        |
| gi 169794130 ref YP_OC | -         | 0.845692632 | 1.068307993 | Ten DAP | 4  | -              | -                                                |
| cassava4.1_002036m PA  | AT2G42490 | 0.847042528 | 1.008793003 | Ten DAP | 2  | 26.07          | misc                                             |
| cassava4.1_001361m PA  | AT3G45140 | 0.849276933 | 1.1170045   | Ten DAP | 19 | 17.7.1.02      | hormone metabolism                               |
| cassava4.1_009089m PA  | AT1G74210 | 0.854154005 | 1.45127023  | Ten DAP | 2  | 11.9.3.03      | lipid metabolism                                 |
| cassava4.1_009051m PA  | AT1G06550 | 0.855146097 | 1.072324464 | Ten DAP | 12 | 11.9.4.03      | lipid metabolism                                 |
| cassava4.1_009051m PA  | AT1G06550 | 0.855146097 | 1.072324464 | Ten DAP | 12 | 13.2.3.5       | amino acid metabolism                            |
| cassava4.1_023507m PA  | AT1G14000 | 0.857084189 | 1.397716259 | Ten DAP | 2  | 29.4           | protein                                          |
| cassava4.1_015876m PA  | AT1G60500 | 0.858316532 | 1.053068761 | Ten DAP | 13 | 26.17          | misc                                             |
| cassava4.1_012898m PA  | AT1G65270 | 0.85842136  | 1.045940826 | Ten DAP | 4  | 35.2           | not assigned                                     |
| cassava4.1_003669m PA  | AT4G11420 | 0.861050818 | 1.010022028 | Ten DAP | 3  | 29.2.3         | protein                                          |
| cassava4.1_015190m PA  | AT1G55900 | 0.861051417 | 1.090299583 | Ten DAP | 3  | 35.1           | not assigned                                     |
| cassava4.1_011533m PA  | AT1G66670 | 0.861368826 | 1.111989252 | Ten DAP | 3  | 29.5.05        | protein                                          |
| cassava4.1_001823m PA  | AT1G76390 | 0.863444048 | 1.059386009 | Ten DAP | 9  | 29.5.11.04.02  | protein                                          |
| cassava4.1_004118m PA  | AT1G72550 | 0.865410372 | 1.045275973 | Ten DAP | 14 | 29.1.020       | protein                                          |
| cassava4.1_002107m PA  | AT5G67360 | 0.867961054 | 1.058448344 | Ten DAP | 16 | 29.5.01        | protein                                          |
| cassava4.1_013169m PA  | AT1G11840 | 0.869139495 | 1.027859689 | Ten DAP | 3  | 13.2.3.2       | amino acid metabolism                            |
| cassava4.1_013169m PA  | AT1G11840 | 0.869139495 | 1.027859689 | Ten DAP | 3  | 24.02          | Biodegradation of Xenobiotics                    |
| cassava4.1_011768m PA  | AT1G05260 | 0.870257763 | 1.306054502 | Ten DAP | 2  | 20.2.2         | stress                                           |
| cassava4.1_010253m PA  | AT5G51970 | 0.870774468 | 1.113205918 | Ten DAP | 2  | 3.3            | minor CHO metabolism                             |
| cassava4.1_008526m PA  | AT5G42740 | 0.874555718 | 1.030880805 | Ten DAP | 6  | 4.03           | glycolysis                                       |
| cassava4.1_002054m PA  | AT1G05520 | 0.874945345 | 1.108407503 | Ten DAP | 4  | 29.3.4.2       | protein                                          |
| cassava4.1_034446m PA  | AT3G14240 | 0.875487027 | 1.266770008 | Ten DAP | 2  | 29.5.01        | protein                                          |
| cassava4.1_007482m PA  | AT4G24550 | 0.879190239 | 1.182458729 | Ten DAP | 6  | 29.3.4.99      | protein                                          |
| cassava4.1_011729m PA  | AT3G07720 | 0.879851035 | 1.000761379 | Ten DAP | 6  | 35.1           | not assigned                                     |
| cassava4.1_011175m PA  | AT3G04120 | 0.88016232  | 1.021336999 | Ten DAP | 23 | 4.09           | glycolysis                                       |
| cassava4.1_017064m PA  | AT1G22450 | 0.880935585 | 1.019902372 | Ten DAP | 6  | 9.07           | mitochondrial electron transport / ATP synthesis |
| cassava4.1_008018m PA  | AT4G23650 | 0.88127098  | 1.041670012 | Ten DAP | 2  | 30.3           | signalling                                       |
| cassava4.1_004762m PA  | AT3G06540 | 0.88187685  | 1.033813675 | Ten DAP | 6  | 30.5           | signalling                                       |

|                        |           |             |             |         |    |              |                                                  |
|------------------------|-----------|-------------|-------------|---------|----|--------------|--------------------------------------------------|
| cassava4.1_015397m PA  | AT5G58590 | 0.884405937 | 1.027954513 | Ten DAP | 4  | 30.5         | signalling                                       |
| cassava4.1_002480m PA  | AT5G26710 | 0.8845848   | 1.00684614  | Ten DAP | 16 | 19.01        | tetrapyrrole synthesis                           |
| cassava4.1_002480m PA  | AT5G26710 | 0.8845848   | 1.00684614  | Ten DAP | 16 | 29.1.017     | protein                                          |
| cassava4.1_005270m PA  | AT3G53950 | 0.887727595 | 1.036932623 | Ten DAP | 4  | 24           | Biodegradation of Xenobiotics                    |
| cassava4.1_001287m PA  | AT1G31730 | 0.887896894 | 1.02517107  | Ten DAP | 4  | 31.4         | cell                                             |
| cassava4.1_004022m PA  | AT5G61970 | 0.889938143 | 1.044585339 | Ten DAP | 8  | 29.3.4.99    | protein                                          |
| cassava4.1_005326m PA  | AT5G38830 | 0.890532791 | 1.0694662   | Ten DAP | 11 | 29.1.016     | protein                                          |
| cassava4.1_024700m PA  | AT1G79870 | 0.892766495 | 1.036069701 | Ten DAP | 4  | 13.2.5.2     | amino acid metabolism                            |
| cassava4.1_024700m PA  | AT1G79870 | 0.892766495 | 1.036069701 | Ten DAP | 4  | 18.10.03     | Co-factor and vitamine metabolism                |
| cassava4.1_024700m PA  | AT1G79870 | 0.892766495 | 1.036069701 | Ten DAP | 4  | 26.01        | misc                                             |
| cassava4.1_031500m PA  | AT1G52340 | 0.894295579 | 1.035450534 | Ten DAP | 2  | 17.1.1.1.011 | hormone metabolism                               |
| cassava4.1_011640m PA  | AT1G04190 | 0.894406273 | 1.091201633 | Ten DAP | 4  | 35.1.5       | not assigned                                     |
| cassava4.1_007701m PA  | AT2G24420 | 0.894655017 | 1.021702849 | Ten DAP | 4  | 28.2         | DNA                                              |
| cassava4.1_003972m PA  | AT1G77180 | 0.895834598 | 1.090270997 | Ten DAP | 2  | 28.1         | DNA                                              |
| cassava4.1_010697m PA  | AT1G50940 | 0.896104715 | 1.013023457 | Ten DAP | 4  | 9.3          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_003319m PA  | AT2G03270 | 0.89894329  | 1.020811888 | Ten DAP | 4  | 28.99        | DNA                                              |
| cassava4.1_003401m PA  | AT5G04590 | 0.899552907 | 1.040374301 | Ten DAP | 16 | 14.03        | S-assimilation                                   |
| cassava4.1_007095m PA  | AT5G11580 | 0.903672726 | 1.062238044 | Ten DAP | 2  | 20.2.5       | stress                                           |
| cassava4.1_007095m PA  | AT5G11580 | 0.903672726 | 1.062238044 | Ten DAP | 2  | 31.2         | cell                                             |
| cassava4.1_012342m PA  | AT5G40810 | 0.903777585 | 1.035702675 | Ten DAP | 2  | 9.6          | mitochondrial electron transport / ATP synthesis |
| cassava4.1_000825m PA  | AT5G17020 | 0.903919563 | 1.031055924 | Ten DAP | 18 | 29.3.1       | protein                                          |
| cassava4.1_006778m PA  | AT4G01070 | 0.904096341 | 1.096099036 | Ten DAP | 4  | 16.8.3       | secondary metabolism                             |
| cassava4.1_006636m PA  | AT2G28100 | 0.905154304 | 1.026157756 | Ten DAP | 3  | 35.1         | not assigned                                     |
| cassava4.1_014920m PA  | AT3G05500 | 0.906836515 | 1.000571935 | Ten DAP | 6  | 35.1         | not assigned                                     |
| cassava4.1_004265m PA  | AT3G04870 | 0.907142824 | 1.044965353 | Ten DAP | 9  | 16.1.4       | secondary metabolism                             |
| cassava4.1_014241m PA  | AT4G34670 | 0.908714985 | 1.011209601 | Ten DAP | 16 | 29.2.2       | protein                                          |
| cassava4.1_009157m PA  | AT3G55010 | 0.909111051 | 1.08264107  | Ten DAP | 8  | 23.1.2.05    | nucleotide metabolism                            |
| cassava4.1_005014m PA  | AT1G51680 | 0.911296815 | 1.039376481 | Ten DAP | 9  | 16.2.1.03    | secondary metabolism                             |
| cassava4.1_010453m PA  | AT5G13560 | 0.911818438 | 1.084131616 | Ten DAP | 2  | 35.1         | not assigned                                     |
| gi 169794116 ref YP_OC | -         | 0.912520534 | 1.481060796 | Ten DAP | 2  | -            | -                                                |
| cassava4.1_001626m PA  | AT3G46970 | 0.912783717 | 1.021786578 | Ten DAP | 21 | 2.2.2.02     | major CHO metabolism                             |
| cassava4.1_004500m PA  | AT1G25350 | 0.913138679 | 1.019277098 | Ten DAP | 5  | 29.1.018     | protein                                          |
| cassava4.1_009444m PA  | AT2G17420 | 0.914900915 | 1.000243002 | Ten DAP | 7  | 21.01        | redox                                            |
| cassava4.1_011917m PA  | AT1G42470 | 0.917742185 | 1.020483856 | Ten DAP | 2  | 35.1         | not assigned                                     |
| cassava4.1_023310m PA  | AT1G80560 | 0.917808443 | 1.322540138 | Ten DAP | 11 | 13.1.4.4.03  | amino acid metabolism                            |
| cassava4.1_000871m PA  | AT1G12930 | 0.91891167  | 1.128782853 | Ten DAP | 2  | 29.3.1       | protein                                          |
| cassava4.1_025676m PA  | AT2G44060 | 0.919257137 | 1.077142045 | Ten DAP | 16 | 33.2         | development                                      |
| cassava4.1_011818m PA  | AT4G12080 | 0.925917257 | 1.081492542 | Ten DAP | 2  | 27.3.67      | RNA                                              |
| cassava4.1_015603m PA  | AT5G53530 | 0.92597934  | 1.041551068 | Ten DAP | 5  | 29.3.4.3     | protein                                          |
| cassava4.1_002838m PA  | AT3G54540 | 0.9275557   | 1.097382113 | Ten DAP | 3  | 34.16        | transport                                        |
| cassava4.1_030393m PA  | AT2G18330 | 0.931851555 | 1.002945845 | Ten DAP | 2  | 29.5.09      | protein                                          |
| cassava4.1_012472m PA  | AT2G30920 | 0.934969877 | 1.078895349 | Ten DAP | 2  | 18.8.01      | Co-factor and vitamine metabolism                |
| cassava4.1_009947m PA  | AT5G14040 | 0.936335311 | 1.031499313 | Ten DAP | 12 | 34.9         | transport                                        |
| cassava4.1_002828m PA  | AT1G79600 | 0.937149053 | 1.125476379 | Ten DAP | 3  | 34.16        | transport                                        |

|                       |           |             |             |         |    |           |                                   |
|-----------------------|-----------|-------------|-------------|---------|----|-----------|-----------------------------------|
| cassava4.1_008076m PA | AT1G35620 | 0.937991733 | 1.13675293  | Ten DAP | 7  | 21.01     | redox                             |
| cassava4.1_018726m PA | AT5G42260 | 0.93822544  | 1.30359566  | Ten DAP | 3  | 26.03     | misc                              |
| cassava4.1_010439m PA | AT5G46630 | 0.939227263 | 1.097894118 | Ten DAP | 3  | 29.3.4.99 | protein                           |
| cassava4.1_003877m PA | AT3G07020 | 0.940480809 | 1.076947347 | Ten DAP | 3  | 11.8.03   | lipid metabolism                  |
| cassava4.1_033251m PA | AT1G79870 | 0.941241635 | 1.071423961 | Ten DAP | 3  | 13.2.5.2  | amino acid metabolism             |
| cassava4.1_033251m PA | AT1G79870 | 0.941241635 | 1.071423961 | Ten DAP | 3  | 18.10.03  | Co-factor and vitamine metabolism |
| cassava4.1_033251m PA | AT1G79870 | 0.941241635 | 1.071423961 | Ten DAP | 3  | 26.01     | misc                              |
| cassava4.1_008225m PA | AT1G16300 | 0.943577617 | 1.053882317 | Ten DAP | 12 | 4.09      | glycolysis                        |
| cassava4.1_004208m PA | AT4G33070 | 0.944812902 | 1.001842493 | Ten DAP | 8  | 5.02      | fermentation                      |
| cassava4.1_009140m PA | AT4G38970 | 0.944833158 | 1.026531768 | Ten DAP | 3  | 1.3.06    | PS                                |
| cassava4.1_003451m PA | AT1G79750 | 0.94556931  | 1.012751815 | Ten DAP | 2  | 8.2.10    | TCA / org                         |
| cassava4.1_008916m PA | AT5G48230 | 0.946903546 | 1.126929249 | Ten DAP | 13 | 13.2.3.5  | amino acid metabolism             |
| cassava4.1_008916m PA | AT5G48230 | 0.946903546 | 1.126929249 | Ten DAP | 13 | 16.1.2.01 | secondary metabolism              |
| cassava4.1_002932m PA | AT1G18260 | 0.9488031   | 1.136339993 | Ten DAP | 3  | 35.1      | not assigned                      |
| cassava4.1_001365m PA | AT2G26570 | 0.951896772 | 1.272082587 | Ten DAP | 3  | 35.2      | not assigned                      |
| cassava4.1_026031m PA | AT2G39040 | 0.95272846  | 1.513151621 | Ten DAP | 2  | 26.12     | misc                              |
| cassava4.1_022298m PA | AT3G18420 | 0.954933636 | 1.003215517 | Ten DAP | 7  | 35.1.5    | not assigned                      |
| cassava4.1_005161m PA | AT1G76160 | 0.956187764 | 1.075713325 | Ten DAP | 3  | 35.1      | not assigned                      |
| cassava4.1_000510m PA | AT1G67230 | 0.956960248 | 1.051648931 | Ten DAP | 3  | 35.2      | not assigned                      |
| cassava4.1_001283m PA | AT1G73370 | 0.957015817 | 1.139926359 | Ten DAP | 7  | 2.2.1.05  | major CHO metabolism              |
| cassava4.1_006181m PA | AT2G24270 | 0.960339583 | 1.019077673 | Ten DAP | 2  | 1.3.04    | PS                                |
| cassava4.1_007379m PA | AT5G39410 | 0.960666921 | 1.012238665 | Ten DAP | 9  | 35.2      | not assigned                      |
| cassava4.1_019445m PA | AT5G17380 | 0.96301834  | 1.012793724 | Ten DAP | 6  | 5.02      | fermentation                      |
| cassava4.1_002823m PA | AT1G71270 | 0.96325834  | 1.02722885  | Ten DAP | 5  | 35.1      | not assigned                      |
| cassava4.1_001078m PA | AT5G62670 | 0.963688071 | 1.003989177 | Ten DAP | 2  | 34.1      | transport                         |
| cassava4.1_002589m PA | AT1G64550 | 0.964985692 | 1.041565149 | Ten DAP | 4  | 34.16     | transport                         |
| cassava4.1_005215m PA | AT4G31180 | 0.965025058 | 1.036477705 | Ten DAP | 9  | 29.1.012  | protein                           |
| cassava4.1_012442m PA | AT4G11980 | 0.966399591 | 1.012905934 | Ten DAP | 3  | 35.1      | not assigned                      |
| cassava4.1_008344m PA | AT1G10670 | 0.968865333 | 1.035513508 | Ten DAP | 4  | 8.2.011   | TCA / org                         |
| cassava4.1_003930m PA | AT5G53370 | 0.968904287 | 1.023597151 | Ten DAP | 9  | 10.8.99   | cell wall                         |
| cassava4.1_011656m PA | AT3G50210 | 0.969733722 | 1.298729463 | Ten DAP | 4  | 16.8.4    | secondary metabolism              |
| cassava4.1_028531m PA | AT4G20360 | 0.970220748 | 1.024446976 | Ten DAP | 4  | 29.2.4    | protein                           |
| cassava4.1_004699m PA | AT1G69340 | 0.973615827 | 1.015049772 | Ten DAP | 3  | 35.1      | not assigned                      |
| cassava4.1_007551m PA | AT5G19780 | 0.976845766 | 1.167138602 | Ten DAP | 4  | 31.1      | cell                              |
| cassava4.1_004713m PA | AT1G49760 | 0.978807367 | 1.041535157 | Ten DAP | 9  | 27.1      | RNA                               |
| cassava4.1_003499m PA | AT2G42520 | 0.983451556 | 1.003401007 | Ten DAP | 6  | 27.1.2    | RNA                               |
| cassava4.1_010309m PA | AT5G23050 | 0.986025795 | 1.185538822 | Ten DAP | 4  | 11.1.08   | lipid metabolism                  |
| cassava4.1_025592m PA | AT4G36760 | 0.986449961 | 1.057797757 | Ten DAP | 3  | 29.5      | protein                           |
| cassava4.1_012267m PA | AT5G11810 | 0.987835079 | 1.001778001 | Ten DAP | 3  | 35.2      | not assigned                      |
| cassava4.1_001703m PA | AT5G51430 | 0.991552847 | 1.007358645 | Ten DAP | 10 | 35.1      | not assigned                      |
| cassava4.1_006591m PA | AT1G01300 | 0.991905745 | 1.007457951 | Ten DAP | 13 | 29.5.04   | protein                           |
| cassava4.1_013813m PA | AT5G23950 | 0.992638664 | 1.0057787   | Ten DAP | 3  | 20.2.2    | stress                            |
| cassava4.1_006574m PA | AT1G32500 | 0.99399441  | 1.082987797 | Ten DAP | 3  | 34.16     | transport                         |
| cassava4.1_009974m PA | AT4G34660 | 0.99429564  | 1.063141195 | Ten DAP | 4  | 35.1      | not assigned                      |

|                       |           |             |              |          |    |              |                       |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------|
| cassava4.1_012176m PA | AT5G28830 | 0.995235636 | 1.072672752  | Ten DAP  | 2  | 30.3         | signalling            |
| cassava4.1_001848m PA | AT3G15730 | 0.995960364 | 1.055613106  | Ten DAP  | 5  | 11.9.3.01    | lipid metabolism      |
| cassava4.1_029912m PA | AT2G19080 | 0.997482544 | 1.110781864  | Ten DAP  | 2  | 35.1         | not assigned          |
| cassava4.1_000033m PA | AT1G36160 | 0.000310268 | -2.372967306 | Zero DAP | 4  | 11.1.01      | lipid metabolism      |
| cassava4.1_005990m PA | AT5G56350 | 0.000651792 | -1.598008389 | Zero DAP | 6  | 4.013        | glycolysis            |
| cassava4.1_004241m PA | AT4G26300 | 0.000953209 | -1.243089333 | Zero DAP | 7  | 29.1.019     | protein               |
| cassava4.1_009231m PA | AT2G38860 | 0.001005953 | -1.360936044 | Zero DAP | 10 | 29.5         | protein               |
| cassava4.1_008506m PA | AT3G50000 | 0.001188611 | -2.277335938 | Zero DAP | 2  | 29.4         | protein               |
| cassava4.1_021312m PA | AT5G49830 | 0.001281753 | -1.449046934 | Zero DAP | 7  | 35.2         | not assigned          |
| cassava4.1_005818m PA | AT5G58860 | 0.001789936 | Infinit      | Zero DAP | 2  | 26.10        | misc                  |
| cassava4.1_013014m PA | AT5G13870 | 0.002361225 | -2.053823582 | Zero DAP | 3  | 10.7         | cell wall             |
| cassava4.1_004106m PA | AT4G00740 | 0.002492005 | -2.374777322 | Zero DAP | 5  | 20.2.3       | stress                |
| cassava4.1_003527m PA | AT5G63120 | 0.002667439 | -2.073351598 | Zero DAP | 2  | 17.5.3       | hormone metabolism    |
| cassava4.1_003527m PA | AT5G63120 | 0.002667439 | -2.073351598 | Zero DAP | 2  | 27.1         | RNA                   |
| cassava4.1_004971m PA | AT5G55230 | 0.002824427 | -1.661939589 | Zero DAP | 5  | 31.1         | cell                  |
| cassava4.1_023409m PA | AT1G79460 | 0.004069604 | -42.83336756 | Zero DAP | 4  | 17.6.1.02    | hormone metabolism    |
| cassava4.1_005817m PA | AT5G57260 | 0.004337957 | -8.255161118 | Zero DAP | 18 | 26.10        | misc                  |
| cassava4.1_000239m PA | AT2G20190 | 0.00456591  | -2.152447807 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_007650m PA | AT5G12250 | 0.004804689 | -2.491595981 | Zero DAP | 11 | 31.1         | cell                  |
| cassava4.1_003389m PA | AT5G27120 | 0.004809163 | -3.165424654 | Zero DAP | 2  | 27.3.67      | RNA                   |
| cassava4.1_004720m PA | AT2G26260 | 0.004870589 | -1.953040311 | Zero DAP | 6  | 11.8.04      | lipid metabolism      |
| cassava4.1_003705m PA | AT5G49720 | 0.005091526 | -4.673208839 | Zero DAP | 4  | 10.2         | cell wall             |
| cassava4.1_003400m PA | AT5G10840 | 0.005521293 | -5.328285484 | Zero DAP | 3  | 35.2         | not assigned          |
| cassava4.1_008088m PA | AT5G41040 | 0.006874662 | -1.63843743  | Zero DAP | 9  | 35.1         | not assigned          |
| cassava4.1_014777m PA | AT3G07030 | 0.007441539 | -1.497351383 | Zero DAP | 2  | 35.2         | not assigned          |
| cassava4.1_009247m PA | AT4G01850 | 0.007795959 | -3.966421553 | Zero DAP | 13 | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_004081m PA | AT5G60790 | 0.008090031 | -2.762754986 | Zero DAP | 10 | 34.16        | transport             |
| cassava4.1_001698m PA | AT1G04080 | 0.008246631 | -1.675107876 | Zero DAP | 5  | 35.1.41      | not assigned          |
| cassava4.1_004221m PA | AT4G34980 | 0.009117001 | -2.534486168 | Zero DAP | 12 | 29.5.01      | protein               |
| cassava4.1_006282m PA | AT1G31070 | 0.009644226 | -1.901909375 | Zero DAP | 6  | 10.1         | cell wall             |
| cassava4.1_009789m PA | AT3G61440 | 0.010016052 | -2.966724969 | Zero DAP | 8  | 13.1.5.3.01  | amino acid metabolism |
| cassava4.1_014594m PA | AT2G38740 | 0.010390822 | -3.204474615 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_005904m PA | AT2G43820 | 0.010485923 | -1.811946214 | Zero DAP | 4  | 26.02        | misc                  |
| cassava4.1_012402m PA | AT5G01410 | 0.010775754 | -4.622868947 | Zero DAP | 11 | 35.1         | not assigned          |
| cassava4.1_005079m PA | AT4G39950 | 0.010893536 | -19.48809178 | Zero DAP | 6  | 26.10        | misc                  |
| cassava4.1_005409m PA | AT4G39210 | 0.011351981 | -2.088975338 | Zero DAP | 28 | 2.1.2.01     | major CHO metabolism  |
| cassava4.1_009245m PA | AT4G01850 | 0.011618162 | -4.153689723 | Zero DAP | 17 | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_005302m PA | AT3G18190 | 0.011719071 | -1.595956909 | Zero DAP | 23 | 29.6         | protein               |
| cassava4.1_000634m PA | AT5G06460 | 0.012333321 | -1.512632939 | Zero DAP | 6  | 29.5.11.02   | protein               |
| cassava4.1_006818m PA | AT2G29560 | 0.013076458 | -1.559280338 | Zero DAP | 6  | 4.012        | glycolysis            |
| cassava4.1_011584m PA | AT3G59480 | 0.013297578 | -1.475956124 | Zero DAP | 22 | 2.2.1.01     | major CHO metabolism  |
| cassava4.1_007409m PA | AT1G14570 | 0.013700506 | -1.67872442  | Zero DAP | 2  | 29.5         | protein               |
| cassava4.1_005057m PA | AT3G03960 | 0.013973317 | -1.244498262 | Zero DAP | 16 | 29.6         | protein               |
| cassava4.1_000150m PA | AT1G71220 | 0.014141749 | -1.661860023 | Zero DAP | 12 | 29.7         | protein               |

|                        |           |             |              |          |    |                |                                                  |
|------------------------|-----------|-------------|--------------|----------|----|----------------|--------------------------------------------------|
| cassava4.1_004579m PA  | AT3G16950 | 0.014414463 | -1.119370463 | Zero DAP | 15 | 8.1.01.03      | TCA / org                                        |
| cassava4.1_000458m PA  | AT3G62360 | 0.014511461 | -1.964032548 | Zero DAP | 5  | 35.2           | not assigned                                     |
| cassava4.1_003857m PA  | AT4G18030 | 0.014679084 | -3.663598697 | Zero DAP | 8  | 20.2.3         | stress                                           |
| cassava4.1_005517m PA  | AT1G75680 | 0.015315386 | -2.472428331 | Zero DAP | 6  | 10.6.1         | cell wall                                        |
| cassava4.1_005517m PA  | AT1G75680 | 0.015315386 | -2.472428331 | Zero DAP | 6  | 26.03          | misc                                             |
| cassava4.1_012023m PA  | AT5G65780 | 0.015473361 | -1.914311188 | Zero DAP | 6  | 13.1.4.1       | amino acid metabolism                            |
| cassava4.1_004314m PA  | AT3G22960 | 0.01550972  | -2.069258196 | Zero DAP | 7  | 11.1.030       | lipid metabolism                                 |
| cassava4.1_000191m PA  | AT5G20490 | 0.015607078 | -1.468547668 | Zero DAP | 9  | 31.1           | cell                                             |
| cassava4.1_026770m PA  | AT5G07720 | 0.015907422 | -7.792853011 | Zero DAP | 2  | 10.3           | cell wall                                        |
| cassava4.1_026770m PA  | AT5G07720 | 0.015907422 | -7.792853011 | Zero DAP | 2  | 26.02          | misc                                             |
| cassava4.1_000688m PA  | AT4G20850 | 0.016587509 | -1.6375074   | Zero DAP | 23 | 29.5.01        | protein                                          |
| cassava4.1_011785m PA  | AT4G14880 | 0.016861007 | -1.470820095 | Zero DAP | 9  | 13.1.5.3.01    | amino acid metabolism                            |
| cassava4.1_012617m PA  | AT1G63000 | 0.017298634 | -2.510786326 | Zero DAP | 9  | 10.1.011       | cell wall                                        |
| cassava4.1_007678m PA  | AT2G36530 | 0.017383169 | -1.184387774 | Zero DAP | 14 | 4.012          | glycolysis                                       |
| cassava4.1_005840m PA  | AT2G03820 | 0.018611319 | -1.931357425 | Zero DAP | 2  | 35.1           | not assigned                                     |
| cassava4.1_001142m PA  | AT2G23520 | 0.020216991 | -1.376081175 | Zero DAP | 2  | 35.2           | not assigned                                     |
| cassava4.1_002126m PA  | AT4G10070 | 0.021320539 | -1.638279498 | Zero DAP | 3  | 27.2           | RNA                                              |
| cassava4.1_000041m PA  | AT1G36160 | 0.022125948 | -4.840405433 | Zero DAP | 2  | 11.1.01        | lipid metabolism                                 |
| gi 169794080 ref YP_OC | -         | 0.02212956  | -3.452999046 | Zero DAP | 5  | -              | -                                                |
| cassava4.1_012699m PA  | AT5G26667 | 0.022295451 | -1.289052438 | Zero DAP | 10 | 23.4.3         | nucleotide metabolism                            |
| cassava4.1_008023m PA  | AT1G17745 | 0.023508802 | -1.721439695 | Zero DAP | 10 | 13.1.5.1.01    | amino acid metabolism                            |
| cassava4.1_007632m PA  | AT5G23860 | 0.023603342 | -1.820090119 | Zero DAP | 14 | 31.1           | cell                                             |
| cassava4.1_003070m PA  | AT1G78570 | 0.024894052 | -1.939766287 | Zero DAP | 13 | 10.1.010       | cell wall                                        |
| cassava4.1_001824m PA  | AT3G13870 | 0.025023618 | -1.969382217 | Zero DAP | 19 | 33.99          | development                                      |
| cassava4.1_001104m PA  | AT2G24520 | 0.025155309 | -1.971669953 | Zero DAP | 2  | 34.1           | transport                                        |
| cassava4.1_009295m PA  | AT5G13930 | 0.025470523 | -8.825291154 | Zero DAP | 5  | 16.8.2.01      | secondary metabolism                             |
| cassava4.1_021147m PA  | AT1G22380 | 0.026194216 | -3.670478589 | Zero DAP | 3  | 26.02          | misc                                             |
| cassava4.1_026874m PA  | AT5G04420 | 0.026369047 | -2.744156117 | Zero DAP | 5  | 35.1           | not assigned                                     |
| cassava4.1_003765m PA  | AT1G26850 | 0.026655555 | -3.301021418 | Zero DAP | 9  | 20.2.3         | stress                                           |
| cassava4.1_000497m PA  | AT5G26570 | 0.026783934 | -1.321020787 | Zero DAP | 9  | 2.2.2.03       | major CHO metabolism                             |
| cassava4.1_000599m PA  | AT5G06600 | 0.027227639 | -1.88056626  | Zero DAP | 10 | 29.5.11.05     | protein                                          |
| cassava4.1_012622m PA  | AT1G63000 | 0.027758688 | -3.058469716 | Zero DAP | 2  | 10.1.011       | cell wall                                        |
| cassava4.1_009402m PA  | AT5G13930 | 0.027894834 | -2.648803168 | Zero DAP | 3  | 16.8.2.01      | secondary metabolism                             |
| cassava4.1_033676m PA  | AT5G54440 | 0.028198395 | -2.50853873  | Zero DAP | 3  | 35.2           | not assigned                                     |
| cassava4.1_009260m PA  | AT4G01850 | 0.028675998 | -7.173982227 | Zero DAP | 4  | 13.1.3.4.011   | amino acid metabolism                            |
| cassava4.1_018147m PA  | AT5G42190 | 0.028928969 | -1.633379408 | Zero DAP | 2  | 29.5.11.4.3.01 | protein                                          |
| cassava4.1_004405m PA  | AT5G52920 | 0.029268563 | -1.460937398 | Zero DAP | 14 | 11.1.030       | lipid metabolism                                 |
| cassava4.1_011800m PA  | AT4G24570 | 0.030038692 | -12.83297076 | Zero DAP | 2  | 9.8            | mitochondrial electron transport / ATP synthesis |
| cassava4.1_006215m PA  | AT3G61490 | 0.030450968 | -6.021070584 | Zero DAP | 2  | 10.6.3         | cell wall                                        |
| cassava4.1_007617m PA  | AT5G23860 | 0.03076012  | -2.338573269 | Zero DAP | 2  | 31.1           | cell                                             |
| cassava4.1_004230m PA  | AT3G22960 | 0.03100602  | -1.581515844 | Zero DAP | 13 | 11.1.030       | lipid metabolism                                 |
| cassava4.1_000103m PA  | AT3G43300 | 0.031779992 | -1.986283503 | Zero DAP | 9  | 30.5           | signalling                                       |
| cassava4.1_023284m PA  | AT3G51160 | 0.032606569 | -1.612490451 | Zero DAP | 7  | 10.1.07        | cell wall                                        |
| cassava4.1_001804m PA  | AT4G30020 | 0.033328303 | -2.780422867 | Zero DAP | 10 | 29.5.01        | protein                                          |

|                       |           |             |              |          |    |              |                       |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------|
| cassava4.1_000195m PA | AT2G38770 | 0.033699088 | -2.771771461 | Zero DAP | 2  | 35.2         | not assigned          |
| cassava4.1_005974m PA | AT5G07990 | 0.03427019  | -4.336721255 | Zero DAP | 2  | 16.8.3.03    | secondary metabolism  |
| cassava4.1_000134m PA | AT3G11130 | 0.034731579 | -1.336794767 | Zero DAP | 62 | 31.4         | cell                  |
| cassava4.1_013011m PA | AT3G23730 | 0.034907825 | -7.078208205 | Zero DAP | 2  | 10.7         | cell wall             |
| cassava4.1_007713m PA | AT5G23860 | 0.035265304 | -1.496295869 | Zero DAP | 26 | 31.1         | cell                  |
| cassava4.1_008405m PA | AT1G51630 | 0.035841927 | -2.036739081 | Zero DAP | 8  | 35.2         | not assigned          |
| cassava4.1_004018m PA | AT1G70770 | 0.036307972 | -2.065843638 | Zero DAP | 9  | 35.2         | not assigned          |
| cassava4.1_006458m PA | AT1G11680 | 0.036350652 | -1.461856026 | Zero DAP | 6  | 17.3.1.2.03  | hormone metabolism    |
| cassava4.1_017972m PA | AT5G24710 | 0.036809768 | -3.734045149 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_008317m PA | AT3G14100 | 0.037345774 | -1.732291775 | Zero DAP | 3  | 35.1         | not assigned          |
| cassava4.1_007339m PA | AT2G17840 | 0.037365356 | -1.769091637 | Zero DAP | 2  | 20.2.3       | stress                |
| cassava4.1_007339m PA | AT2G17840 | 0.037365356 | -1.769091637 | Zero DAP | 2  | 33.99        | development           |
| cassava4.1_003361m PA | AT2G07360 | 0.038436759 | -2.255211256 | Zero DAP | 6  | 35.1         | not assigned          |
| cassava4.1_032535m PA | AT4G10320 | 0.038778811 | -1.554065116 | Zero DAP | 7  | 29.1.05      | protein               |
| cassava4.1_011197m PA | AT1G43670 | 0.039438558 | -1.217314961 | Zero DAP | 2  | 2.1.1.03     | major CHO metabolism  |
| cassava4.1_000306m PA | AT2G36910 | 0.040240115 | -2.828121082 | Zero DAP | 7  | 17.2.2       | hormone metabolism    |
| cassava4.1_000306m PA | AT2G36910 | 0.040240115 | -2.828121082 | Zero DAP | 7  | 34.16        | transport             |
| cassava4.1_005410m PA | AT5G53850 | 0.041451637 | -1.357691971 | Zero DAP | 14 | 3.5          | minor CHO metabolism  |
| cassava4.1_000656m PA | AT1G09620 | 0.041876401 | -5.828654907 | Zero DAP | 2  | 29.1.04      | protein               |
| cassava4.1_009169m PA | AT5G12470 | 0.042465397 | -1.544540869 | Zero DAP | 8  | 35.2         | not assigned          |
| cassava4.1_010021m PA | AT3G61440 | 0.042572879 | -3.602058257 | Zero DAP | 2  | 13.1.5.3.01  | amino acid metabolism |
| cassava4.1_009783m PA | AT2G37620 | 0.042603938 | -1.252216472 | Zero DAP | 2  | 31.1         | cell                  |
| cassava4.1_000607m PA | AT4G32640 | 0.042648559 | -2.028837774 | Zero DAP | 6  | 31.4         | cell                  |
| cassava4.1_005510m PA | AT2G26170 | 0.04271988  | -2.30123054  | Zero DAP | 3  | 17.2.2       | hormone metabolism    |
| cassava4.1_016634m PA | AT5G04420 | 0.042765794 | -2.610155898 | Zero DAP | 9  | 35.1         | not assigned          |
| cassava4.1_009356m PA | AT2G36880 | 0.042766793 | -3.634983363 | Zero DAP | 4  | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_009356m PA | AT2G36880 | 0.042766793 | -3.634983363 | Zero DAP | 4  | 15.2         | metal handling        |
| cassava4.1_000716m PA | AT1G07670 | 0.042935125 | -2.185440264 | Zero DAP | 8  | 34.21        | transport             |
| cassava4.1_006951m PA | AT2G03640 | 0.043776796 | -2.356099306 | Zero DAP | 5  | 29.3.1       | protein               |
| cassava4.1_003746m PA | AT1G14830 | 0.043944445 | -3.043660566 | Zero DAP | 6  | 26.17        | misc                  |
| cassava4.1_006550m PA | AT4G36480 | 0.044136398 | -1.299408497 | Zero DAP | 5  | 11.8.1       | lipid metabolism      |
| cassava4.1_003858m PA | AT5G42080 | 0.04414592  | -1.979692128 | Zero DAP | 30 | 26.17        | misc                  |
| cassava4.1_003858m PA | AT5G42080 | 0.04414592  | -1.979692128 | Zero DAP | 30 | 30.5         | signalling            |
| cassava4.1_001902m PA | AT1G16780 | 0.044297493 | -4.250299323 | Zero DAP | 2  | 34.30        | transport             |
| cassava4.1_011715m PA | AT5G61240 | 0.044740061 | -3.867016975 | Zero DAP | 2  | 20.1         | stress                |
| cassava4.1_005134m PA | AT4G32810 | 0.044837868 | -9.38927503  | Zero DAP | 2  | 17.1.1       | hormone metabolism    |
| cassava4.1_032325m PA | AT5G67360 | 0.045000855 | -5.267634152 | Zero DAP | 9  | 29.5.01      | protein               |
| cassava4.1_001780m PA | AT2G34300 | 0.045419222 | -2.306619469 | Zero DAP | 3  | 20.2.3       | stress                |
| cassava4.1_000375m PA | AT5G37830 | 0.045496398 | -1.906025628 | Zero DAP | 6  | 35.1         | not assigned          |
| cassava4.1_001585m PA | AT3G07100 | 0.045976556 | -2.204258055 | Zero DAP | 3  | 29.3.4.2     | protein               |
| cassava4.1_007645m PA | AT3G62830 | 0.046882028 | -2.474521947 | Zero DAP | 8  | 10.1.05      | cell wall             |
| cassava4.1_000264m PA | AT1G74260 | 0.046941495 | -1.157447776 | Zero DAP | 21 | 23.1.2.04    | nucleotide metabolism |
| cassava4.1_014182m PA | AT3G49720 | 0.047532797 | -4.068148662 | Zero DAP | 7  | 35.2         | not assigned          |
| cassava4.1_033822m PA | AT3G48770 | 0.047699234 | -3.658330681 | Zero DAP | 3  | 35.2         | not assigned          |

|                       |           |             |              |          |    |             |                       |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_004109m PA | AT2G01970 | 0.049269555 | -2.583957311 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_002730m PA | AT2G36910 | 0.050451189 | -3.675274833 | Zero DAP | 3  | 17.2.2      | hormone metabolism    |
| cassava4.1_002730m PA | AT2G36910 | 0.050451189 | -3.675274833 | Zero DAP | 3  | 34.16       | transport             |
| cassava4.1_006307m PA | AT4G04950 | 0.050937731 | -1.83056123  | Zero DAP | 9  | 21.01       | redox                 |
| cassava4.1_023396m PA | AT2G07050 | 0.051325251 | -2.283239733 | Zero DAP | 5  | 11.8.06     | lipid metabolism      |
| cassava4.1_023396m PA | AT2G07050 | 0.051325251 | -2.283239733 | Zero DAP | 5  | 17.3.1.2.99 | hormone metabolism    |
| cassava4.1_000052m PA | AT2G22125 | 0.051706843 | -16.0134457  | Zero DAP | 2  | 35.1.19     | not assigned          |
| cassava4.1_032962m PA | AT1G18270 | 0.051744173 | -1.171850871 | Zero DAP | 15 | 35.1        | not assigned          |
| cassava4.1_001312m PA | AT2G27040 | 0.052267146 | -1.440044705 | Zero DAP | 3  | 27.3.36     | RNA                   |
| cassava4.1_002709m PA | AT2G37040 | 0.052401432 | -2.095994966 | Zero DAP | 17 | 16.2.1.01   | secondary metabolism  |
| cassava4.1_034463m PA | AT3G14920 | 0.052498334 | -3.447677257 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_012773m PA | AT5G18900 | 0.052876726 | -2.150868383 | Zero DAP | 6  | 13.2.2.2    | amino acid metabolism |
| cassava4.1_012773m PA | AT5G18900 | 0.052876726 | -2.150868383 | Zero DAP | 6  | 26.07       | misc                  |
| cassava4.1_032346m PA | AT1G20960 | 0.05390777  | -1.832553584 | Zero DAP | 9  | 27.1        | RNA                   |
| cassava4.1_032346m PA | AT1G20960 | 0.05390777  | -1.832553584 | Zero DAP | 9  | 28.1        | DNA                   |
| cassava4.1_034377m PA | AT2G37040 | 0.054267515 | -1.608598285 | Zero DAP | 6  | 16.2.1.01   | secondary metabolism  |
| cassava4.1_006663m PA | AT5G15490 | 0.054689925 | -1.457182957 | Zero DAP | 20 | 10.1.04     | cell wall             |
| cassava4.1_011998m PA | AT1G23740 | 0.055736084 | -2.436151941 | Zero DAP | 3  | 26.07       | misc                  |
| cassava4.1_003754m PA | AT3G60190 | 0.056264621 | -1.346231081 | Zero DAP | 3  | 26.17       | misc                  |
| cassava4.1_002192m PA | AT3G23640 | 0.057483302 | -1.94043044  | Zero DAP | 10 | 2.2.2.1     | major CHO metabolism  |
| cassava4.1_002192m PA | AT3G23640 | 0.057483302 | -1.94043044  | Zero DAP | 10 | 26.03       | misc                  |
| cassava4.1_001389m PA | AT4G34450 | 0.058157801 | -1.287754848 | Zero DAP | 8  | 31.4        | cell                  |
| cassava4.1_000434m PA | AT1G62020 | 0.058574141 | -1.877474651 | Zero DAP | 32 | 31.4        | cell                  |
| cassava4.1_001326m PA | AT4G23460 | 0.05857614  | -1.467404395 | Zero DAP | 14 | 31.4        | cell                  |
| cassava4.1_000038m PA | AT5G53460 | 0.058828253 | -7.706148959 | Zero DAP | 3  | 12.2.1.02   | N-metabolism          |
| cassava4.1_006870m PA | AT3G61650 | 0.060474719 | -1.579779411 | Zero DAP | 3  | 31.1        | cell                  |
| cassava4.1_029686m PA | AT1G49390 | 0.06048357  | -1.17010131  | Zero DAP | 2  | 16.8.4      | secondary metabolism  |
| cassava4.1_004027m PA | AT4G19120 | 0.060929571 | -8.062795674 | Zero DAP | 2  | 20.2.3      | stress                |
| cassava4.1_007323m PA | AT3G23820 | 0.061733816 | -15.72300044 | Zero DAP | 2  | 10.1.06     | cell wall             |
| cassava4.1_021804m PA | AT3G13330 | 0.062960187 | -2.103215518 | Zero DAP | 16 | 35.2        | not assigned          |
| cassava4.1_000652m PA | AT5G64740 | 0.063005955 | -2.986672643 | Zero DAP | 4  | 10.2.01     | cell wall             |
| cassava4.1_005759m PA | AT3G10410 | 0.063532231 | -2.58039141  | Zero DAP | 2  | 29.5.05     | protein               |
| cassava4.1_001251m PA | AT1G59610 | 0.064036318 | -2.539874139 | Zero DAP | 5  | 26.17       | misc                  |
| cassava4.1_001864m PA | AT3G43190 | 0.065106092 | -1.258455378 | Zero DAP | 50 | 2.2.1.05    | major CHO metabolism  |
| cassava4.1_006293m PA | AT4G00660 | 0.065764637 | -1.591317734 | Zero DAP | 7  | 28.1        | DNA                   |
| cassava4.1_029365m PA | AT5G42150 | 0.06606291  | -2.714370986 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_004967m PA | AT1G30760 | 0.066200613 | -1.666273188 | Zero DAP | 2  | 16.4.1      | secondary metabolism  |
| cassava4.1_004967m PA | AT1G30760 | 0.066200613 | -1.666273188 | Zero DAP | 2  | 26.08       | misc                  |
| cassava4.1_006969m PA | AT2G03640 | 0.067146794 | -2.096579845 | Zero DAP | 5  | 29.3.1      | protein               |
| cassava4.1_005241m PA | AT1G24510 | 0.067817827 | -1.211263315 | Zero DAP | 6  | 29.4        | protein               |
| cassava4.1_000725m PA | AT1G68750 | 0.068246242 | -1.212412649 | Zero DAP | 34 | 4.014       | glycolysis            |
| cassava4.1_015161m PA | AT1G76010 | 0.069121746 | -1.468106207 | Zero DAP | 2  | 27.3.67     | RNA                   |
| cassava4.1_009942m PA | AT4G39090 | 0.069440431 | -3.69365985  | Zero DAP | 2  | 29.5.03     | protein               |
| cassava4.1_014988m PA | AT2G45140 | 0.069574499 | -1.949695903 | Zero DAP | 3  | 35.1        | not assigned          |



|                        |           |             |              |          |    |             |                       |
|------------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_001132m PA  | AT4G31480 | 0.069632903 | -1.854285982 | Zero DAP | 7  | 31.4        | cell                  |
| cassava4.1_000949m PA  | AT5G07350 | 0.069838559 | -2.011650145 | Zero DAP | 35 | 27.3.73     | RNA                   |
| cassava4.1_007598m PA  | AT5G12250 | 0.070172113 | -2.826278516 | Zero DAP | 2  | 31.1        | cell                  |
| cassava4.1_001924m PA  | AT3G07770 | 0.071621094 | -1.435444439 | Zero DAP | 14 | 20.2.1      | stress                |
| cassava4.1_001905m PA  | AT2G04030 | 0.07189603  | -1.555413393 | Zero DAP | 7  | 20.2.1      | stress                |
| cassava4.1_004733m PA  | AT3G08590 | 0.072270475 | -1.145879222 | Zero DAP | 8  | 4.011       | glycolysis            |
| cassava4.1_006558m PA  | AT1G22380 | 0.072869444 | -2.065889988 | Zero DAP | 7  | 26.02       | misc                  |
| cassava4.1_010212m PA  | AT3G51240 | 0.072870901 | -3.099376688 | Zero DAP | 17 | 16.8.3.02   | secondary metabolism  |
| cassava4.1_001713m PA  | AT4G33650 | 0.072925083 | -1.348971109 | Zero DAP | 25 | 26.17       | misc                  |
| cassava4.1_015079m PA  | AT5G59950 | 0.073174992 | -1.389881818 | Zero DAP | 8  | 27.4        | RNA                   |
| cassava4.1_009083m PA  | AT3G26040 | 0.073362278 | -1.612906459 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_011447m PA  | AT2G20760 | 0.073539254 | -1.849118077 | Zero DAP | 4  | 35.2        | not assigned          |
| cassava4.1_003221m PA  | AT5G35160 | 0.07423579  | -2.577936106 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_006916m PA  | AT3G53710 | 0.076032207 | -2.268139014 | Zero DAP | 5  | 27.3.99     | RNA                   |
| cassava4.1_005978m PA  | AT2G30490 | 0.076038917 | -1.686021432 | Zero DAP | 16 | 16.2.1.02   | secondary metabolism  |
| cassava4.1_000133m PA  | AT3G11130 | 0.078319383 | -1.501883747 | Zero DAP | 18 | 31.4        | cell                  |
| cassava4.1_000439m PA  | AT1G62020 | 0.078384275 | -1.948669591 | Zero DAP | 15 | 31.4        | cell                  |
| cassava4.1_002591m PA  | AT2G37040 | 0.078457509 | -2.079302935 | Zero DAP | 4  | 16.2.1.01   | secondary metabolism  |
| cassava4.1_001612m PA  | AT1G79930 | 0.079273281 | -1.450938522 | Zero DAP | 33 | 20.2.1      | stress                |
| cassava4.1_023162m PA  | AT1G22410 | 0.079371208 | -2.474108116 | Zero DAP | 7  | 13.1.6.1.01 | amino acid metabolism |
| cassava4.1_006852m PA  | AT4G32720 | 0.079634542 | -2.04250915  | Zero DAP | 4  | 27.3.99     | RNA                   |
| cassava4.1_015620m PA  | AT5G59240 | 0.079939539 | -1.266170537 | Zero DAP | 5  | 29.2.2      | protein               |
| cassava4.1_011616m PA  | AT5G14790 | 0.080114724 | -1.547288808 | Zero DAP | 4  | 35.2        | not assigned          |
| cassava4.1_005636m PA  | AT5G03070 | 0.080493635 | -1.742289734 | Zero DAP | 3  | 29.3.1      | protein               |
| gi 169794114 ref YP_OC | -         | 0.081117879 | -1.245412229 | Zero DAP | 2  | -           | -                     |
| cassava4.1_006174m PA  | AT4G31500 | 0.081818635 | -2.755157503 | Zero DAP | 11 | 26.10       | misc                  |
| cassava4.1_012531m PA  | AT1G30910 | 0.082023184 | -2.487124039 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_002190m PA  | AT2G21630 | 0.082384323 | -1.194759377 | Zero DAP | 3  | 29.3.4.2    | protein               |
| cassava4.1_023808m PA  | AT4G18060 | 0.08258256  | -6.048926736 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_000109m PA  | AT3G60860 | 0.084386708 | -2.383048761 | Zero DAP | 5  | 30.5        | signalling            |
| cassava4.1_002291m PA  | AT4G01400 | 0.084628252 | -2.559372295 | Zero DAP | 2  | 35.1.5      | not assigned          |
| cassava4.1_002970m PA  | AT5G06600 | 0.084756188 | -1.786200253 | Zero DAP | 6  | 29.5.11.05  | protein               |
| cassava4.1_001477m PA  | AT3G08947 | 0.08495288  | -1.368632025 | Zero DAP | 16 | 29.3.1      | protein               |
| cassava4.1_005303m PA  | AT3G06720 | 0.085963972 | -2.257961636 | Zero DAP | 2  | 29.3.1      | protein               |
| cassava4.1_020642m PA  | AT1G04820 | 0.087067372 | -3.040400543 | Zero DAP | 2  | 31.1        | cell                  |
| cassava4.1_003015m PA  | AT3G05420 | 0.089463985 | -1.817427059 | Zero DAP | 12 | 11.1.013    | lipid metabolism      |
| cassava4.1_001793m PA  | AT1G50500 | 0.08975524  | -1.690577435 | Zero DAP | 7  | 31.4        | cell                  |
| cassava4.1_000865m PA  | AT4G11420 | 0.090161722 | -1.196171848 | Zero DAP | 13 | 29.2.3      | protein               |
| cassava4.1_015437m PA  | AT5G58590 | 0.09103674  | -1.258089102 | Zero DAP | 8  | 30.5        | signalling            |
| cassava4.1_006760m PA  | AT1G09020 | 0.091367694 | -1.4026111   | Zero DAP | 3  | 29.4        | protein               |
| cassava4.1_000994m PA  | AT2G30110 | 0.091971719 | -1.507985879 | Zero DAP | 23 | 29.5.11.02  | protein               |
| cassava4.1_016908m PA  | AT4G26910 | 0.093036114 | -1.310853517 | Zero DAP | 4  | 35.1        | not assigned          |
| cassava4.1_010431m PA  | AT1G20330 | 0.097023816 | -2.235505532 | Zero DAP | 3  | 17.3.1.2.02 | hormone metabolism    |
| cassava4.1_001508m PA  | AT5G65700 | 0.097765045 | -2.867744863 | Zero DAP | 2  | 30.2.11     | signalling            |

|                       |           |             |              |          |    |            |                      |
|-----------------------|-----------|-------------|--------------|----------|----|------------|----------------------|
| cassava4.1_005625m PA | AT3G10410 | 0.099803555 | -1.611033817 | Zero DAP | 4  | 29.5.05    | protein              |
| cassava4.1_008671m PA | AT1G06070 | 0.100032945 | -1.387773873 | Zero DAP | 2  | 27.3.35    | RNA                  |
| cassava4.1_006491m PA | AT1G60170 | 0.100055472 | -1.643872473 | Zero DAP | 2  | 27.1       | RNA                  |
| cassava4.1_003721m PA | AT1G04430 | 0.100761703 | -9.068010148 | Zero DAP | 2  | 20.2.3     | stress               |
| cassava4.1_013512m PA | AT3G51140 | 0.101577055 | -1.175635697 | Zero DAP | 2  | 35.2       | not assigned         |
| cassava4.1_003290m PA | AT1G13900 | 0.105466169 | -2.566813481 | Zero DAP | 3  | 26.13      | misc                 |
| cassava4.1_032947m PA | AT4G30160 | 0.105915202 | -1.342317055 | Zero DAP | 3  | 31.1       | cell                 |
| cassava4.1_001980m PA | AT5G26742 | 0.10807087  | -7.844273698 | Zero DAP | 3  | 27.1.2     | RNA                  |
| cassava4.1_004797m PA | AT5G58440 | 0.110277653 | -1.149929618 | Zero DAP | 2  | 35.1       | not assigned         |
| cassava4.1_003222m PA | AT4G12650 | 0.110329636 | -1.594832305 | Zero DAP | 2  | 35.2       | not assigned         |
| cassava4.1_000440m PA | AT1G62020 | 0.111246532 | -2.922815281 | Zero DAP | 2  | 31.4       | cell                 |
| cassava4.1_009478m PA | AT5G45620 | 0.111660934 | -1.129471775 | Zero DAP | 6  | 29.5.11.20 | protein              |
| cassava4.1_017157m PA | AT5G58070 | 0.112040016 | -2.296720027 | Zero DAP | 2  | 35.1       | not assigned         |
| cassava4.1_010852m PA | AT3G13224 | 0.112315629 | -1.384551058 | Zero DAP | 7  | 35.1       | not assigned         |
| cassava4.1_031963m PA | AT3G63460 | 0.112933811 | -1.6120225   | Zero DAP | 14 | 35.1       | not assigned         |
| cassava4.1_003449m PA | AT3G13772 | 0.113512858 | -1.955864771 | Zero DAP | 5  | 35.2       | not assigned         |
| cassava4.1_001449m PA | AT1G60070 | 0.114134389 | -1.354192059 | Zero DAP | 5  | 31.4       | cell                 |
| cassava4.1_001607m PA | AT1G79920 | 0.114584689 | -1.768400216 | Zero DAP | 23 | 20.2.1     | stress               |
| cassava4.1_012525m PA | AT4G05320 | 0.11640596  | -1.201344302 | Zero DAP | 6  | 29.5.11.01 | protein              |
| cassava4.1_027656m PA | AT5G24710 | 0.117798534 | -1.471117978 | Zero DAP | 3  | 35.1       | not assigned         |
| cassava4.1_002180m PA | AT3G19720 | 0.117890691 | -1.540618657 | Zero DAP | 2  | 26.17      | misc                 |
| cassava4.1_009199m PA | AT2G38860 | 0.118047526 | -1.563518764 | Zero DAP | 12 | 29.5       | protein              |
| cassava4.1_000554m PA | AT1G15750 | 0.118173515 | -2.902186386 | Zero DAP | 5  | 35.1       | not assigned         |
| cassava4.1_012494m PA | AT2G19590 | 0.118297569 | -3.316619024 | Zero DAP | 5  | 17.5.1.02  | hormone metabolism   |
| cassava4.1_001229m PA | AT5G50920 | 0.118929068 | -1.271468742 | Zero DAP | 40 | 29.5.05    | protein              |
| cassava4.1_008118m PA | AT1G05350 | 0.119580607 | -1.358755153 | Zero DAP | 5  | 35.1       | not assigned         |
| cassava4.1_005668m PA | AT5G07990 | 0.119827044 | -2.21899024  | Zero DAP | 5  | 16.8.3.03  | secondary metabolism |
| cassava4.1_006080m PA | AT3G16760 | 0.120472478 | -2.856264717 | Zero DAP | 2  | 35.1.5     | not assigned         |
| cassava4.1_001815m PA | AT3G13870 | 0.120689931 | -2.228153322 | Zero DAP | 3  | 33.99      | development          |
| cassava4.1_029240m PA | AT1G61790 | 0.120798119 | -2.045090452 | Zero DAP | 3  | 34.3       | transport            |
| cassava4.1_000444m PA | AT3G55200 | 0.121329403 | -1.948516064 | Zero DAP | 7  | 27.1.1     | RNA                  |
| cassava4.1_001480m PA | AT5G53480 | 0.125371776 | -1.524350855 | Zero DAP | 5  | 29.3.1     | protein              |
| cassava4.1_011499m PA | AT1G77470 | 0.128009951 | -2.141876915 | Zero DAP | 2  | 28.1       | DNA                  |
| cassava4.1_001232m PA | AT5G50920 | 0.128090013 | -1.240039682 | Zero DAP | 16 | 29.5.05    | protein              |
| cassava4.1_001434m PA | AT3G02760 | 0.128444198 | -1.178789582 | Zero DAP | 30 | 29.1.021   | protein              |
| cassava4.1_004814m PA | AT5G60640 | 0.128786431 | -1.051798155 | Zero DAP | 16 | 21.01      | redox                |
| cassava4.1_014781m PA | AT1G10510 | 0.129305678 | -1.243120042 | Zero DAP | 2  | 33.99      | development          |
| cassava4.1_011773m PA | AT2G46290 | 0.12968384  | -1.139854529 | Zero DAP | 11 | 29.2.3     | protein              |
| cassava4.1_023691m PA | AT2G44060 | 0.130300953 | -1.319418858 | Zero DAP | 3  | 33.2       | development          |
| cassava4.1_012246m PA | AT4G23420 | 0.130825845 | -1.707989569 | Zero DAP | 5  | 26.22      | misc                 |
| cassava4.1_005412m PA | AT5G05010 | 0.131514634 | -1.387211294 | Zero DAP | 16 | 31.4       | cell                 |
| cassava4.1_001943m PA | AT1G25350 | 0.133228633 | -1.108658488 | Zero DAP | 19 | 29.1.018   | protein              |
| cassava4.1_007640m PA | AT5G12250 | 0.133797057 | -1.403905509 | Zero DAP | 2  | 31.1       | cell                 |
| cassava4.1_005236m PA | AT4G05420 | 0.134489575 | -1.406391523 | Zero DAP | 8  | 20.2.5     | stress               |

|                       |           |             |              |          |    |              |                       |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------|
| cassava4.1_005236m PA | AT4G05420 | 0.134489575 | -1.406391523 | Zero DAP | 8  | 29.5.11.4.06 | protein               |
| cassava4.1_000940m PA | AT2G27880 | 0.134544783 | -1.83663712  | Zero DAP | 5  | 27.3.36      | RNA                   |
| cassava4.1_000940m PA | AT2G27880 | 0.134544783 | -1.83663712  | Zero DAP | 5  | 33.99        | development           |
| cassava4.1_002411m PA | AT2G45000 | 0.134562973 | -1.573681723 | Zero DAP | 3  | 35.2         | not assigned          |
| cassava4.1_011524m PA | AT2G20760 | 0.13630996  | -1.232886514 | Zero DAP | 3  | 35.2         | not assigned          |
| cassava4.1_000381m PA | AT5G64270 | 0.137181746 | -1.826199668 | Zero DAP | 6  | 27.1.1       | RNA                   |
| cassava4.1_018507m PA | AT3G51030 | 0.13733635  | -2.05086728  | Zero DAP | 5  | 21.01        | redox                 |
| cassava4.1_009143m PA | AT4G38970 | 0.137753418 | -1.391879393 | Zero DAP | 7  | 1.3.06       | PS                    |
| cassava4.1_001424m PA | AT1G52360 | 0.138747233 | -1.500370263 | Zero DAP | 18 | 31.4         | cell                  |
| cassava4.1_012192m PA | AT5G12380 | 0.141518046 | -1.514301967 | Zero DAP | 15 | 31.1         | cell                  |
| cassava4.1_001931m PA | AT2G40730 | 0.142233519 | -1.271409941 | Zero DAP | 4  | 29.4         | protein               |
| cassava4.1_001869m PA | AT4G13780 | 0.144364779 | -1.29059591  | Zero DAP | 12 | 29.1.010     | protein               |
| cassava4.1_007330m PA | AT1G32440 | 0.145350852 | -1.355625365 | Zero DAP | 7  | 11.1.030     | lipid metabolism      |
| cassava4.1_014620m PA | AT3G57650 | 0.148190655 | -2.745739887 | Zero DAP | 2  | 11.3.01      | lipid metabolism      |
| cassava4.1_000048m PA | AT2G22125 | 0.148935796 | -2.031665254 | Zero DAP | 10 | 35.1.19      | not assigned          |
| cassava4.1_001100m PA | AT5G42220 | 0.149072629 | -3.043880986 | Zero DAP | 3  | 29.2.3       | protein               |
| cassava4.1_006899m PA | AT5G62790 | 0.150722774 | -1.53472782  | Zero DAP | 7  | 16.1.1.02    | secondary metabolism  |
| cassava4.1_000611m PA | AT3G03380 | 0.15094663  | -3.052047343 | Zero DAP | 4  | 29.5.05      | protein               |
| cassava4.1_018340m PA | -         | 0.150958293 | -3.564582787 | Zero DAP | 2  | -            | -                     |
| cassava4.1_010414m PA | AT3G08900 | 0.151645903 | -1.395277088 | Zero DAP | 3  | 10.5.5       | cell wall             |
| cassava4.1_009041m PA | AT5G63180 | 0.152614979 | -11.18212258 | Zero DAP | 2  | 10.6.3       | cell wall             |
| cassava4.1_015453m PA | AT5G41210 | 0.152957014 | -1.713153327 | Zero DAP | 2  | 26.09        | misc                  |
| cassava4.1_003552m PA | AT3G18670 | 0.153263822 | -1.390270974 | Zero DAP | 3  | 31.1         | cell                  |
| cassava4.1_007502m PA | AT1G50010 | 0.154095892 | -1.32270652  | Zero DAP | 25 | 31.1         | cell                  |
| cassava4.1_018011m PA | AT3G59440 | 0.15474751  | -4.664042421 | Zero DAP | 2  | 30.3         | signalling            |
| cassava4.1_002425m PA | AT2G34680 | 0.155243465 | -1.571500894 | Zero DAP | 6  | 35.1         | not assigned          |
| cassava4.1_010782m PA | AT3G57560 | 0.155515992 | -1.130361263 | Zero DAP | 4  | 13.1.2.3.02  | amino acid metabolism |
| cassava4.1_010782m PA | AT3G57560 | 0.155515992 | -1.130361263 | Zero DAP | 4  | 23.4.99      | nucleotide metabolism |
| cassava4.1_009544m PA | AT1G48420 | 0.156454004 | -1.142350167 | Zero DAP | 7  | 17.5.1       | hormone metabolism    |
| cassava4.1_008836m PA | AT2G34560 | 0.157047562 | -5.203690154 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_011926m PA | AT5G06570 | 0.157682544 | -1.212155658 | Zero DAP | 5  | 35.1         | not assigned          |
| cassava4.1_002830m PA | AT4G24520 | 0.158050628 | -1.621137992 | Zero DAP | 6  | 16.2         | secondary metabolism  |
| cassava4.1_002830m PA | AT4G24520 | 0.158050628 | -1.621137992 | Zero DAP | 6  | 26.10        | misc                  |
| cassava4.1_008057m PA | AT5G67150 | 0.158706658 | -2.809675125 | Zero DAP | 2  | 16.2         | secondary metabolism  |
| cassava4.1_004670m PA | AT3G19820 | 0.160897762 | -1.333037762 | Zero DAP | 20 | 17.3.1.2.08  | hormone metabolism    |
| cassava4.1_009917m PA | AT1G79460 | 0.161307928 | -4.098475722 | Zero DAP | 4  | 17.6.1.02    | hormone metabolism    |
| cassava4.1_001388m PA | AT4G34450 | 0.161666381 | -1.230983914 | Zero DAP | 29 | 31.4         | cell                  |
| cassava4.1_016503m PA | AT1G17730 | 0.161797139 | -1.308875945 | Zero DAP | 4  | 27.3.71      | RNA                   |
| cassava4.1_012474m PA | AT2G40060 | 0.164207855 | -1.982885352 | Zero DAP | 2  | 35.2         | not assigned          |
| cassava4.1_000550m PA | AT3G15880 | 0.164494254 | -1.839507307 | Zero DAP | 3  | 33.99        | development           |
| cassava4.1_005440m PA | AT3G52990 | 0.165048789 | -1.227373338 | Zero DAP | 27 | 4.013        | glycolysis            |
| cassava4.1_006418m PA | AT4G38510 | 0.165759715 | -1.207238125 | Zero DAP | 7  | 34.1.01      | transport             |
| cassava4.1_009273m PA | AT2G34510 | 0.167148444 | -1.851998178 | Zero DAP | 3  | 35.2         | not assigned          |
| cassava4.1_002873m PA | AT5G64390 | 0.168803621 | -1.575077873 | Zero DAP | 3  | 27.4         | RNA                   |

|                       |           |             |               |          |    |               |                       |
|-----------------------|-----------|-------------|---------------|----------|----|---------------|-----------------------|
| cassava4.1_005700m PA | AT3G01090 | 0.168848088 | -1.181970191  | Zero DAP | 15 | 29.4          | protein               |
| cassava4.1_005700m PA | AT3G01090 | 0.168848088 | -1.181970191  | Zero DAP | 15 | 30.1          | signalling            |
| cassava4.1_026724m PA | AT3G53180 | 0.170053256 | -1.2548219873 | Zero DAP | 4  | 12.2.02       | N-metabolism          |
| cassava4.1_032172m PA | AT2G37230 | 0.170713847 | -1.342185483  | Zero DAP | 3  | 35.1.5        | not assigned          |
| cassava4.1_000807m PA | AT1G20760 | 0.171396572 | -1.421182322  | Zero DAP | 2  | 30.3          | signalling            |
| cassava4.1_027898m PA | AT3G44330 | 0.172686971 | -1.464103018  | Zero DAP | 2  | 35.2          | not assigned          |
| cassava4.1_029890m PA | AT2G34680 | 0.173017946 | -1.712428922  | Zero DAP | 2  | 35.1          | not assigned          |
| cassava4.1_001879m PA | AT5G03340 | 0.175433659 | -1.154708067  | Zero DAP | 45 | 31.2          | cell                  |
| cassava4.1_006979m PA | AT5G17310 | 0.17657924  | -1.139212866  | Zero DAP | 6  | 4.01          | glycolysis            |
| cassava4.1_009442m PA | AT3G15010 | 0.176983182 | -2.701525944  | Zero DAP | 5  | 27.4          | RNA                   |
| cassava4.1_006154m PA | AT5G23630 | 0.177095447 | -1.398042048  | Zero DAP | 2  | 34.14         | transport             |
| cassava4.1_000768m PA | AT2G32730 | 0.178686751 | -1.359203907  | Zero DAP | 17 | 29.5.11.20    | protein               |
| cassava4.1_001650m PA | AT5G11490 | 0.178930633 | -1.11602864   | Zero DAP | 8  | 31.4          | cell                  |
| cassava4.1_005320m PA | AT4G23650 | 0.179076187 | -1.283838051  | Zero DAP | 4  | 30.3          | signalling            |
| cassava4.1_000300m PA | AT4G30100 | 0.179378173 | -1.704186746  | Zero DAP | 2  | 28.1          | DNA                   |
| cassava4.1_007263m PA | AT5G43960 | 0.179497947 | -1.426903179  | Zero DAP | 4  | 29.3.1        | protein               |
| cassava4.1_007614m PA | AT5G23860 | 0.18042207  | -1.490929643  | Zero DAP | 11 | 31.1          | cell                  |
| cassava4.1_005209m PA | AT1G22410 | 0.183508087 | -2.040400684  | Zero DAP | 2  | 13.1.6.1.01   | amino acid metabolism |
| cassava4.1_012174m PA | AT1G62290 | 0.183874945 | -1.357770277  | Zero DAP | 5  | 29.5.04       | protein               |
| cassava4.1_004928m PA | AT2G34040 | 0.184594777 | -1.469667419  | Zero DAP | 15 | 33.99         | development           |
| cassava4.1_000160m PA | AT3G50590 | 0.185483159 | -1.377757597  | Zero DAP | 9  | 35.1          | not assigned          |
| cassava4.1_006336m PA | AT5G36890 | 0.186046191 | -1.511666104  | Zero DAP | 4  | 26.03         | misc                  |
| cassava4.1_034227m PA | AT2G36770 | 0.1864669   | -3.537260731  | Zero DAP | 3  | 26.02         | misc                  |
| cassava4.1_002236m PA | AT4G24840 | 0.187132771 | -1.484578297  | Zero DAP | 7  | 35.2          | not assigned          |
| cassava4.1_003255m PA | AT5G23570 | 0.18738984  | -2.575520242  | Zero DAP | 3  | 27.3.99       | RNA                   |
| cassava4.1_000597m PA | AT1G29900 | 0.189301558 | -1.149953736  | Zero DAP | 18 | 13.1.2.3.011  | amino acid metabolism |
| cassava4.1_000597m PA | AT1G29900 | 0.189301558 | -1.149953736  | Zero DAP | 18 | 23.1.1.01     | nucleotide metabolism |
| cassava4.1_001871m PA | AT3G43190 | 0.190463169 | -1.15987115   | Zero DAP | 82 | 2.2.1.05      | major CHO metabolism  |
| cassava4.1_023285m PA | AT4G26000 | 0.191609761 | -1.628176037  | Zero DAP | 3  | 27.3.99       | RNA                   |
| cassava4.1_003785m PA | AT1G26850 | 0.192042495 | -3.440469102  | Zero DAP | 3  | 20.2.3        | stress                |
| cassava4.1_001669m PA | AT2G23140 | 0.192209488 | -1.385969451  | Zero DAP | 4  | 29.5.11.04.02 | protein               |
| cassava4.1_003934m PA | AT4G33070 | 0.192713043 | -1.283526861  | Zero DAP | 10 | 5.02          | fermentation          |
| cassava4.1_028367m PA | AT2G28760 | 0.192730085 | -1.428537111  | Zero DAP | 14 | 10.1.05       | cell wall             |
| cassava4.1_003067m PA | AT1G78570 | 0.194482964 | -3.682132649  | Zero DAP | 4  | 10.1.010      | cell wall             |
| cassava4.1_005183m PA | AT1G71440 | 0.195370789 | -1.348707413  | Zero DAP | 6  | 31.1          | cell                  |
| cassava4.1_007612m PA | AT1G06570 | 0.195572251 | -2.091793481  | Zero DAP | 2  | 13.2.6.2      | amino acid metabolism |
| cassava4.1_007612m PA | AT1G06570 | 0.195572251 | -2.091793481  | Zero DAP | 2  | 16.1.3.01     | secondary metabolism  |
| cassava4.1_009173m PA | AT2G43710 | 0.195830622 | -1.705688301  | Zero DAP | 6  | 11.1.015      | lipid metabolism      |
| cassava4.1_012653m PA | AT3G53970 | 0.196201459 | -1.359692197  | Zero DAP | 3  | 29.5.11.20    | protein               |
| cassava4.1_012020m PA | AT5G40150 | 0.197270192 | -2.269539678  | Zero DAP | 9  | 26.12         | misc                  |
| cassava4.1_002328m PA | AT2G27460 | 0.197789123 | -1.436845564  | Zero DAP | 6  | 29.3.4.2      | protein               |
| cassava4.1_001227m PA | AT1G59610 | 0.197986575 | -1.795119919  | Zero DAP | 5  | 26.17         | misc                  |
| cassava4.1_010265m PA | AT5G51970 | 0.199056082 | -1.29567229   | Zero DAP | 6  | 3.3           | minor CHO metabolism  |
| cassava4.1_000030m PA | AT1G80070 | 0.199707833 | -1.435209516  | Zero DAP | 5  | 27.1.1        | RNA                   |

|                       |           |             |              |          |    |             |                       |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_004602m PA | AT1G12000 | 0.199752268 | -1.274753778 | Zero DAP | 18 | 4.05        | glycolysis            |
| cassava4.1_005714m PA | AT1G17720 | 0.199930828 | -1.346232339 | Zero DAP | 9  | 29.4        | protein               |
| cassava4.1_034367m PA | AT1G62730 | 0.200169606 | -1.189748199 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_026237m PA | AT5G41040 | 0.200599264 | -1.402533889 | Zero DAP | 4  | 35.1        | not assigned          |
| cassava4.1_006625m PA | AT4G26270 | 0.200778451 | -1.371593061 | Zero DAP | 2  | 4.04        | glycolysis            |
| cassava4.1_011984m PA | AT2G40010 | 0.200941812 | -1.129150903 | Zero DAP | 8  | 29.2.2      | protein               |
| cassava4.1_010169m PA | AT5G02890 | 0.201058565 | -1.985887814 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_003814m PA | AT1G20950 | 0.201283797 | -1.352083741 | Zero DAP | 13 | 4.05        | glycolysis            |
| cassava4.1_001228m PA | AT2G41740 | 0.203114372 | -1.852213017 | Zero DAP | 10 | 31.1        | cell                  |
| cassava4.1_004227m PA | AT3G58610 | 0.204035797 | -1.111224656 | Zero DAP | 24 | 13.1.4.1    | amino acid metabolism |
| cassava4.1_005249m PA | AT1G24510 | 0.204298987 | -1.31056531  | Zero DAP | 13 | 29.4        | protein               |
| cassava4.1_005439m PA | AT5G20890 | 0.204394373 | -1.391873271 | Zero DAP | 23 | 29.6        | protein               |
| cassava4.1_006134m PA | AT3G62120 | 0.205193741 | -1.54972133  | Zero DAP | 13 | 29.1.040    | protein               |
| cassava4.1_011566m PA | AT1G60650 | 0.20529678  | -1.802531438 | Zero DAP | 2  | 27.4        | RNA                   |
| cassava4.1_011578m PA | AT3G59480 | 0.20581161  | -1.514375705 | Zero DAP | 10 | 2.2.1.01    | major CHO metabolism  |
| cassava4.1_004457m PA | AT3G22960 | 0.207034125 | -1.396069182 | Zero DAP | 4  | 11.1.030    | lipid metabolism      |
| cassava4.1_000834m PA | AT5G22780 | 0.207105807 | -2.30384455  | Zero DAP | 12 | 31.4        | cell                  |
| cassava4.1_006013m PA | AT5G56350 | 0.208583385 | -1.299462423 | Zero DAP | 8  | 4.013       | glycolysis            |
| cassava4.1_000953m PA | AT5G07350 | 0.211352088 | -10.93597897 | Zero DAP | 2  | 27.3.73     | RNA                   |
| cassava4.1_008135m PA | AT1G60780 | 0.211897339 | -1.201202497 | Zero DAP | 4  | 29.3.4.99   | protein               |
| cassava4.1_006498m PA | AT4G13940 | 0.212321039 | -1.402188106 | Zero DAP | 14 | 13.2.3.4    | amino acid metabolism |
| cassava4.1_003461m PA | AT5G57460 | 0.212824778 | -1.353412852 | Zero DAP | 9  | 35.2        | not assigned          |
| cassava4.1_001134m PA | AT4G31480 | 0.214756289 | -1.301132204 | Zero DAP | 26 | 31.4        | cell                  |
| cassava4.1_009487m PA | AT2G33840 | 0.215969314 | -1.484034669 | Zero DAP | 2  | 29.1.01     | protein               |
| cassava4.1_001702m PA | AT5G22780 | 0.216769327 | -5.251768037 | Zero DAP | 2  | 31.4        | cell                  |
| cassava4.1_006168m PA | AT1G09870 | 0.217372962 | -1.985626681 | Zero DAP | 3  | 26.13       | misc                  |
| cassava4.1_001093m PA | AT1G50200 | 0.219440275 | -1.421112712 | Zero DAP | 30 | 29.1.07     | protein               |
| cassava4.1_002175m PA | AT1G78950 | 0.220116202 | -199.8256438 | Zero DAP | 2  | 16.1.5      | secondary metabolism  |
| cassava4.1_006145m PA | AT5G46570 | 0.221731178 | -1.227336239 | Zero DAP | 4  | 29.4        | protein               |
| cassava4.1_012958m PA | AT3G48750 | 0.222334258 | -1.690177755 | Zero DAP | 4  | 31.3        | cell                  |
| cassava4.1_001000m PA | AT5G26860 | 0.222545455 | -1.246617507 | Zero DAP | 7  | 29.5.05     | protein               |
| cassava4.1_013358m PA | AT2G36870 | 0.223033528 | -1.615915878 | Zero DAP | 4  | 10.7        | cell wall             |
| cassava4.1_002768m PA | AT3G51050 | 0.224300663 | -1.389298313 | Zero DAP | 7  | 35.1        | not assigned          |
| cassava4.1_003825m PA | AT3G14240 | 0.224400237 | -1.541947741 | Zero DAP | 7  | 29.5.01     | protein               |
| cassava4.1_002221m PA | AT1G45332 | 0.225998227 | -2.491900698 | Zero DAP | 4  | 29.2.4      | protein               |
| cassava4.1_002371m PA | AT3G55610 | 0.22724297  | -1.650303001 | Zero DAP | 5  | 13.1.2.2.01 | amino acid metabolism |
| cassava4.1_008895m PA | AT1G62640 | 0.228149083 | -1.08681315  | Zero DAP | 3  | 11.1.03     | lipid metabolism      |
| cassava4.1_000821m PA | AT5G43470 | 0.228755922 | -1.245165899 | Zero DAP | 4  | 20.1        | stress                |
| cassava4.1_007694m PA | AT4G29040 | 0.230710714 | -1.37875563  | Zero DAP | 24 | 29.5.11.20  | protein               |
| cassava4.1_005251m PA | AT1G56110 | 0.231646073 | -1.348690326 | Zero DAP | 12 | 27.3.67     | RNA                   |
| cassava4.1_001191m PA | AT5G63840 | 0.231910911 | -1.622777138 | Zero DAP | 5  | 2.2.2.1     | major CHO metabolism  |
| cassava4.1_001191m PA | AT5G63840 | 0.231910911 | -1.622777138 | Zero DAP | 5  | 26.03       | misc                  |
| cassava4.1_000563m PA | AT3G63460 | 0.23499536  | -1.826582502 | Zero DAP | 11 | 35.1        | not assigned          |
| cassava4.1_008456m PA | AT1G63660 | 0.235604958 | -1.519950626 | Zero DAP | 3  | 23.1.2.031  | nucleotide metabolism |

|                       |           |             |              |          |    |              |                                   |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------------------|
| cassava4.1_000389m PA | AT2G16640 | 0.235725678 | -1.436239604 | Zero DAP | 8  | 29.3.3       | protein                           |
| cassava4.1_001463m PA | AT1G55020 | 0.237088458 | -1.297116648 | Zero DAP | 9  | 17.7.1.02    | hormone metabolism                |
| cassava4.1_001878m PA | AT3G53230 | 0.238249901 | -1.596671695 | Zero DAP | 21 | 31.2         | cell                              |
| cassava4.1_000767m PA | AT5G19820 | 0.238911655 | -1.196456026 | Zero DAP | 7  | 35.1         | not assigned                      |
| cassava4.1_006561m PA | AT1G22380 | 0.238925273 | -1.159010735 | Zero DAP | 16 | 26.02        | misc                              |
| cassava4.1_001379m PA | AT4G34450 | 0.239453354 | -1.74553958  | Zero DAP | 5  | 31.4         | cell                              |
| cassava4.1_000649m PA | AT1G21630 | 0.240366981 | -1.389288747 | Zero DAP | 2  | 30.3         | signalling                        |
| cassava4.1_000515m PA | AT3G01780 | 0.243266216 | -1.579602406 | Zero DAP | 4  | 35.2         | not assigned                      |
| cassava4.1_003870m PA | AT2G44160 | 0.243319965 | -1.332936552 | Zero DAP | 17 | 25.06        | C1-metabolism                     |
| cassava4.1_002853m PA | AT5G55230 | 0.244521017 | -1.433043746 | Zero DAP | 7  | 31.1         | cell                              |
| cassava4.1_017924m PA | AT1G77120 | 0.245352276 | -1.280134892 | Zero DAP | 3  | 5.03         | fermentation                      |
| cassava4.1_005262m PA | AT4G39980 | 0.246187446 | -2.943840189 | Zero DAP | 2  | 13.1.6.1.01  | amino acid metabolism             |
| cassava4.1_002006m PA | AT1G73430 | 0.247874896 | -1.707889235 | Zero DAP | 3  | 29.3.4.2     | protein                           |
| cassava4.1_030986m PA | AT1G65810 | 0.249427138 | -1.578963638 | Zero DAP | 2  | 28.1         | DNA                               |
| cassava4.1_000654m PA | AT1G09620 | 0.25010725  | -1.24862701  | Zero DAP | 26 | 29.1.04      | protein                           |
| cassava4.1_004725m PA | AT3G25140 | 0.251031865 | -6.077008193 | Zero DAP | 2  | 10.4         | cell wall                         |
| cassava4.1_000885m PA | AT2G32730 | 0.252078337 | -1.547671435 | Zero DAP | 4  | 29.5.11.20   | protein                           |
| cassava4.1_003908m PA | AT5G49460 | 0.252250075 | -1.104198522 | Zero DAP | 6  | 8.2.011      | TCA / org                         |
| cassava4.1_004682m PA | AT3G10380 | 0.252476691 | -3.226190744 | Zero DAP | 2  | 31.4         | cell                              |
| cassava4.1_005784m PA | AT1G21380 | 0.252775032 | -1.094178875 | Zero DAP | 2  | 31.4         | cell                              |
| cassava4.1_026305m PA | AT4G33790 | 0.2549199   | -1.797641338 | Zero DAP | 4  | 11.9.4.013   | lipid metabolism                  |
| cassava4.1_004982m PA | AT5G49020 | 0.25528538  | -1.7310998   | Zero DAP | 4  | 26.06        | misc                              |
| cassava4.1_004065m PA | AT3G54860 | 0.255425181 | -1.392578982 | Zero DAP | 4  | 29.3.4.3     | protein                           |
| cassava4.1_015786m PA | AT1G04760 | 0.257468854 | -1.690005813 | Zero DAP | 7  | 31.4         | cell                              |
| cassava4.1_008185m PA | AT1G15200 | 0.259637505 | -1.285463348 | Zero DAP | 3  | 35.1         | not assigned                      |
| cassava4.1_000279m PA | AT2G32240 | 0.260738828 | -1.198183402 | Zero DAP | 80 | 35.2         | not assigned                      |
| cassava4.1_006672m PA | AT5G34850 | 0.261518859 | -1.538427908 | Zero DAP | 2  | 26.13        | misc                              |
| cassava4.1_005313m PA | AT5G20860 | 0.262365491 | -1.258576374 | Zero DAP | 3  | 10.8.01      | cell wall                         |
| cassava4.1_013178m PA | AT1G31860 | 0.263045671 | -1.367502031 | Zero DAP | 2  | 13.1.7.02    | amino acid metabolism             |
| cassava4.1_001370m PA | AT3G51550 | 0.263188641 | -3.07717149  | Zero DAP | 3  | 30.2.16      | signalling                        |
| cassava4.1_008061m PA | AT5G07030 | 0.263401797 | -1.037561364 | Zero DAP | 15 | 27.3.67      | RNA                               |
| cassava4.1_002129m PA | AT2G38040 | 0.263784161 | -1.722290621 | Zero DAP | 10 | 16.99        | secondary metabolism              |
| cassava4.1_000665m PA | AT5G49810 | 0.264332214 | -1.200698529 | Zero DAP | 5  | 13.1.3.4.011 | amino acid metabolism             |
| cassava4.1_003557m PA | AT2G14740 | 0.265111518 | -1.912701255 | Zero DAP | 2  | 29.3.4.3     | protein                           |
| cassava4.1_002974m PA | AT2G25970 | 0.265481579 | -1.147835231 | Zero DAP | 14 | 35.1         | not assigned                      |
| cassava4.1_010114m PA | AT4G35360 | 0.267501656 | -1.215661666 | Zero DAP | 6  | 18.4.01      | Co-factor and vitamine metabolism |
| cassava4.1_010334m PA | AT3G08900 | 0.26827522  | -1.039515748 | Zero DAP | 17 | 10.5.5       | cell wall                         |
| cassava4.1_005989m PA | AT1G55150 | 0.268806172 | -1.228147207 | Zero DAP | 4  | 17.5.3       | hormone metabolism                |
| cassava4.1_007010m PA | AT2G41540 | 0.269642611 | -2.070434811 | Zero DAP | 2  | 11.5.02      | lipid metabolism                  |
| cassava4.1_007854m PA | AT4G00090 | 0.270231492 | -1.483635555 | Zero DAP | 2  | 33.99        | development                       |
| cassava4.1_004898m PA | AT5G12370 | 0.270695696 | -1.131926227 | Zero DAP | 8  | 35.1         | not assigned                      |
| cassava4.1_034217m PA | AT2G17980 | 0.270892357 | -1.379714656 | Zero DAP | 4  | 29.3.4.4     | protein                           |
| cassava4.1_001603m PA | AT1G67930 | 0.272193318 | -1.670543317 | Zero DAP | 7  | 31.4         | cell                              |
| cassava4.1_002657m PA | AT3G01680 | 0.273286148 | -1.081422376 | Zero DAP | 17 | 35.2         | not assigned                      |

|                       |           |             |              |          |    |              |                       |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------|
| cassava4.1_002628m PA | AT3G53260 | 0.274821949 | -2.263289102 | Zero DAP | 4  | 16.2.1.01    | secondary metabolism  |
| cassava4.1_013475m PA | AT5G17770 | 0.275931945 | -1.404083477 | Zero DAP | 9  | 21.99        | redox                 |
| cassava4.1_002048m PA | AT3G14067 | 0.276882501 | -1.294899475 | Zero DAP | 4  | 29.5.01      | protein               |
| cassava4.1_013884m PA | AT4G17720 | 0.278089603 | -1.06640368  | Zero DAP | 5  | 27.4         | RNA                   |
| cassava4.1_007549m PA | AT1G75780 | 0.28133407  | -1.335809098 | Zero DAP | 19 | 31.1         | cell                  |
| cassava4.1_008778m PA | AT2G34590 | 0.283120183 | -1.064644164 | Zero DAP | 6  | 11.1.031     | lipid metabolism      |
| cassava4.1_013875m PA | AT3G02870 | 0.283929068 | -1.203425686 | Zero DAP | 8  | 3.4.05       | minor CHO metabolism  |
| cassava4.1_001841m PA | AT2G21790 | 0.292408753 | -1.809298426 | Zero DAP | 2  | 23.5.04      | nucleotide metabolism |
| cassava4.1_003281m PA | AT2G25430 | 0.2928494   | -1.313091884 | Zero DAP | 8  | 35.1.21      | not assigned          |
| cassava4.1_004789m PA | AT4G26270 | 0.295388041 | -1.119311497 | Zero DAP | 15 | 4.04         | glycolysis            |
| cassava4.1_013410m PA | AT2G30620 | 0.299232771 | -1.169984478 | Zero DAP | 2  | 28.1.3       | DNA                   |
| cassava4.1_004686m PA | AT3G11830 | 0.299246034 | -1.162748818 | Zero DAP | 23 | 29.6         | protein               |
| cassava4.1_015822m PA | AT4G20410 | 0.299700864 | -1.082045723 | Zero DAP | 3  | 29.3.4.99    | protein               |
| cassava4.1_030760m PA | AT5G51550 | 0.300249279 | -1.313388773 | Zero DAP | 4  | 35.1         | not assigned          |
| cassava4.1_001026m PA | AT2G46520 | 0.301022394 | -1.153183263 | Zero DAP | 21 | 20.1         | stress                |
| cassava4.1_001026m PA | AT2G46520 | 0.301022394 | -1.153183263 | Zero DAP | 21 | 29.3.1       | protein               |
| cassava4.1_011702m PA | AT1G04690 | 0.301895713 | -1.078487653 | Zero DAP | 13 | 34.15        | transport             |
| cassava4.1_003876m PA | AT5G66020 | 0.302417297 | -1.294814849 | Zero DAP | 7  | 29.5         | protein               |
| cassava4.1_003876m PA | AT5G66020 | 0.302417297 | -1.294814849 | Zero DAP | 7  | 30.4         | signalling            |
| cassava4.1_014364m PA | AT5G02050 | 0.304107908 | -1.258769008 | Zero DAP | 4  | 35.1         | not assigned          |
| cassava4.1_002445m PA | AT3G62310 | 0.304908875 | -1.662332328 | Zero DAP | 3  | 27.1.2       | RNA                   |
| cassava4.1_001063m PA | AT2G05170 | 0.306275274 | -1.516262443 | Zero DAP | 2  | 29.3.4.3     | protein               |
| cassava4.1_014680m PA | AT5G10360 | 0.306893425 | -1.075013806 | Zero DAP | 14 | 29.2.2       | protein               |
| cassava4.1_001045m PA | AT3G14940 | 0.308350079 | -1.068919865 | Zero DAP | 42 | 4.014        | glycolysis            |
| cassava4.1_016203m PA | AT2G30860 | 0.309842199 | -1.61549477  | Zero DAP | 2  | 26.09        | misc                  |
| cassava4.1_005730m PA | AT1G18070 | 0.311176237 | -1.149838522 | Zero DAP | 20 | 30.5         | signalling            |
| cassava4.1_029503m PA | AT3G14470 | 0.311442231 | -1.434785994 | Zero DAP | 2  | 20.1         | stress                |
| cassava4.1_006585m PA | AT1G51710 | 0.313187601 | -1.376430101 | Zero DAP | 8  | 29.5.11.05   | protein               |
| cassava4.1_023600m PA | AT1G79690 | 0.314274331 | -1.897170296 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_013077m PA | AT2G25280 | 0.317490481 | -1.187822134 | Zero DAP | 9  | 35.2         | not assigned          |
| cassava4.1_005890m PA | AT3G18080 | 0.318592933 | -1.433046893 | Zero DAP | 2  | 26.03        | misc                  |
| cassava4.1_017618m PA | AT5G03840 | 0.318728147 | -3.198649693 | Zero DAP | 2  | 33.99        | development           |
| cassava4.1_006331m PA | AT4G14300 | 0.31956071  | -1.626441938 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_001487m PA | AT5G53480 | 0.319746418 | -1.119414225 | Zero DAP | 6  | 29.3.1       | protein               |
| cassava4.1_007561m PA | AT1G27090 | 0.320488404 | -10.08051037 | Zero DAP | 2  | 35.1.40      | not assigned          |
| cassava4.1_003275m PA | AT5G55860 | 0.321028776 | -1.308936832 | Zero DAP | 4  | 35.2         | not assigned          |
| cassava4.1_000886m PA | AT5G15400 | 0.323036765 | -1.673602724 | Zero DAP | 7  | 29.5.11      | protein               |
| cassava4.1_010218m PA | AT3G08030 | 0.323427213 | -1.526502448 | Zero DAP | 6  | 35.2         | not assigned          |
| cassava4.1_009985m PA | AT1G75330 | 0.324421766 | -1.190018734 | Zero DAP | 8  | 13.1.2.3.021 | amino acid metabolism |
| cassava4.1_011838m PA | AT5G19150 | 0.325308231 | -1.102633246 | Zero DAP | 2  | 3.5          | minor CHO metabolism  |
| cassava4.1_015465m PA | AT1G12520 | 0.325549013 | -1.157559274 | Zero DAP | 4  | 15.2         | metal handling        |
| cassava4.1_015465m PA | AT1G12520 | 0.325549013 | -1.157559274 | Zero DAP | 4  | 21.6         | redox                 |
| cassava4.1_007513m PA | AT5G19780 | 0.326323057 | -1.298802962 | Zero DAP | 10 | 31.1         | cell                  |
| cassava4.1_033198m PA | AT3G44330 | 0.327466163 | -1.471952597 | Zero DAP | 4  | 35.2         | not assigned          |

|                       |           |             |              |          |    |             |                       |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_018324m PA | AT3G60770 | 0.329562244 | -1.326923764 | Zero DAP | 7  | 29.2.2      | protein               |
| cassava4.1_008841m PA | AT1G51410 | 0.332597375 | -1.380525873 | Zero DAP | 2  | 26.11       | misc                  |
| cassava4.1_015377m PA | AT3G27310 | 0.332663541 | -1.56580164  | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_005819m PA | AT3G61050 | 0.332711437 | -1.495360313 | Zero DAP | 4  | 30.3        | signalling            |
| cassava4.1_004591m PA | AT5G11710 | 0.334291677 | -2.485800471 | Zero DAP | 2  | 35.1.21     | not assigned          |
| cassava4.1_012957m PA | AT5G26667 | 0.337281857 | -1.242398634 | Zero DAP | 11 | 23.4.3      | nucleotide metabolism |
| cassava4.1_011693m PA | AT1G59960 | 0.33866683  | -1.261127283 | Zero DAP | 17 | 16.8.2      | secondary metabolism  |
| cassava4.1_005850m PA | AT5G08570 | 0.341091752 | -1.065878932 | Zero DAP | 30 | 4.013       | glycolysis            |
| cassava4.1_001141m PA | AT2G36810 | 0.341663472 | -1.536059747 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_000863m PA | AT1G50200 | 0.345706112 | -1.732394586 | Zero DAP | 3  | 29.1.07     | protein               |
| cassava4.1_006409m PA | AT4G38510 | 0.349462983 | -1.191834226 | Zero DAP | 25 | 34.1.01     | transport             |
| cassava4.1_015404m PA | AT5G66410 | 0.350208928 | -1.320775254 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_010809m PA | AT4G22880 | 0.351992526 | -1.650067301 | Zero DAP | 5  | 16.8.1.01   | secondary metabolism  |
| cassava4.1_007973m PA | AT1G01090 | 0.355072418 | -1.509752752 | Zero DAP | 3  | 11.1.031    | lipid metabolism      |
| cassava4.1_003859m PA | AT5G42080 | 0.356007274 | -1.404716505 | Zero DAP | 2  | 26.17       | misc                  |
| cassava4.1_003859m PA | AT5G42080 | 0.356007274 | -1.404716505 | Zero DAP | 2  | 30.5        | signalling            |
| cassava4.1_016211m PA | AT5G05270 | 0.356095699 | -1.755159688 | Zero DAP | 2  | 16.8.2      | secondary metabolism  |
| cassava4.1_013996m PA | AT3G10350 | 0.358191338 | -1.391483131 | Zero DAP | 3  | 34.18.01    | transport             |
| cassava4.1_022761m PA | AT3G51160 | 0.3586267   | -1.588993815 | Zero DAP | 3  | 10.1.07     | cell wall             |
| cassava4.1_000948m PA | AT1G06220 | 0.359104038 | -1.28220935  | Zero DAP | 5  | 29.2.4      | protein               |
| cassava4.1_005735m PA | AT1G11910 | 0.359381312 | -1.389786971 | Zero DAP | 6  | 29.5.04     | protein               |
| cassava4.1_003760m PA | AT1G80270 | 0.360462179 | -1.175225196 | Zero DAP | 5  | 27.3.67     | RNA                   |
| cassava4.1_011727m PA | AT2G16760 | 0.361575973 | -1.953429017 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_034199m PA | AT3G14470 | 0.361610931 | -2.585759417 | Zero DAP | 2  | 20.1        | stress                |
| cassava4.1_021056m PA | AT2G13540 | 0.362806936 | -1.156989244 | Zero DAP | 9  | 29.2.3      | protein               |
| cassava4.1_011632m PA | AT1G18080 | 0.366769296 | -1.125578461 | Zero DAP | 8  | 27.3.99     | RNA                   |
| cassava4.1_011632m PA | AT1G18080 | 0.366769296 | -1.125578461 | Zero DAP | 8  | 33.99       | development           |
| cassava4.1_005423m PA | AT1G04510 | 0.367366596 | -1.495533348 | Zero DAP | 4  | 35.1        | not assigned          |
| cassava4.1_016645m PA | AT5G58060 | 0.369008325 | -1.409195547 | Zero DAP | 2  | 31.4        | cell                  |
| cassava4.1_024927m PA | AT1G14000 | 0.369381231 | -1.423565477 | Zero DAP | 2  | 29.4        | protein               |
| cassava4.1_026844m PA | AT1G05180 | 0.370278938 | -1.56561562  | Zero DAP | 5  | 17.2.2      | hormone metabolism    |
| cassava4.1_009036m PA | AT5G42130 | 0.372757022 | -1.465266073 | Zero DAP | 2  | 34.9        | transport             |
| cassava4.1_013677m PA | AT4G24380 | 0.37285605  | -1.279984755 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_004360m PA | AT2G41790 | 0.373177571 | -1.121934451 | Zero DAP | 13 | 29.5.07     | protein               |
| cassava4.1_008280m PA | AT4G01320 | 0.374217066 | -1.313340943 | Zero DAP | 7  | 29.5        | protein               |
| cassava4.1_007902m PA | AT3G26618 | 0.37749923  | -1.105923509 | Zero DAP | 5  | 29.2.5      | protein               |
| cassava4.1_004712m PA | AT3G02350 | 0.380484087 | -1.680493785 | Zero DAP | 2  | 26.02       | misc                  |
| cassava4.1_006196m PA | AT5G17330 | 0.380767679 | -1.243052761 | Zero DAP | 8  | 13.1.1.1.01 | amino acid metabolism |
| cassava4.1_001528m PA | AT2G41620 | 0.38099804  | -1.280869392 | Zero DAP | 8  | 29.3.1      | protein               |
| cassava4.1_009410m PA | AT1G08200 | 0.382178588 | -1.202317572 | Zero DAP | 19 | 10.1.03     | cell wall             |
| cassava4.1_001401m PA | AT4G30190 | 0.382205224 | -1.093456093 | Zero DAP | 13 | 34.1.02     | transport             |
| cassava4.1_001047m PA | AT1G53310 | 0.383411372 | -1.062839258 | Zero DAP | 23 | 4.014       | glycolysis            |
| cassava4.1_005309m PA | AT3G18190 | 0.386876873 | -1.74718242  | Zero DAP | 3  | 29.6        | protein               |
| cassava4.1_007343m PA | AT5G59420 | 0.387352367 | -1.225906247 | Zero DAP | 8  | 31.4        | cell                  |



|                       |           |             |              |          |    |             |                                                  |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|--------------------------------------------------|
| cassava4.1_003328m PA | AT5G02500 | 0.389293787 | -1.208234007 | Zero DAP | 21 | 29.6        | protein                                          |
| cassava4.1_004403m PA | AT3G25680 | 0.390068924 | -1.341044213 | Zero DAP | 2  | 35.2        | not assigned                                     |
| cassava4.1_006253m PA | AT3G30841 | 0.390347043 | -1.113577426 | Zero DAP | 6  | 4.011       | glycolysis                                       |
| cassava4.1_001050m PA | AT3G14940 | 0.390648545 | -1.27557415  | Zero DAP | 6  | 4.014       | glycolysis                                       |
| cassava4.1_028537m PA | AT1G48410 | 0.393191568 | -1.179995708 | Zero DAP | 16 | 27.3.36     | RNA                                              |
| cassava4.1_028537m PA | AT1G48410 | 0.393191568 | -1.179995708 | Zero DAP | 16 | 33.99       | development                                      |
| cassava4.1_002964m PA | AT5G09590 | 0.394458618 | -1.179584849 | Zero DAP | 21 | 20.2.1      | stress                                           |
| cassava4.1_013208m PA | AT5G66060 | 0.395424583 | -1.250118822 | Zero DAP | 2  | 13.2.2.2    | amino acid metabolism                            |
| cassava4.1_013208m PA | AT5G66060 | 0.395424583 | -1.250118822 | Zero DAP | 2  | 26.07       | misc                                             |
| cassava4.1_003823m PA | AT1G76400 | 0.399971611 | -1.387515247 | Zero DAP | 12 | 29.7        | protein                                          |
| cassava4.1_007337m PA | AT1G55810 | 0.400816571 | -1.318786401 | Zero DAP | 4  | 23.3.1.03   | nucleotide metabolism                            |
| cassava4.1_003606m PA | AT5G56360 | 0.400948077 | -1.256956479 | Zero DAP | 2  | 30.3        | signalling                                       |
| cassava4.1_005523m PA | AT5G26830 | 0.401015679 | -1.029141049 | Zero DAP | 6  | 29.1.03     | protein                                          |
| cassava4.1_003674m PA | AT1G04430 | 0.401377902 | -1.987146994 | Zero DAP | 7  | 20.2.3      | stress                                           |
| cassava4.1_003364m PA | AT5G03540 | 0.402078974 | -1.171669621 | Zero DAP | 9  | 31.4        | cell                                             |
| cassava4.1_005424m PA | AT5G05010 | 0.402874184 | -1.166319338 | Zero DAP | 4  | 31.4        | cell                                             |
| cassava4.1_001052m PA | AT2G41740 | 0.403363523 | -1.234160649 | Zero DAP | 27 | 31.1        | cell                                             |
| cassava4.1_011120m PA | AT4G17190 | 0.403844751 | -1.042731718 | Zero DAP | 4  | 16.1.2.09   | secondary metabolism                             |
| cassava4.1_001066m PA | AT5G62600 | 0.40529779  | -1.285508515 | Zero DAP | 6  | 35.1        | not assigned                                     |
| cassava4.1_000966m PA | AT5G11560 | 0.406420542 | -1.204626124 | Zero DAP | 22 | 35.1        | not assigned                                     |
| cassava4.1_008665m PA | AT1G04850 | 0.407695673 | -1.055592527 | Zero DAP | 8  | 29.5.11     | protein                                          |
| cassava4.1_001712m PA | AT5G17920 | 0.407750196 | -1.135022493 | Zero DAP | 39 | 13.1.3.4    | amino acid metabolism                            |
| cassava4.1_014072m PA | AT4G13720 | 0.408721601 | -1.153231284 | Zero DAP | 3  | 35.1        | not assigned                                     |
| cassava4.1_012129m PA | AT3G20000 | 0.409630437 | -1.089005795 | Zero DAP | 6  | 35.1        | not assigned                                     |
| cassava4.1_004004m PA | AT5G13110 | 0.411092798 | -1.212963212 | Zero DAP | 11 | 7.1.01      | OPP                                              |
| cassava4.1_029649m PA | AT2G22475 | 0.411205804 | -1.28159669  | Zero DAP | 2  | 17.1.3      | hormone metabolism                               |
| cassava4.1_012725m PA | AT2G30050 | 0.411350659 | -1.542821595 | Zero DAP | 5  | 29.3.4.4    | protein                                          |
| cassava4.1_001558m PA | AT4G17770 | 0.411586018 | -1.112607291 | Zero DAP | 4  | 3.2.3       | minor CHO metabolism                             |
| cassava4.1_010822m PA | AT5G64210 | 0.412436031 | -1.499607584 | Zero DAP | 9  | 9.04        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_011551m PA | AT2G02400 | 0.41250551  | -1.191342295 | Zero DAP | 2  | 16.2        | secondary metabolism                             |
| cassava4.1_030187m PA | AT5G13560 | 0.413273053 | -1.341765658 | Zero DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_005682m PA | AT5G26780 | 0.416283326 | -1.144529228 | Zero DAP | 23 | 1.2.05      | PS                                               |
| cassava4.1_005682m PA | AT5G26780 | 0.416283326 | -1.144529228 | Zero DAP | 23 | 13.1.5.2.01 | amino acid metabolism                            |
| cassava4.1_005682m PA | AT5G26780 | 0.416283326 | -1.144529228 | Zero DAP | 23 | 25.01       | C1-metabolism                                    |
| cassava4.1_002487m PA | AT5G26710 | 0.417541    | -1.232816738 | Zero DAP | 3  | 19.01       | tetrapyrrole synthesis                           |
| cassava4.1_002487m PA | AT5G26710 | 0.417541    | -1.232816738 | Zero DAP | 3  | 29.1.017    | protein                                          |
| cassava4.1_001827m PA | AT4G24190 | 0.418897312 | -1.121090683 | Zero DAP | 38 | 20.2.1      | stress                                           |
| cassava4.1_006090m PA | AT3G53110 | 0.420521138 | -1.156170938 | Zero DAP | 10 | 28.1        | DNA                                              |
| cassava4.1_012200m PA | AT5G12380 | 0.420534687 | -1.161570533 | Zero DAP | 15 | 31.1        | cell                                             |
| cassava4.1_000843m PA | AT4G30160 | 0.423147933 | -1.006061037 | Zero DAP | 17 | 31.1        | cell                                             |
| cassava4.1_002253m PA | AT1G71820 | 0.423765376 | -1.181517897 | Zero DAP | 11 | 35.2        | not assigned                                     |
| cassava4.1_002723m PA | AT3G54440 | 0.425044901 | -1.264260688 | Zero DAP | 5  | 26.03       | misc                                             |
| cassava4.1_011870m PA | AT2G33040 | 0.426437989 | -1.142911431 | Zero DAP | 14 | 9.09        | mitochondrial electron transport / ATP synthesis |
| cassava4.1_001498m PA | AT4G33090 | 0.42860693  | -1.033619268 | Zero DAP | 27 | 29.5        | protein                                          |

|                       |           |             |              |          |    |              |                       |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------|
| cassava4.1_011652m PA | AT4G13010 | 0.428660383 | -1.081153386 | Zero DAP | 8  | 26.07        | misc                  |
| cassava4.1_005485m PA | AT5G53850 | 0.42934528  | -1.353157179 | Zero DAP | 2  | 3.5          | minor CHO metabolism  |
| cassava4.1_013135m PA | AT3G56190 | 0.430001422 | -1.131644581 | Zero DAP | 10 | 35.1         | not assigned          |
| cassava4.1_001085m PA | AT3G19170 | 0.431309893 | -1.196022223 | Zero DAP | 3  | 29.5.07      | protein               |
| cassava4.1_012002m PA | AT1G67730 | 0.433201771 | -1.305924124 | Zero DAP | 2  | 16.7         | secondary metabolism  |
| cassava4.1_004975m PA | AT3G20050 | 0.436868269 | -1.179412798 | Zero DAP | 29 | 29.6         | protein               |
| cassava4.1_026366m PA | AT4G30160 | 0.437237253 | -1.139650676 | Zero DAP | 6  | 31.1         | cell                  |
| cassava4.1_004280m PA | AT4G30920 | 0.437642316 | -1.144480085 | Zero DAP | 34 | 29.5         | protein               |
| cassava4.1_001499m PA | AT4G33090 | 0.437893414 | -1.120546965 | Zero DAP | 2  | 29.5         | protein               |
| cassava4.1_014268m PA | AT2G39700 | 0.438817473 | -2.276195669 | Zero DAP | 2  | 10.7         | cell wall             |
| cassava4.1_014223m PA | AT1G78300 | 0.438991444 | -1.071094582 | Zero DAP | 19 | 30.7         | signalling            |
| cassava4.1_015407m PA | AT4G26410 | 0.439749    | -1.671168833 | Zero DAP | 2  | 35.2         | not assigned          |
| cassava4.1_006150m PA | AT2G46630 | 0.439843239 | -1.11931876  | Zero DAP | 3  | 10.5.3       | cell wall             |
| cassava4.1_024698m PA | AT1G48430 | 0.441257123 | -1.061243226 | Zero DAP | 9  | 3.5          | minor CHO metabolism  |
| cassava4.1_006725m PA | AT5G43600 | 0.446741171 | -1.335932162 | Zero DAP | 7  | 29.5         | protein               |
| cassava4.1_005252m PA | AT3G02530 | 0.44708314  | -1.100207447 | Zero DAP | 26 | 29.6         | protein               |
| cassava4.1_006082m PA | AT5G41950 | 0.45117335  | -1.218274937 | Zero DAP | 3  | 35.2         | not assigned          |
| cassava4.1_010881m PA | AT4G17260 | 0.452112962 | -1.189213506 | Zero DAP | 5  | 5.01         | fermentation          |
| cassava4.1_011283m PA | AT1G15950 | 0.45409929  | -1.264073793 | Zero DAP | 6  | 16.2.1.07    | secondary metabolism  |
| cassava4.1_007374m PA | AT3G02100 | 0.45724081  | -1.208806097 | Zero DAP | 2  | 26.02        | misc                  |
| cassava4.1_002009m PA | AT1G62870 | 0.458125728 | -1.447893233 | Zero DAP | 4  | 35.2         | not assigned          |
| cassava4.1_007673m PA | AT2G36530 | 0.45845483  | -1.079144809 | Zero DAP | 34 | 4.012        | glycolysis            |
| cassava4.1_005355m PA | AT4G29840 | 0.459243254 | -1.114509738 | Zero DAP | 5  | 13.1.3.2.01  | amino acid metabolism |
| cassava4.1_008669m PA | AT5G15610 | 0.460135842 | -1.170322807 | Zero DAP | 8  | 29.5.11.20   | protein               |
| cassava4.1_003946m PA | AT4G33070 | 0.461665621 | -1.279396032 | Zero DAP | 5  | 5.02         | fermentation          |
| cassava4.1_010463m PA | AT2G47470 | 0.462245665 | -1.084267749 | Zero DAP | 10 | 21.01        | redox                 |
| cassava4.1_006526m PA | AT1G54990 | 0.465431485 | -1.394625466 | Zero DAP | 2  | 35.2         | not assigned          |
| cassava4.1_007464m PA | AT1G06950 | 0.466186401 | -1.091960835 | Zero DAP | 8  | 29.3.3       | protein               |
| cassava4.1_007039m PA | AT5G25754 | 0.466558832 | -1.090340689 | Zero DAP | 10 | 35.2         | not assigned          |
| cassava4.1_029767m PA | AT4G36760 | 0.469127989 | -1.234557746 | Zero DAP | 8  | 29.5         | protein               |
| cassava4.1_007915m PA | AT5G57020 | 0.472652568 | -1.33749787  | Zero DAP | 2  | 29.4         | protein               |
| cassava4.1_000019m PA | AT1G64790 | 0.473657928 | -1.184126653 | Zero DAP | 17 | 35.1         | not assigned          |
| cassava4.1_030093m PA | AT4G17140 | 0.475642953 | -1.483846532 | Zero DAP | 2  | 35.1         | not assigned          |
| cassava4.1_028263m PA | AT2G27100 | 0.475929562 | -1.467793077 | Zero DAP | 2  | 27.3.11      | RNA                   |
| cassava4.1_005332m PA | AT3G06350 | 0.479010327 | -1.139028065 | Zero DAP | 13 | 13.1.6.1.010 | amino acid metabolism |
| cassava4.1_003735m PA | AT1G26460 | 0.479749972 | -1.22345825  | Zero DAP | 2  | 35.1.5       | not assigned          |
| cassava4.1_001564m PA | AT4G16660 | 0.481895612 | -1.297312266 | Zero DAP | 7  | 20.2.1       | stress                |
| cassava4.1_001865m PA | AT4G02350 | 0.482308664 | -1.149624934 | Zero DAP | 8  | 31.4         | cell                  |
| cassava4.1_004547m PA | AT2G26890 | 0.482759726 | -1.228681796 | Zero DAP | 2  | 20.2.1       | stress                |
| cassava4.1_003346m PA | AT5G02500 | 0.483978736 | -1.193985118 | Zero DAP | 4  | 29.6         | protein               |
| cassava4.1_014376m PA | AT4G23420 | 0.484583369 | -1.23280006  | Zero DAP | 6  | 26.22        | misc                  |
| cassava4.1_004292m PA | AT1G70570 | 0.485057013 | -1.251498577 | Zero DAP | 10 | 13.1.6.5.02  | amino acid metabolism |
| cassava4.1_007686m PA | AT2G29550 | 0.485965217 | -1.238020874 | Zero DAP | 2  | 31.1         | cell                  |
| cassava4.1_008198m PA | AT4G14300 | 0.488683047 | -1.328080859 | Zero DAP | 2  | 35.1         | not assigned          |

|                       |           |             |              |          |    |             |                                                  |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|--------------------------------------------------|
| cassava4.1_013025m PA | AT5G13870 | 0.488981095 | -1.08612867  | Zero DAP | 11 | 10.7        | cell wall                                        |
| cassava4.1_001644m PA | AT1G56070 | 0.489178888 | -1.083692364 | Zero DAP | 53 | 29.2.4      | protein                                          |
| cassava4.1_004939m PA | AT2G17200 | 0.489758327 | -1.356043491 | Zero DAP | 8  | 29.5.11     | protein                                          |
| cassava4.1_008510m PA | AT5G22060 | 0.492867645 | -1.125498    | Zero DAP | 7  | 20.2.1      | stress                                           |
| cassava4.1_004778m PA | AT5G49650 | 0.495559932 | -1.255844843 | Zero DAP | 2  | 3.7         | minor CHO metabolism                             |
| cassava4.1_003630m PA | AT3G52850 | 0.495583903 | -1.397439734 | Zero DAP | 5  | 29.3.4.3    | protein                                          |
| cassava4.1_005997m PA | AT3G26280 | 0.495595754 | -1.053691982 | Zero DAP | 4  | 26.10       | misc                                             |
| cassava4.1_008743m PA | AT3G19760 | 0.495913476 | -1.14516534  | Zero DAP | 10 | 27.1        | RNA                                              |
| cassava4.1_008743m PA | AT3G19760 | 0.495913476 | -1.14516534  | Zero DAP | 10 | 29.2.3      | protein                                          |
| cassava4.1_009269m PA | AT2G14260 | 0.496296404 | -1.116599333 | Zero DAP | 11 | 29.5        | protein                                          |
| cassava4.1_000897m PA | AT1G08420 | 0.497180086 | -1.299981769 | Zero DAP | 3  | 29.4        | protein                                          |
| cassava4.1_005341m PA | AT5G62810 | 0.498266798 | -1.080082082 | Zero DAP | 2  | 35.1        | not assigned                                     |
| cassava4.1_003880m PA | AT4G27500 | 0.501039343 | -1.196663317 | Zero DAP | 8  | 35.2        | not assigned                                     |
| cassava4.1_023107m PA | AT3G04940 | 0.50364659  | -1.127952046 | Zero DAP | 5  | 13.1.5.3.01 | amino acid metabolism                            |
| cassava4.1_002598m PA | AT5G37510 | 0.504930896 | -1.180475567 | Zero DAP | 12 | 9.1.2       | mitochondrial electron transport / ATP synthesis |
| cassava4.1_013239m PA | AT1G09760 | 0.507060735 | -1.068148157 | Zero DAP | 9  | 27.1        | RNA                                              |
| cassava4.1_007596m PA | AT1G36730 | 0.508772172 | -1.060465487 | Zero DAP | 2  | 29.2.3      | protein                                          |
| cassava4.1_003953m PA | AT4G19210 | 0.510132817 | -1.013285348 | Zero DAP | 10 | 27.2        | RNA                                              |
| cassava4.1_003402m PA | AT2G23350 | 0.510414542 | -1.847299698 | Zero DAP | 4  | 27.1        | RNA                                              |
| cassava4.1_013854m PA | AT5G46860 | 0.510524446 | -1.190235709 | Zero DAP | 3  | 31.4        | cell                                             |
| cassava4.1_012693m PA | AT3G02780 | 0.511975614 | -1.353690093 | Zero DAP | 3  | 16.1.2.07   | secondary metabolism                             |
| cassava4.1_027170m PA | AT2G21270 | 0.5175008   | -1.086472359 | Zero DAP | 9  | 29.5.11     | protein                                          |
| cassava4.1_001451m PA | AT1G60070 | 0.517980536 | -1.117163473 | Zero DAP | 11 | 31.4        | cell                                             |
| cassava4.1_002990m PA | AT2G25970 | 0.519236817 | -1.156477221 | Zero DAP | 4  | 35.1        | not assigned                                     |
| cassava4.1_001476m PA | AT5G53480 | 0.520398396 | -1.087313701 | Zero DAP | 15 | 29.3.1      | protein                                          |
| cassava4.1_008685m PA | AT5G36230 | 0.52115836  | -1.119418338 | Zero DAP | 6  | 29.2.3      | protein                                          |
| cassava4.1_018737m PA | AT5G59890 | 0.525597951 | -1.021344296 | Zero DAP | 3  | 31.1        | cell                                             |
| cassava4.1_016210m PA | AT5G26667 | 0.525807945 | -1.246533278 | Zero DAP | 8  | 23.4.3      | nucleotide metabolism                            |
| cassava4.1_001919m PA | AT3G07160 | 0.529533403 | -1.415717625 | Zero DAP | 6  | 3.6         | minor CHO metabolism                             |
| cassava4.1_001272m PA | AT1G59610 | 0.529922255 | -1.254674441 | Zero DAP | 19 | 26.17       | misc                                             |
| cassava4.1_011608m PA | AT1G65650 | 0.531082476 | -1.09524201  | Zero DAP | 3  | 29.5.11     | protein                                          |
| cassava4.1_010490m PA | AT5G42240 | 0.531611881 | -1.152313176 | Zero DAP | 3  | 29.5.05     | protein                                          |
| cassava4.1_018854m PA | AT2G32720 | 0.535075076 | -1.509680834 | Zero DAP | 3  | 21.2        | redox                                            |
| cassava4.1_002775m PA | AT4G21150 | 0.536594597 | -1.261474547 | Zero DAP | 13 | 35.1        | not assigned                                     |
| cassava4.1_000788m PA | AT2G31660 | 0.537390874 | -1.328448422 | Zero DAP | 3  | 29.3.1      | protein                                          |
| cassava4.1_011882m PA | AT4G33670 | 0.541088996 | -1.148245068 | Zero DAP | 7  | 3.5         | minor CHO metabolism                             |
| cassava4.1_008655m PA | AT4G32330 | 0.541594278 | -1.152370301 | Zero DAP | 2  | 35.2        | not assigned                                     |
| cassava4.1_005119m PA | AT1G63770 | 0.548999285 | -1.038928669 | Zero DAP | 16 | 29.5        | protein                                          |
| cassava4.1_002407m PA | AT5G04130 | 0.551654941 | -1.417696558 | Zero DAP | 5  | 28.1        | DNA                                              |
| cassava4.1_002156m PA | AT5G17920 | 0.552295333 | -1.067753004 | Zero DAP | 51 | 13.1.3.4    | amino acid metabolism                            |
| cassava4.1_028954m PA | AT2G20580 | 0.553479272 | -1.111408971 | Zero DAP | 2  | 29.5.11.20  | protein                                          |
| cassava4.1_013760m PA | AT5G58100 | 0.555120744 | -1.349866149 | Zero DAP | 4  | 35.2        | not assigned                                     |
| cassava4.1_004471m PA | AT3G23990 | 0.555843831 | -1.111148723 | Zero DAP | 18 | 20.2.1      | stress                                           |
| cassava4.1_004471m PA | AT3G23990 | 0.555843831 | -1.111148723 | Zero DAP | 18 | 29.6        | protein                                          |

|                       |           |             |              |          |    |             |                       |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_008072m PA | AT5G41040 | 0.560878265 | -1.184426239 | Zero DAP | 3  | 35.1        | not assigned          |
| cassava4.1_002933m PA | AT4G31200 | 0.564372638 | -1.166209384 | Zero DAP | 3  | 27.1        | RNA                   |
| cassava4.1_015707m PA | AT3G53620 | 0.566303611 | -1.117540223 | Zero DAP | 5  | 23.4.99     | nucleotide metabolism |
| cassava4.1_016651m PA | AT2G18110 | 0.567530529 | -1.427567852 | Zero DAP | 3  | 29.2.4      | protein               |
| cassava4.1_010473m PA | AT1G50380 | 0.571251993 | -1.175792928 | Zero DAP | 3  | 29.5        | protein               |
| cassava4.1_010183m PA | AT3G02230 | 0.572600682 | -1.066954552 | Zero DAP | 4  | 10.5.5      | cell wall             |
| cassava4.1_004848m PA | AT1G56110 | 0.572845542 | -1.053482682 | Zero DAP | 14 | 27.3.67     | RNA                   |
| cassava4.1_005418m PA | AT3G52990 | 0.574000528 | -1.180918455 | Zero DAP | 10 | 4.013       | glycolysis            |
| cassava4.1_005232m PA | AT5G61790 | 0.574131326 | -1.159980559 | Zero DAP | 8  | 30.3        | signalling            |
| cassava4.1_008232m PA | AT1G56050 | 0.575337723 | -1.170977119 | Zero DAP | 3  | 30.5        | signalling            |
| cassava4.1_008598m PA | AT1G78850 | 0.575394331 | -1.178610058 | Zero DAP | 2  | 26.16       | misc                  |
| cassava4.1_031246m PA | AT4G24620 | 0.575652002 | -1.049204492 | Zero DAP | 13 | 4.03        | glycolysis            |
| cassava4.1_004679m PA | AT2G01600 | 0.577171946 | -1.320591698 | Zero DAP | 5  | 35.1.21     | not assigned          |
| cassava4.1_013860m PA | AT3G02870 | 0.577959141 | -1.189042612 | Zero DAP | 3  | 3.4.05      | minor CHO metabolism  |
| cassava4.1_005543m PA | AT4G29840 | 0.579925663 | -1.156119166 | Zero DAP | 4  | 13.1.3.2.01 | amino acid metabolism |
| cassava4.1_007313m PA | AT5G65720 | 0.580742666 | -1.110701417 | Zero DAP | 5  | 13.1.5.3    | amino acid metabolism |
| cassava4.1_003648m PA | AT5G46190 | 0.581708154 | -1.247264846 | Zero DAP | 3  | 35.1        | not assigned          |
| cassava4.1_024269m PA | AT4G34050 | 0.584206955 | -1.094517158 | Zero DAP | 5  | 16.2.1.06   | secondary metabolism  |
| cassava4.1_003988m PA | AT1G43690 | 0.584969562 | -1.074185995 | Zero DAP | 5  | 29.5.11     | protein               |
| cassava4.1_021580m PA | AT1G79280 | 0.59010117  | -1.199072719 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_018524m PA | AT1G70600 | 0.590549539 | -1.01690311  | Zero DAP | 2  | 29.2.2      | protein               |
| cassava4.1_000064m PA | AT1G49340 | 0.592626001 | -1.259616288 | Zero DAP | 2  | 30.4        | signalling            |
| cassava4.1_011059m PA | AT2G37400 | 0.593528861 | -1.168903755 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_001080m PA | AT5G62670 | 0.593612742 | -1.061933419 | Zero DAP | 30 | 34.1        | transport             |
| cassava4.1_029678m PA | AT2G39960 | 0.59468313  | -1.123840225 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_032692m PA | AT1G07250 | 0.595074248 | -1.291104853 | Zero DAP | 2  | 26.02       | misc                  |
| cassava4.1_006251m PA | AT2G19860 | 0.595756134 | -1.043431483 | Zero DAP | 3  | 2.2.1.04    | major CHO metabolism  |
| cassava4.1_004516m PA | AT4G35470 | 0.597741779 | -1.082742701 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_001342m PA | AT1G80410 | 0.599828662 | -1.082317738 | Zero DAP | 11 | 26.05       | misc                  |
| cassava4.1_002586m PA | AT2G26140 | 0.601829259 | -1.365354519 | Zero DAP | 2  | 29.5.07     | protein               |
| cassava4.1_004160m PA | AT3G25800 | 0.602866902 | -1.090856724 | Zero DAP | 16 | 29.4        | protein               |
| cassava4.1_011738m PA | AT5G66390 | 0.602878467 | -1.283657347 | Zero DAP | 3  | 26.12       | misc                  |
| cassava4.1_001305m PA | AT2G27040 | 0.603255578 | -1.103854015 | Zero DAP | 15 | 27.3.36     | RNA                   |
| cassava4.1_019781m PA | AT2G37600 | 0.603563575 | -1.796524137 | Zero DAP | 2  | 29.2.2      | protein               |
| cassava4.1_008355m PA | AT1G21750 | 0.603916353 | -1.070835714 | Zero DAP | 27 | 21.01       | redox                 |
| cassava4.1_001982m PA | AT1G62750 | 0.604009616 | -1.014931801 | Zero DAP | 7  | 29.2.4      | protein               |
| cassava4.1_015803m PA | AT5G39510 | 0.606598593 | -1.013854268 | Zero DAP | 4  | 31.4        | cell                  |
| cassava4.1_005461m PA | AT4G33510 | 0.607900321 | -1.163312533 | Zero DAP | 6  | 13.1.6.1.01 | amino acid metabolism |
| cassava4.1_003902m PA | AT5G49460 | 0.608377652 | -1.166261625 | Zero DAP | 34 | 8.2.011     | TCA / org             |
| cassava4.1_010098m PA | AT3G04830 | 0.608588648 | -1.124313961 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_014378m PA | AT2G16595 | 0.608675063 | -1.068552209 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_016070m PA | AT2G37270 | 0.60941872  | -1.004864247 | Zero DAP | 12 | 29.2.2      | protein               |
| cassava4.1_004327m PA | AT5G23880 | 0.609631928 | -1.162469887 | Zero DAP | 3  | 27.1        | RNA                   |
| cassava4.1_015675m PA | AT2G27450 | 0.60966991  | -1.091507394 | Zero DAP | 2  | 22.1.05     | polyamine metabolism  |

|                       |           |             |              |          |    |            |                                        |
|-----------------------|-----------|-------------|--------------|----------|----|------------|----------------------------------------|
| cassava4.1_006347m PA | AT2G43980 | 0.610519811 | -1.124505525 | Zero DAP | 2  | 30.4.05    | signalling                             |
| cassava4.1_010947m PA | AT2G01350 | 0.612800331 | -1.267079412 | Zero DAP | 3  | 35.1       | not assigned                           |
| cassava4.1_019252m PA | AT3G05560 | 0.615520788 | -1.002716316 | Zero DAP | 7  | 29.2.2     | protein                                |
| cassava4.1_032996m PA | AT3G10670 | 0.616056192 | -1.053823759 | Zero DAP | 5  | 29.8       | protein assembly and cofactor ligation |
| cassava4.1_003331m PA | AT5G02500 | 0.61684202  | -1.090969044 | Zero DAP | 53 | 29.6       | protein                                |
| cassava4.1_017908m PA | AT2G36620 | 0.616995621 | -1.033218712 | Zero DAP | 7  | 29.2.2     | protein                                |
| cassava4.1_013798m PA | AT3G51680 | 0.618953853 | -1.303362009 | Zero DAP | 3  | 26.22      | misc                                   |
| cassava4.1_004836m PA | AT3G18480 | 0.619484014 | -1.830465272 | Zero DAP | 2  | 35.1       | not assigned                           |
| cassava4.1_003986m PA | AT2G01910 | 0.619650281 | -1.149371137 | Zero DAP | 2  | 31.1       | cell                                   |
| cassava4.1_028726m PA | AT1G73180 | 0.619866963 | -1.13484413  | Zero DAP | 2  | 29.2.3     | protein                                |
| cassava4.1_019405m PA | AT2G27730 | 0.621782426 | -1.02443259  | Zero DAP | 4  | 35.2       | not assigned                           |
| cassava4.1_008633m PA | AT3G13920 | 0.626686438 | -1.14440779  | Zero DAP | 6  | 29.2.3     | protein                                |
| cassava4.1_016888m PA | AT4G31580 | 0.628713652 | -1.164226887 | Zero DAP | 2  | 27.1.1     | RNA                                    |
| cassava4.1_010230m PA | AT4G00620 | 0.6295953   | -1.054787243 | Zero DAP | 3  | 25.05      | C1-metabolism                          |
| cassava4.1_001032m PA | AT5G15450 | 0.63171075  | -1.035181507 | Zero DAP | 22 | 20.2.1     | stress                                 |
| cassava4.1_024426m PA | AT3G52990 | 0.631881142 | -1.109999738 | Zero DAP | 7  | 4.013      | glycolysis                             |
| cassava4.1_006084m PA | AT1G30690 | 0.631979513 | -1.261925638 | Zero DAP | 3  | 28.99      | DNA                                    |
| cassava4.1_006084m PA | AT1G30690 | 0.631979513 | -1.261925638 | Zero DAP | 3  | 34.99      | transport                              |
| cassava4.1_018508m PA | AT1G70600 | 0.632285357 | -1.017575834 | Zero DAP | 6  | 29.2.2     | protein                                |
| cassava4.1_017568m PA | AT2G42610 | 0.633284728 | -1.46544828  | Zero DAP | 2  | 35.2       | not assigned                           |
| cassava4.1_005264m PA | AT1G09270 | 0.634364279 | -1.280355769 | Zero DAP | 2  | 29.3.1     | protein                                |
| cassava4.1_005008m PA | AT3G08960 | 0.635762021 | -1.158161064 | Zero DAP | 3  | 27.3.99    | RNA                                    |
| cassava4.1_005588m PA | AT3G04610 | 0.637558951 | -1.107623126 | Zero DAP | 8  | 27.4       | RNA                                    |
| cassava4.1_023817m PA | AT4G37990 | 0.639725726 | -1.135561876 | Zero DAP | 3  | 16.2.1.010 | secondary metabolism                   |
| cassava4.1_001268m PA | AT1G59610 | 0.640291451 | -1.354897331 | Zero DAP | 4  | 26.17      | misc                                   |
| cassava4.1_013696m PA | AT3G01280 | 0.641500055 | -1.134589248 | Zero DAP | 4  | 34.20      | transport                              |
| cassava4.1_034369m PA | AT1G12310 | 0.641552951 | -1.0103102   | Zero DAP | 7  | 29.4       | protein                                |
| cassava4.1_004800m PA | AT5G26360 | 0.642287461 | -1.065212332 | Zero DAP | 25 | 29.6       | protein                                |
| cassava4.1_002554m PA | AT2G04350 | 0.643048373 | -1.052011594 | Zero DAP | 6  | 11.1.09    | lipid metabolism                       |
| cassava4.1_000575m PA | AT1G09570 | 0.643256733 | -1.052679317 | Zero DAP | 15 | 30.11      | signalling                             |
| cassava4.1_015611m PA | AT5G63400 | 0.644435521 | -1.15242761  | Zero DAP | 3  | 23.4.01    | nucleotide metabolism                  |
| cassava4.1_014410m PA | AT5G20720 | 0.64537249  | -1.251694825 | Zero DAP | 4  | 29.6       | protein                                |
| cassava4.1_016383m PA | AT1G14000 | 0.647688303 | -1.065004451 | Zero DAP | 8  | 29.4       | protein                                |
| cassava4.1_015969m PA | AT5G60860 | 0.650461079 | -1.373089382 | Zero DAP | 8  | 30.5       | signalling                             |
| cassava4.1_000662m PA | AT5G19820 | 0.651342616 | -1.176875413 | Zero DAP | 8  | 35.1       | not assigned                           |
| cassava4.1_030765m PA | AT1G45000 | 0.6518382   | -1.046591163 | Zero DAP | 4  | 29.5.11.20 | protein                                |
| cassava4.1_002016m PA | AT3G51550 | 0.653551924 | -1.217189809 | Zero DAP | 2  | 30.2.16    | signalling                             |
| cassava4.1_011550m PA | AT5G43330 | 0.654595793 | -1.100062179 | Zero DAP | 7  | 8.2.09     | TCA / org                              |
| cassava4.1_016374m PA | AT5G14030 | 0.65543719  | -1.079724173 | Zero DAP | 3  | 35.1       | not assigned                           |
| cassava4.1_001837m PA | AT1G07510 | 0.655619946 | -1.178647513 | Zero DAP | 3  | 29.5.07    | protein                                |
| cassava4.1_004255m PA | AT5G15270 | 0.655677187 | -1.258801734 | Zero DAP | 3  | 35.1       | not assigned                           |
| cassava4.1_012731m PA | AT1G14130 | 0.656718694 | -1.111931521 | Zero DAP | 3  | 26.07      | misc                                   |
| cassava4.1_002625m PA | AT4G21150 | 0.657946532 | -1.258453169 | Zero DAP | 8  | 35.1       | not assigned                           |
| cassava4.1_017263m PA | AT3G58700 | 0.658291406 | -1.001854709 | Zero DAP | 9  | 29.2.2     | protein                                |

|                       |           |             |              |          |    |              |                                   |
|-----------------------|-----------|-------------|--------------|----------|----|--------------|-----------------------------------|
| cassava4.1_004989m PA | AT3G20290 | 0.65982264  | -1.092954118 | Zero DAP | 13 | 30.3         | signalling                        |
| cassava4.1_001470m PA | AT2G20580 | 0.661534649 | -1.030329033 | Zero DAP | 31 | 29.5.11.20   | protein                           |
| cassava4.1_006316m PA | AT3G52990 | 0.663237038 | -1.195295281 | Zero DAP | 3  | 4.013        | glycolysis                        |
| cassava4.1_003743m PA | AT3G60190 | 0.663701188 | -1.115084078 | Zero DAP | 10 | 26.17        | misc                              |
| cassava4.1_007683m PA | AT2G44100 | 0.6644146   | -1.065682    | Zero DAP | 20 | 30.5         | signalling                        |
| cassava4.1_016297m PA | AT1G69370 | 0.665004502 | -1.070509534 | Zero DAP | 5  | 13.1.6.2.01  | amino acid metabolism             |
| cassava4.1_022174m PA | AT1G70670 | 0.666520646 | -1.068992843 | Zero DAP | 2  | 33.99        | development                       |
| cassava4.1_006224m PA | AT1G48900 | 0.672531868 | -1.028565759 | Zero DAP | 7  | 29.3.4.99    | protein                           |
| cassava4.1_003149m PA | AT5G42020 | 0.675749186 | -1.108346124 | Zero DAP | 4  | 20.2.1       | stress                            |
| cassava4.1_011997m PA | AT1G67730 | 0.676743682 | -1.026534222 | Zero DAP | 7  | 16.7         | secondary metabolism              |
| cassava4.1_019619m PA | AT1G36240 | 0.67824011  | -1.003487817 | Zero DAP | 6  | 29.2.2       | protein                           |
| cassava4.1_009401m PA | AT1G10070 | 0.678949103 | -1.521023598 | Zero DAP | 2  | 13.1.4.1     | amino acid metabolism             |
| cassava4.1_006123m PA | AT3G06580 | 0.680003457 | -1.020619452 | Zero DAP | 5  | 3.8.01       | minor CHO metabolism              |
| cassava4.1_031388m PA | AT1G55210 | 0.680506831 | -1.064324778 | Zero DAP | 2  | 20.1         | stress                            |
| cassava4.1_003623m PA | AT1G76850 | 0.6805749   | -1.141281763 | Zero DAP | 3  | 35.2         | not assigned                      |
| cassava4.1_003999m PA | AT4G10840 | 0.681641392 | -1.072237386 | Zero DAP | 2  | 31.1         | cell                              |
| cassava4.1_016951m PA | AT1G48830 | 0.68326232  | -1.017928554 | Zero DAP | 2  | 29.2.2       | protein                           |
| cassava4.1_030256m PA | AT3G06350 | 0.687013946 | -1.149068908 | Zero DAP | 8  | 13.1.6.1.010 | amino acid metabolism             |
| cassava4.1_002186m PA | AT3G18860 | 0.687082196 | -1.081569182 | Zero DAP | 7  | 11.9.3       | lipid metabolism                  |
| cassava4.1_003931m PA | AT1G72160 | 0.69106505  | -1.061554539 | Zero DAP | 14 | 28.99        | DNA                               |
| cassava4.1_003931m PA | AT1G72160 | 0.69106505  | -1.061554539 | Zero DAP | 14 | 34.99        | transport                         |
| cassava4.1_000707m PA | AT5G46070 | 0.692006244 | -1.049657034 | Zero DAP | 5  | 30.5         | signalling                        |
| cassava4.1_016430m PA | AT5G11680 | 0.692661857 | -1.051853468 | Zero DAP | 3  | 35.2         | not assigned                      |
| cassava4.1_006542m PA | AT3G52750 | 0.693419537 | -1.070500839 | Zero DAP | 5  | 31.2         | cell                              |
| cassava4.1_016480m PA | AT1G02130 | 0.693957561 | -1.400653352 | Zero DAP | 3  | 30.5         | signalling                        |
| cassava4.1_002706m PA | AT5G49910 | 0.694156219 | -1.059178252 | Zero DAP | 10 | 20.2.1       | stress                            |
| cassava4.1_005136m PA | AT5G61790 | 0.699838856 | -1.070370644 | Zero DAP | 15 | 30.3         | signalling                        |
| cassava4.1_018188m PA | AT3G11780 | 0.700304925 | -1.036835505 | Zero DAP | 2  | 35.1         | not assigned                      |
| cassava4.1_001019m PA | AT1G14610 | 0.70135224  | -1.009034734 | Zero DAP | 6  | 29.1.09      | protein                           |
| cassava4.1_006031m PA | AT1G63770 | 0.706578758 | -1.059451262 | Zero DAP | 24 | 29.5         | protein                           |
| cassava4.1_017984m PA | AT3G07480 | 0.707462266 | -1.050371603 | Zero DAP | 3  | 35.2         | not assigned                      |
| cassava4.1_001866m PA | AT3G20630 | 0.711067217 | -1.038249963 | Zero DAP | 12 | 29.5.11.05   | protein                           |
| cassava4.1_002121m PA | AT1G78060 | 0.711544467 | -1.035141982 | Zero DAP | 14 | 10.6.2       | cell wall                         |
| cassava4.1_001324m PA | AT4G32180 | 0.711943487 | -1.068751243 | Zero DAP | 4  | 18.4.01      | Co-factor and vitamine metabolism |
| cassava4.1_006635m PA | AT5G40870 | 0.712216077 | -1.062657295 | Zero DAP | 6  | 23.3.1.03    | nucleotide metabolism             |
| cassava4.1_012623m PA | AT3G02780 | 0.71248215  | -1.032250163 | Zero DAP | 4  | 16.1.2.07    | secondary metabolism              |
| cassava4.1_012845m PA | AT1G02280 | 0.712541211 | -1.042018158 | Zero DAP | 7  | 30.5         | signalling                        |
| cassava4.1_008915m PA | AT3G58060 | 0.713275436 | -1.01368093  | Zero DAP | 2  | 34.12        | transport                         |
| cassava4.1_018797m PA | AT5G20500 | 0.718749622 | -1.064678304 | Zero DAP | 3  | 21.04        | redox                             |
| cassava4.1_019018m PA | AT5G42850 | 0.723016919 | -1.086719011 | Zero DAP | 3  | 35.2         | not assigned                      |
| cassava4.1_004824m PA | AT5G56680 | 0.723857107 | -1.04082886  | Zero DAP | 19 | 29.1.022     | protein                           |
| cassava4.1_016331m PA | AT5G48760 | 0.729594498 | -1.05550915  | Zero DAP | 12 | 29.2.2       | protein                           |
| cassava4.1_018638m PA | AT3G10610 | 0.730070879 | -1.035324416 | Zero DAP | 8  | 29.2.2       | protein                           |
| cassava4.1_004743m PA | AT1G09780 | 0.730918309 | -1.057283359 | Zero DAP | 34 | 4.011        | glycolysis                        |

|                       |           |             |              |          |    |                |                       |
|-----------------------|-----------|-------------|--------------|----------|----|----------------|-----------------------|
| cassava4.1_001768m PA | AT5G46210 | 0.733103606 | -1.072883399 | Zero DAP | 2  | 29.5.11.4.3.03 | protein               |
| cassava4.1_016578m PA | AT3G11730 | 0.733383536 | -1.056871811 | Zero DAP | 2  | 30.5           | signalling            |
| cassava4.1_001575m PA | AT1G68020 | 0.73746868  | -1.024050971 | Zero DAP | 11 | 3.2.3          | minor CHO metabolism  |
| cassava4.1_010997m PA | AT1G61720 | 0.738097773 | -1.115827922 | Zero DAP | 6  | 16.8.3.01      | secondary metabolism  |
| cassava4.1_031560m PA | -         | 0.738340991 | -1.195034102 | Zero DAP | 2  | -              | -                     |
| cassava4.1_018513m PA | AT2G28900 | 0.739488949 | -1.001756878 | Zero DAP | 7  | 29.3.2         | protein               |
| cassava4.1_004336m PA | AT1G70730 | 0.739905026 | -1.03259972  | Zero DAP | 9  | 4.02           | glycolysis            |
| cassava4.1_034068m PA | AT4G16640 | 0.740850052 | -1.060133564 | Zero DAP | 4  | 29.5.07        | protein               |
| cassava4.1_016353m PA | AT5G37720 | 0.741009054 | -1.050454072 | Zero DAP | 2  | 27.4           | RNA                   |
| cassava4.1_014479m PA | AT2G45140 | 0.743173762 | -1.060402738 | Zero DAP | 2  | 35.1           | not assigned          |
| cassava4.1_006318m PA | AT4G39280 | 0.743504582 | -1.066077741 | Zero DAP | 9  | 29.1.020       | protein               |
| cassava4.1_031313m PA | AT3G50950 | 0.745094213 | -1.018528036 | Zero DAP | 2  | 20.1           | stress                |
| cassava4.1_018289m PA | AT1G08830 | 0.746269362 | -1.161778488 | Zero DAP | 5  | 21.6           | redox                 |
| cassava4.1_013862m PA | AT5G20920 | 0.751093549 | -1.054278766 | Zero DAP | 9  | 29.2.3         | protein               |
| cassava4.1_017871m PA | AT1G53540 | 0.751586678 | -1.039906298 | Zero DAP | 13 | 20.2.1         | stress                |
| cassava4.1_005369m PA | AT4G16143 | 0.752604183 | -1.036850828 | Zero DAP | 6  | 29.3.1         | protein               |
| cassava4.1_018472m PA | AT5G18380 | 0.754625812 | -1.078600409 | Zero DAP | 9  | 29.2.2         | protein               |
| cassava4.1_017802m PA | AT3G53430 | 0.754664143 | -1.077615668 | Zero DAP | 4  | 29.2.2         | protein               |
| cassava4.1_009786m PA | AT4G34460 | 0.754799007 | -1.185330324 | Zero DAP | 3  | 30.5           | signalling            |
| cassava4.1_010728m PA | AT4G18060 | 0.754891026 | -1.103886035 | Zero DAP | 2  | 35.1           | not assigned          |
| cassava4.1_003425m PA | AT1G74040 | 0.757968636 | -1.236044876 | Zero DAP | 3  | 13.1.4.4.01    | amino acid metabolism |
| cassava4.1_005643m PA | AT5G01220 | 0.759558072 | -1.138846893 | Zero DAP | 2  | 11.10.04       | lipid metabolism      |
| cassava4.1_000741m PA | AT5G06120 | 0.762053258 | -1.063660183 | Zero DAP | 11 | 30.5           | signalling            |
| cassava4.1_018150m PA | AT2G47110 | 0.770198225 | -1.022081778 | Zero DAP | 3  | 29.5.11        | protein               |
| cassava4.1_015846m PA | AT4G14710 | 0.770220711 | -1.065665217 | Zero DAP | 2  | 15.3           | metal handling        |
| cassava4.1_008560m PA | AT5G55280 | 0.771327815 | -1.066558056 | Zero DAP | 2  | 31.2           | cell                  |
| cassava4.1_002955m PA | AT5G09590 | 0.773928072 | -1.072246697 | Zero DAP | 32 | 20.2.1         | stress                |
| cassava4.1_015287m PA | AT4G25740 | 0.77408131  | -1.098547884 | Zero DAP | 2  | 29.2.2         | protein               |
| cassava4.1_014774m PA | AT3G57290 | 0.774441696 | -1.075015706 | Zero DAP | 6  | 29.2.3         | protein               |
| cassava4.1_013839m PA | AT1G19580 | 0.775069781 | -1.03167981  | Zero DAP | 5  | 35.1           | not assigned          |
| cassava4.1_000722m PA | AT4G33010 | 0.777156134 | -1.093886258 | Zero DAP | 26 | 13.2.5.2       | amino acid metabolism |
| cassava4.1_009504m PA | AT1G36050 | 0.778040507 | -1.070307735 | Zero DAP | 2  | 35.2           | not assigned          |
| cassava4.1_005863m PA | AT1G04280 | 0.778699262 | -1.106297919 | Zero DAP | 2  | 35.2           | not assigned          |
| cassava4.1_010996m PA | AT2G28760 | 0.779115209 | -1.125097902 | Zero DAP | 5  | 10.1.05        | cell wall             |
| cassava4.1_010707m PA | AT1G60690 | 0.782796979 | -1.06650103  | Zero DAP | 2  | 17.2.3         | hormone metabolism    |
| cassava4.1_006487m PA | AT4G24330 | 0.783717098 | -1.259666449 | Zero DAP | 5  | 35.2           | not assigned          |
| cassava4.1_003872m PA | AT3G18060 | 0.785140085 | -1.012666823 | Zero DAP | 10 | 33.99          | development           |
| cassava4.1_007132m PA | AT4G04720 | 0.786610935 | -1.104828065 | Zero DAP | 3  | 29.4           | protein               |
| cassava4.1_007132m PA | AT4G04720 | 0.786610935 | -1.104828065 | Zero DAP | 3  | 30.3           | signalling            |
| cassava4.1_008222m PA | AT1G53750 | 0.788981654 | -1.027469885 | Zero DAP | 10 | 29.5.11.20     | protein               |
| cassava4.1_014631m PA | AT5G43830 | 0.789034299 | -1.063943012 | Zero DAP | 2  | 15             | metal handling        |
| cassava4.1_014631m PA | AT5G43830 | 0.789034299 | -1.063943012 | Zero DAP | 2  | 17.2.3         | hormone metabolism    |
| cassava4.1_000960m PA | AT1G12470 | 0.789141445 | -1.14749796  | Zero DAP | 5  | 29.3.4.3       | protein               |
| cassava4.1_012142m PA | AT5G42180 | 0.789197853 | -1.000919552 | Zero DAP | 3  | 26.12          | misc                  |

|                       |           |             |              |          |    |             |                       |
|-----------------------|-----------|-------------|--------------|----------|----|-------------|-----------------------|
| cassava4.1_018275m PA | AT2G33845 | 0.790043987 | -1.106565938 | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_018974m PA | AT4G18100 | 0.790918518 | -1.091781078 | Zero DAP | 3  | 29.2.2      | protein               |
| cassava4.1_004530m PA | AT5G10240 | 0.792050369 | -1.084029213 | Zero DAP | 3  | 13.1.3.1.01 | amino acid metabolism |
| cassava4.1_031613m PA | AT5G16660 | 0.792186422 | -1.031148153 | Zero DAP | 3  | 35.2        | not assigned          |
| cassava4.1_016326m PA | AT3G18820 | 0.793525947 | -1.099527485 | Zero DAP | 7  | 30.5        | signalling            |
| cassava4.1_010620m PA | AT5G54770 | 0.794735243 | -1.078985858 | Zero DAP | 11 | 35.1        | not assigned          |
| cassava4.1_018404m PA | AT4G29410 | 0.795569073 | -1.061441727 | Zero DAP | 9  | 29.2.2      | protein               |
| cassava4.1_027425m PA | AT1G29970 | 0.797086965 | -1.172286949 | Zero DAP | 2  | 29.2.2      | protein               |
| cassava4.1_013047m PA | AT4G20440 | 0.798376067 | -1.029560388 | Zero DAP | 6  | 27.1        | RNA                   |
| cassava4.1_003284m PA | AT5G27540 | 0.799407435 | -1.048154708 | Zero DAP | 19 | 30.5        | signalling            |
| cassava4.1_010185m PA | AT4G31530 | 0.799784821 | -1.020781322 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_024940m PA | AT5G48230 | 0.802076688 | -1.03841     | Zero DAP | 8  | 13.2.3.5    | amino acid metabolism |
| cassava4.1_024940m PA | AT5G48230 | 0.802076688 | -1.03841     | Zero DAP | 8  | 16.1.2.01   | secondary metabolism  |
| cassava4.1_022819m PA | AT2G13560 | 0.804328142 | -1.031511126 | Zero DAP | 2  | 8.2.10      | TCA / org             |
| cassava4.1_005089m PA | AT4G22010 | 0.804486087 | -1.074986296 | Zero DAP | 2  | 26.07       | misc                  |
| cassava4.1_012974m PA | AT3G11400 | 0.809446208 | -1.026795499 | Zero DAP | 10 | 29.2.3      | protein               |
| cassava4.1_019294m PA | AT4G39260 | 0.810580776 | -1.337980166 | Zero DAP | 3  | 27.3.75     | RNA                   |
| cassava4.1_001880m PA | AT5G03340 | 0.81096391  | -1.071021305 | Zero DAP | 8  | 31.2        | cell                  |
| cassava4.1_019281m PA | AT3G45030 | 0.812034311 | -1.098348787 | Zero DAP | 3  | 29.2.2      | protein               |
| cassava4.1_011050m PA | AT2G40290 | 0.813987118 | -1.038512487 | Zero DAP | 7  | 29.2.3      | protein               |
| cassava4.1_018000m PA | AT2G47710 | 0.814209812 | -1.254685819 | Zero DAP | 2  | 20.2.99     | stress                |
| cassava4.1_006917m PA | AT2G41060 | 0.814985576 | -1.065192443 | Zero DAP | 3  | 35.1        | not assigned          |
| cassava4.1_021540m PA | AT5G19690 | 0.81521602  | -1.1653705   | Zero DAP | 2  | 35.1        | not assigned          |
| cassava4.1_016870m PA | AT4G02080 | 0.815319434 | -1.143645746 | Zero DAP | 2  | 30.5        | signalling            |
| cassava4.1_006118m PA | AT5G17330 | 0.815555798 | -1.004858014 | Zero DAP | 23 | 13.1.1.1.01 | amino acid metabolism |
| cassava4.1_005842m PA | AT4G13430 | 0.818529098 | -1.031419884 | Zero DAP | 7  | 8.1.03      | TCA / org             |
| cassava4.1_019289m PA | AT5G02610 | 0.818585242 | -1.097407187 | Zero DAP | 8  | 29.2.2      | protein               |
| cassava4.1_008690m PA | AT5G18170 | 0.820176997 | -1.011774886 | Zero DAP | 3  | 12.3.01     | N-metabolism          |
| cassava4.1_004656m PA | AT5G27410 | 0.820639054 | -1.017606692 | Zero DAP | 15 | 26.26.1     | misc                  |
| cassava4.1_009823m PA | AT5G28840 | 0.820661221 | -1.04347875  | Zero DAP | 11 | 21.2.1.01   | redox                 |
| cassava4.1_015884m PA | AT3G16780 | 0.821863752 | -1.15739432  | Zero DAP | 2  | 29.2.2      | protein               |
| cassava4.1_000283m PA | AT1G76810 | 0.822165757 | -1.046585502 | Zero DAP | 2  | 29.2.3      | protein               |
| cassava4.1_002959m PA | AT2G32910 | 0.823366077 | -1.255009431 | Zero DAP | 2  | 35.2        | not assigned          |
| cassava4.1_005107m PA | AT1G77140 | 0.826151661 | -1.476743153 | Zero DAP | 4  | 29.3.4.3    | protein               |
| cassava4.1_018266m PA | AT1G78870 | 0.826395346 | -1.204896573 | Zero DAP | 6  | 29.5.11.03  | protein               |
| cassava4.1_013619m PA | AT3G01280 | 0.827340691 | -1.054546477 | Zero DAP | 17 | 34.20       | transport             |
| cassava4.1_017738m PA | AT3G16640 | 0.830492973 | -1.12531354  | Zero DAP | 6  | 35.1        | not assigned          |
| cassava4.1_009493m PA | AT4G24820 | 0.831193249 | -1.041134446 | Zero DAP | 2  | 29.5.11.20  | protein               |
| cassava4.1_019754m PA | AT4G39200 | 0.831844716 | -1.099653136 | Zero DAP | 5  | 29.2.2      | protein               |
| cassava4.1_017755m PA | AT4G11600 | 0.832195606 | -1.108873896 | Zero DAP | 7  | 21.2.2      | redox                 |
| cassava4.1_020156m PA | AT1G66240 | 0.833088122 | -1.368581884 | Zero DAP | 4  | 15.2        | metal handling        |
| cassava4.1_014235m PA | AT2G42590 | 0.833164584 | -1.100207461 | Zero DAP | 12 | 30.7        | signalling            |
| cassava4.1_001686m PA | AT5G03650 | 0.83351531  | -1.02787995  | Zero DAP | 14 | 2.1.2.03    | major CHO metabolism  |
| cassava4.1_013147m PA | AT1G30630 | 0.834135941 | -1.020801709 | Zero DAP | 3  | 31.4        | cell                  |



|                       |           |             |              |          |    |               |                       |
|-----------------------|-----------|-------------|--------------|----------|----|---------------|-----------------------|
| cassava4.1_022905m PA | AT2G44610 | 0.834369774 | -1.419990679 | Zero DAP | 4  | 30.5          | signalling            |
| cassava4.1_018827m PA | AT5G28060 | 0.834568656 | -1.106104471 | Zero DAP | 5  | 29.2.2        | protein               |
| cassava4.1_016963m PA | AT5G61640 | 0.834938944 | -1.080209615 | Zero DAP | 3  | 29.4          | protein               |
| cassava4.1_018530m PA | AT3G52560 | 0.836494591 | -1.347919227 | Zero DAP | 2  | 29.5.11.03    | protein               |
| cassava4.1_019020m PA | AT3G12260 | 0.839690149 | -1.07025467  | Zero DAP | 5  | 35.1          | not assigned          |
| cassava4.1_006634m PA | AT3G29360 | 0.843345713 | -1.972698757 | Zero DAP | 3  | 10.1.04       | cell wall             |
| cassava4.1_003147m PA | AT1G67680 | 0.844128752 | -1.078178212 | Zero DAP | 5  | 29.3.4.99     | protein               |
| cassava4.1_008945m PA | AT4G09020 | 0.847019054 | -1.031881174 | Zero DAP | 2  | 2.1.2.04      | major CHO metabolism  |
| cassava4.1_025609m PA | AT1G23010 | 0.849558336 | -1.460857641 | Zero DAP | 2  | 35.1          | not assigned          |
| cassava4.1_015938m PA | AT5G59840 | 0.853251782 | -1.287548413 | Zero DAP | 4  | 30.5          | signalling            |
| cassava4.1_023450m PA | AT2G33740 | 0.853400654 | -1.404724761 | Zero DAP | 2  | 15.2          | metal handling        |
| cassava4.1_001591m PA | AT1G06410 | 0.855903364 | -1.013465401 | Zero DAP | 6  | 3.2.3         | minor CHO metabolism  |
| cassava4.1_004003m PA | AT4G10840 | 0.85707322  | -1.033583341 | Zero DAP | 11 | 31.1          | cell                  |
| cassava4.1_002533m PA | AT5G27640 | 0.857716858 | -1.000262322 | Zero DAP | 6  | 29.2.3        | protein               |
| cassava4.1_013128m PA | AT2G39990 | 0.85795894  | -1.013952058 | Zero DAP | 8  | 29.5.11.20    | protein               |
| cassava4.1_015054m PA | AT5G63310 | 0.857973727 | -1.091983977 | Zero DAP | 2  | 23.4.010      | nucleotide metabolism |
| cassava4.1_011700m PA | AT1G04690 | 0.858435359 | -1.018198845 | Zero DAP | 3  | 34.15         | transport             |
| cassava4.1_018076m PA | AT5G23740 | 0.858613448 | -1.140373752 | Zero DAP | 5  | 29.2.2        | protein               |
| cassava4.1_018516m PA | AT3G49910 | 0.8592907   | -1.094552092 | Zero DAP | 7  | 29.2.2        | protein               |
| cassava4.1_027526m PA | AT1G24140 | 0.861432824 | -1.153670211 | Zero DAP | 4  | 29.5.07       | protein               |
| cassava4.1_033213m PA | AT4G26900 | 0.862600176 | -1.089705818 | Zero DAP | 6  | 35.1          | not assigned          |
| cassava4.1_019080m PA | AT2G34160 | 0.862754441 | -1.06675325  | Zero DAP | 4  | 35.2          | not assigned          |
| cassava4.1_034152m PA | AT4G12700 | 0.863528224 | -2.063722    | Zero DAP | 3  | 35.2          | not assigned          |
| cassava4.1_009545m PA | AT5G12470 | 0.865660101 | -1.037309466 | Zero DAP | 4  | 35.2          | not assigned          |
| cassava4.1_028353m PA | AT3G57880 | 0.866062293 | -1.071714891 | Zero DAP | 5  | 35.1.19       | not assigned          |
| cassava4.1_011133m PA | AT1G53240 | 0.866486145 | -1.003611309 | Zero DAP | 22 | 8.1.09        | TCA / org             |
| cassava4.1_019824m PA | AT5G22580 | 0.867344269 | -1.109344136 | Zero DAP | 3  | 35.2          | not assigned          |
| cassava4.1_000163m PA | AT5G13530 | 0.867448111 | -1.137003086 | Zero DAP | 3  | 29.4          | protein               |
| cassava4.1_009811m PA | AT5G21060 | 0.869991817 | -1.160810847 | Zero DAP | 3  | 13.1.3.6.1.03 | amino acid metabolism |
| cassava4.1_018824m PA | AT5G28060 | 0.871534416 | -1.176830674 | Zero DAP | 2  | 29.2.2        | protein               |
| cassava4.1_014275m PA | AT2G42590 | 0.872616918 | -1.032857253 | Zero DAP | 5  | 30.7          | signalling            |
| cassava4.1_017511m PA | AT5G14680 | 0.873221134 | -1.104659027 | Zero DAP | 2  | 20.2.99       | stress                |
| cassava4.1_002469m PA | AT3G54540 | 0.875352727 | -1.056228621 | Zero DAP | 6  | 34.16         | transport             |
| cassava4.1_001875m PA | AT3G53230 | 0.876348076 | -1.022241289 | Zero DAP | 11 | 31.2          | cell                  |
| cassava4.1_026154m PA | AT4G36760 | 0.87732223  | -1.015449632 | Zero DAP | 5  | 29.5          | protein               |
| cassava4.1_019777m PA | AT5G40370 | 0.878184487 | -1.135900298 | Zero DAP | 4  | 21.04         | redox                 |
| cassava4.1_001803m PA | AT4G16170 | 0.879944471 | -1.194503881 | Zero DAP | 4  | 35.2          | not assigned          |
| cassava4.1_008467m PA | AT3G49680 | 0.880176067 | -1.011412885 | Zero DAP | 11 | 13.1.4.1      | amino acid metabolism |
| cassava4.1_000211m PA | AT1G14850 | 0.880737153 | -1.051367869 | Zero DAP | 4  | 29.3.1        | protein               |
| cassava4.1_013428m PA | AT3G18940 | 0.883609925 | -1.0017564   | Zero DAP | 2  | 35.1          | not assigned          |
| cassava4.1_017240m PA | AT4G12440 | 0.886605722 | -1.394754235 | Zero DAP | 2  | 23.3.1.01     | nucleotide metabolism |
| cassava4.1_018059m PA | AT1G13950 | 0.888724901 | -1.079785729 | Zero DAP | 3  | 29.2.3        | protein               |
| cassava4.1_003374m PA | AT2G36850 | 0.890196254 | -1.172940123 | Zero DAP | 2  | 3.6           | minor CHO metabolism  |
| cassava4.1_014767m PA | AT2G25810 | 0.891579343 | -1.054684736 | Zero DAP | 3  | 34.19.2       | transport             |

|                       |           |             |              |          |    |           |                                        |
|-----------------------|-----------|-------------|--------------|----------|----|-----------|----------------------------------------|
| cassava4.1_032573m PA | AT1G21680 | 0.894040088 | -1.002540004 | Zero DAP | 10 | 35.1      | not assigned                           |
| cassava4.1_006508m PA | AT4G13940 | 0.894087246 | -1.042388558 | Zero DAP | 26 | 13.2.3.4  | amino acid metabolism                  |
| cassava4.1_003676m PA | AT1G78900 | 0.894497998 | -1.023123435 | Zero DAP | 24 | 34.1      | transport                              |
| cassava4.1_001428m PA | AT3G16290 | 0.895956494 | -1.037397394 | Zero DAP | 3  | 29.5.07   | protein                                |
| cassava4.1_033996m PA | AT4G17100 | 0.898097531 | -1.019019886 | Zero DAP | 3  | 29.5.05   | protein                                |
| cassava4.1_008693m PA | AT5G07440 | 0.898345069 | -1.942628999 | Zero DAP | 2  | 12.3.01   | N-metabolism                           |
| cassava4.1_020032m PA | AT3G25220 | 0.900248928 | -1.181539038 | Zero DAP | 2  | 31.3.01   | cell                                   |
| cassava4.1_020203m PA | AT3G10950 | 0.900507216 | -1.741051985 | Zero DAP | 2  | 29.2.2    | protein                                |
| cassava4.1_010865m PA | AT2G26060 | 0.901157406 | -1.038165349 | Zero DAP | 2  | 35.1      | not assigned                           |
| cassava4.1_009374m PA | AT1G61580 | 0.908271217 | -1.039110146 | Zero DAP | 11 | 29.2.2    | protein                                |
| cassava4.1_018226m PA | AT3G55280 | 0.9098995   | -1.077098053 | Zero DAP | 2  | 29.2.2    | protein                                |
| cassava4.1_018064m PA | AT1G13950 | 0.910054106 | -1.184316026 | Zero DAP | 9  | 29.2.3    | protein                                |
| cassava4.1_006109m PA | AT1G21750 | 0.910059016 | -1.007350353 | Zero DAP | 15 | 21.01     | redox                                  |
| cassava4.1_000001m PA | AT3G02260 | 0.910780508 | -1.014635911 | Zero DAP | 23 | 17.2.2    | hormone metabolism                     |
| cassava4.1_020226m PA | AT1G31812 | 0.912657397 | -1.14799133  | Zero DAP | 3  | 11.1.013  | lipid metabolism                       |
| cassava4.1_014328m PA | AT5G27470 | 0.91421691  | -1.027409962 | Zero DAP | 13 | 29.1.011  | protein                                |
| cassava4.1_018965m PA | AT4G27090 | 0.914807216 | -1.121559225 | Zero DAP | 8  | 29.2.2    | protein                                |
| cassava4.1_002530m PA | AT5G27640 | 0.916050716 | -1.04285271  | Zero DAP | 6  | 29.2.3    | protein                                |
| cassava4.1_018644m PA | AT5G61170 | 0.921528089 | -1.211332485 | Zero DAP | 7  | 29.2.2    | protein                                |
| cassava4.1_019181m PA | AT3G01640 | 0.923790002 | -1.008479021 | Zero DAP | 2  | 35.1      | not assigned                           |
| cassava4.1_002747m PA | AT5G56000 | 0.923932914 | -1.008130963 | Zero DAP | 63 | 20.2.1    | stress                                 |
| cassava4.1_006165m PA | AT3G18490 | 0.924311119 | -1.028462355 | Zero DAP | 3  | 27.3.99   | RNA                                    |
| cassava4.1_004852m PA | AT4G04770 | 0.924344863 | -1.612030446 | Zero DAP | 3  | 29.8      | protein assembly and cofactor ligation |
| cassava4.1_005092m PA | AT3G48000 | 0.927184482 | -1.007019521 | Zero DAP | 6  | 5.10      | fermentation                           |
| cassava4.1_014161m PA | AT4G38800 | 0.927227896 | -1.07487983  | Zero DAP | 5  | 35.1      | not assigned                           |
| cassava4.1_018235m PA | AT3G55280 | 0.929921895 | -1.125763698 | Zero DAP | 5  | 29.2.2    | protein                                |
| cassava4.1_003658m PA | AT3G46740 | 0.929935222 | -1.03270995  | Zero DAP | 24 | 29.3.3    | protein                                |
| cassava4.1_015621m PA | AT2G16600 | 0.931327282 | -1.169077009 | Zero DAP | 10 | 26.01     | misc                                   |
| cassava4.1_015621m PA | AT2G16600 | 0.931327282 | -1.169077009 | Zero DAP | 10 | 31.3      | cell                                   |
| cassava4.1_019041m PA | AT1G20580 | 0.931378554 | -1.143712503 | Zero DAP | 2  | 27.1      | RNA                                    |
| cassava4.1_010874m PA | AT1G17890 | 0.931435795 | -1.011014388 | Zero DAP | 6  | 10.1.08   | cell wall                              |
| cassava4.1_005454m PA | AT1G34220 | 0.932143946 | -1.03954866  | Zero DAP | 2  | 35.2      | not assigned                           |
| cassava4.1_011340m PA | AT3G04120 | 0.939211246 | -1.039678525 | Zero DAP | 24 | 4.09      | glycolysis                             |
| cassava4.1_003144m PA | AT5G42020 | 0.93961833  | -1.010445613 | Zero DAP | 28 | 20.2.1    | stress                                 |
| cassava4.1_013330m PA | AT1G03210 | 0.940211195 | -1.018619436 | Zero DAP | 2  | 35.1      | not assigned                           |
| cassava4.1_005006m PA | AT3G21240 | 0.940864767 | -1.124427639 | Zero DAP | 3  | 16.2.1.03 | secondary metabolism                   |
| cassava4.1_008453m PA | AT1G09210 | 0.940998571 | -1.024385786 | Zero DAP | 9  | 30.3      | signalling                             |
| cassava4.1_010805m PA | AT3G28715 | 0.9410944   | -1.013199217 | Zero DAP | 5  | 34.1.01   | transport                              |
| cassava4.1_005003m PA | AT3G03960 | 0.941403113 | -1.009108095 | Zero DAP | 21 | 29.6      | protein                                |
| cassava4.1_009860m PA | AT1G79230 | 0.941819257 | -1.018021457 | Zero DAP | 4  | 13.2.5.3  | amino acid metabolism                  |
| cassava4.1_012149m PA | AT3G23620 | 0.947824576 | -1.113877867 | Zero DAP | 2  | 29.2.2.50 | protein                                |
| cassava4.1_001255m PA | AT3G27530 | 0.95105048  | -1.026785378 | Zero DAP | 7  | 35.1      | not assigned                           |
| cassava4.1_011245m PA | AT5G16760 | 0.951513692 | -1.000379422 | Zero DAP | 5  | 30.4.05   | signalling                             |
| cassava4.1_009027m PA | AT5G63980 | 0.952851638 | -1.033983039 | Zero DAP | 6  | 23.2      | nucleotide metabolism                  |

|                       |           |             |              |          |    |           |               |
|-----------------------|-----------|-------------|--------------|----------|----|-----------|---------------|
| cassava4.1_005352m PA | AT3G06720 | 0.953544275 | -1.006986093 | Zero DAP | 19 | 29.3.1    | protein       |
| cassava4.1_029194m PA | AT3G55200 | 0.953943764 | -1.761314429 | Zero DAP | 3  | 27.1.1    | RNA           |
| cassava4.1_013644m PA | AT1G55170 | 0.960514266 | -1.074557229 | Zero DAP | 5  | 35.2      | not assigned  |
| cassava4.1_014383m PA | AT2G16595 | 0.963301014 | -1.157732591 | Zero DAP | 3  | 35.1      | not assigned  |
| cassava4.1_004315m PA | AT2G32910 | 0.963506411 | -1.044690144 | Zero DAP | 3  | 35.2      | not assigned  |
| cassava4.1_000124m PA | AT1G20960 | 0.966302182 | -1.047239788 | Zero DAP | 2  | 27.1      | RNA           |
| cassava4.1_000124m PA | AT1G20960 | 0.966302182 | -1.047239788 | Zero DAP | 2  | 28.1      | DNA           |
| cassava4.1_029952m PA | AT5G40382 | 0.971605413 | -1.208473509 | Zero DAP | 4  | -         | -             |
| cassava4.1_001828m PA | AT5G05980 | 0.974159869 | -1.005713309 | Zero DAP | 3  | 25.08     | C1-metabolism |
| cassava4.1_016219m PA | AT3G27080 | 0.977011728 | -1.372029237 | Zero DAP | 4  | 29.3.2    | protein       |
| cassava4.1_001614m PA | AT2G38020 | 0.980070521 | -1.043276032 | Zero DAP | 6  | 29.3.4.3  | protein       |
| cassava4.1_001455m PA | AT4G01810 | 0.980258185 | -1.976975904 | Zero DAP | 2  | 29.3.4.2  | protein       |
| cassava4.1_006533m PA | AT5G60160 | 0.980487439 | -1.007219108 | Zero DAP | 13 | 29.5.04   | protein       |
| cassava4.1_018143m PA | AT4G14420 | 0.981630068 | -1.357586735 | Zero DAP | 2  | 35.1      | not assigned  |
| cassava4.1_014276m PA | AT5G05670 | 0.984425502 | -1.073948286 | Zero DAP | 2  | 29.3.4.99 | protein       |
| cassava4.1_019037m PA | AT5G56600 | 0.987534441 | -1.150670792 | Zero DAP | 4  | 31.1      | cell          |
| cassava4.1_019435m PA | AT2G18040 | 0.989499853 | -1.251841314 | Zero DAP | 5  | 31.3.01   | cell          |
| cassava4.1_018964m PA | AT2G32720 | 0.989928031 | -1.228327572 | Zero DAP | 2  | 21.2      | redox         |
| cassava4.1_010964m PA | AT2G47970 | 0.994719908 | -1.007357975 | Zero DAP | 6  | 35.1      | not assigned  |
| cassava4.1_034288m PA | AT1G50180 | 0.99491094  | -1.00106679  | Zero DAP | 19 | 20.1      | stress        |
| cassava4.1_011307m PA | AT4G21450 | 0.996118829 | -1.006848597 | Zero DAP | 2  | 31.4      | cell          |
| cassava4.1_017098m PA | AT3G05590 | 0.998356961 | -1.277587784 | Zero DAP | 7  | 29.2.2    | protein       |
| cassava4.1_017792m PA | AT1G42960 | 0.999246856 | -1.33112505  | Zero DAP | 2  | 35.2      | not assigned  |

**Supplemental Table 4.2. Significantly differentially regulated proteins during pruning.** Proteins significantly more abundant zero or ten days after pruning (DAP) were defined as having an ANCOVA

| Cassava accession     | Arabidopsis Accession | Anova (p)  | Progenesis      |                        | Mapman      |                                                  |
|-----------------------|-----------------------|------------|-----------------|------------------------|-------------|--------------------------------------------------|
|                       |                       |            | Max fold change | Highest mean condition | BINCODE     | NAME                                             |
| cassava4.1_006617m PA | AT1G70580             | 0.00735146 | 1.926825688     | Ten DAP                | 1.2.3       | photosynthesis                                   |
| cassava4.1_010126m PA | AT3G14420             | 0.04241707 | 1.750614151     | Ten DAP                | 1.2.02      | photosynthesis                                   |
| cassava4.1_009233m PA | AT2G01140             | 0.01661161 | 1.31375782      | Ten DAP                | 1.3.06      | photosynthesis                                   |
| cassava4.1_006596m PA | AT3G12780             | 0.00917201 | 1.745902602     | Ten DAP                | 1.3.03      | photosynthesis                                   |
| cassava4.1_006605m PA | AT3G12780             | 0.02459507 | 1.61206826      | Ten DAP                | 1.3.03      | photosynthesis                                   |
| cassava4.1_007221m PA | AT1G47840             | 0.03804542 | 1.512944971     | Ten DAP                | 2.2.1.04    | major CHO metabolism                             |
| cassava4.1_005201m PA | AT4G09510             | 0.03593139 | 1.279162588     | Ten DAP                | 2.2.1.03.01 | major CHO metabolism                             |
| cassava4.1_009614m PA | AT4G32400             | 0.0418598  | 1.204114911     | Ten DAP                | 2.1.2.05    | major CHO metabolism                             |
| cassava4.1_001233m PA | AT1G68560             | 0.03975045 | 1.79339563      | Ten DAP                | 2.2.2.1     | major CHO metabolism                             |
| cassava4.1_001244m PA | AT1G68560             | 0.04287359 | 1.296483626     | Ten DAP                | 2.2.2.1     | major CHO metabolism                             |
| cassava4.1_006138m PA | AT4G29130             | 0.03929927 | 1.559017046     | Ten DAP                | 2.2.1.04    | major CHO metabolism                             |
| cassava4.1_001362m PA | AT1G69830             | 0.02387379 | 6.271784075     | Ten DAP                | 2.2.2.1     | major CHO metabolism                             |
| cassava4.1_004771m PA | AT5G04360             | 0.02213155 | 1.490585736     | Ten DAP                | 2.1.2.04    | major CHO metabolism                             |
| cassava4.1_004619m PA | AT5G24300             | 0.02716323 | 1.244623178     | Ten DAP                | 2.1.2.02    | major CHO metabolism                             |
| cassava4.1_008949m PA | AT5G08370             | 0.00616051 | 1.484106938     | Ten DAP                | 3.8.2       | minor CHO metabolism                             |
| cassava4.1_006596m PA | AT3G12780             | 0.00917201 | 1.745902602     | Ten DAP                | 4.010       | glycolysis                                       |
| cassava4.1_006605m PA | AT3G12780             | 0.02459507 | 1.61206826      | Ten DAP                | 4.010       | glycolysis                                       |
| cassava4.1_006252m PA | AT1G44170             | 0.03025212 | 1.392279242     | Ten DAP                | 5.10        | fermentation                                     |
| cassava4.1_005123m PA | AT3G48000             | 0.00810057 | 1.519328287     | Ten DAP                | 5.10        | fermentation                                     |
| cassava4.1_004362m PA | AT4G37870             | 0.01579413 | 2.842536127     | Ten DAP                | 6.04        | gluconeogenesis/ glyoxylate cycle                |
| cassava4.1_030131m PA | AT4G37870             | 0.02949767 | 2.560662975     | Ten DAP                | 6.04        | gluconeogenesis/ glyoxylate cycle                |
| cassava4.1_010585m PA | AT2G22780             | 0.02813043 | 1.768182403     | Ten DAP                | 6.03        | gluconeogenesis                                  |
| cassava4.1_003566m PA | AT1G09420             | 0.0006559  | 1.233848973     | Ten DAP                | 7.1.01      | OPP                                              |
| cassava4.1_010105m PA | AT5G43330             | 0.00239901 | 1.203658368     | Ten DAP                | 8.2.09      | TCA / org                                        |
| cassava4.1_009952m PA | AT4G35650             | 0.04786744 | 1.431034228     | Ten DAP                | 8.2.04      | TCA / org                                        |
| cassava4.1_000903m PA | AT2G05710             | 0.0176247  | 1.241465409     | Ten DAP                | 8.1.03      | TCA / org                                        |
| cassava4.1_006853m PA | AT2G44350             | 0.03377405 | 1.613845419     | Ten DAP                | 8.1.02      | TCA / org                                        |
| cassava4.1_007540m PA | AT1G59900             | 0.01135208 | 1.398320386     | Ten DAP                | 8.1.01.01   | TCA / org                                        |
| cassava4.1_004864m PA | AT3G13930             | 0.04236915 | 1.298227513     | Ten DAP                | 8.1.01.02   | TCA / org                                        |
| cassava4.1_003490m PA | AT3G52200             | 0.03490738 | 1.263526843     | Ten DAP                | 8.1.01.02   | TCA / org                                        |
| cassava4.1_008387m PA | AT2G20420             | 0.04327857 | 1.300115518     | Ten DAP                | 8.1.06      | TCA / org                                        |
| cassava4.1_007889m PA | AT5G58330             | 0.0092062  | 2.283125624     | Ten DAP                | 8.2.09      | TCA / org                                        |
| cassava4.1_009175m PA | AT2G20360             | 0.02925598 | 1.374081152     | Ten DAP                | 9.1.2       | mitochondrial electron transport / ATP synthesis |
| cassava4.1_004821m PA | AT4G02320             | 0.0288791  | 1.689617201     | Ten DAP                | 10.8.99     | cell wall                                        |

|                       |           |            |             |         |               |                       |
|-----------------------|-----------|------------|-------------|---------|---------------|-----------------------|
| cassava4.1_003278m PA | AT1G62440 | 0.00596309 | 1.599930163 | Ten DAP | 10.5.3        | cell wall             |
| cassava4.1_001252m PA | AT1G58370 | 0.00725663 | 1.475983414 | Ten DAP | 10.6.2        | cell wall             |
| cassava4.1_014262m PA | AT3G45970 | 0.03298087 | 1.465024207 | Ten DAP | 10.7          | cell wall             |
| cassava4.1_004339m PA | AT3G14310 | 0.00338657 | 1.481605442 | Ten DAP | 10.8.01       | cell wall             |
| cassava4.1_002966m PA | AT1G06290 | 0.00324872 | 2.249492405 | Ten DAP | 11.9.4.02     | lipid metabolism      |
| cassava4.1_005575m PA | AT4G29010 | 0.00838385 | 1.577977396 | Ten DAP | 11.9.4.09     | lipid metabolism      |
| cassava4.1_002951m PA | AT5G13640 | 0.02307906 | 1.178699477 | Ten DAP | 11.8.10       | lipid metabolism      |
| cassava4.1_007181m PA | AT2G33150 | 0.02495669 | 2.286929361 | Ten DAP | 11.9.4.05     | lipid metabolism      |
| cassava4.1_019325m PA | AT5G42890 | 0.04040195 | 1.561863456 | Ten DAP | 11.8          | lipid metabolism      |
| cassava4.1_014036m PA | AT4G16210 | 0.00748564 | 1.643172336 | Ten DAP | 11.9.4.04     | lipid metabolism      |
| cassava4.1_002479m PA | AT3G06860 | 0.02641783 | 1.959534388 | Ten DAP | 11.9.4.09     | lipid metabolism      |
| cassava4.1_001538m PA | AT4G35790 | 0.0183763  | 1.58413125  | Ten DAP | 11.9.3.01     | lipid metabolism      |
| cassava4.1_006559m PA | AT2G18730 | 0.03827546 | 1.306196433 | Ten DAP | 11.3.05       | lipid metabolism      |
| cassava4.1_028937m PA | AT5G35360 | 0.00162604 | 1.636998465 | Ten DAP | 11.1.01       | lipid metabolism      |
| cassava4.1_008713m PA | AT5G18170 | 0.00200684 | 2.03320298  | Ten DAP | 12.3.01       | N-metabolism          |
| cassava4.1_012571m PA | AT5G54080 | 0.00819589 | 3.560328754 | Ten DAP | 13.2.6.2      | amino acid metabolism |
| cassava4.1_008396m PA | AT1G12050 | 0.00411318 | 1.602166851 | Ten DAP | 13.2.6.2      | amino acid metabolism |
| cassava4.1_006859m PA | AT5G46180 | 0.0271597  | 1.838310247 | Ten DAP | 13.2.2.3      | amino acid metabolism |
| cassava4.1_008844m PA | AT5G19550 | 0.04100868 | 1.260339511 | Ten DAP | 13.1.1.2.01   | amino acid metabolism |
| cassava4.1_022406m PA | AT4G34030 | 0.01046407 | 1.115867703 | Ten DAP | 13.2.4.4      | amino acid metabolism |
| cassava4.1_007181m PA | AT2G33150 | 0.02495669 | 2.286929361 | Ten DAP | 13.2.4.1      | amino acid metabolism |
| cassava4.1_004831m PA | AT5G62530 | 0.01272154 | 1.369684373 | Ten DAP | 13.2.2.2      | amino acid metabolism |
| cassava4.1_005703m PA | AT3G22200 | 0.03252997 | 1.768579566 | Ten DAP | 13.1.1.1.02   | amino acid metabolism |
| cassava4.1_014036m PA | AT4G16210 | 0.00748564 | 1.643172336 | Ten DAP | 13.2.3.5      | amino acid metabolism |
| cassava4.1_006617m PA | AT1G70580 | 0.00735146 | 1.926825688 | Ten DAP | 13.1.1.3.01   | amino acid metabolism |
| cassava4.1_008563m PA | AT2G24580 | 0.02640825 | 1.497246038 | Ten DAP | 13.1.5.2.041  | amino acid metabolism |
| cassava4.1_011138m PA | AT3G22740 | 0.01149975 | 9.158163652 | Ten DAP | 13.1.3.4.012  | amino acid metabolism |
| cassava4.1_006432m PA | AT5G11880 | 0.02236041 | 1.417256807 | Ten DAP | 13.1.3.5.05   | amino acid metabolism |
| cassava4.1_007019m PA | AT3G57050 | 0.04777276 | 1.5727444   | Ten DAP | 13.1.3.4.02   | amino acid metabolism |
| cassava4.1_010180m PA | AT2G17265 | 0.01133639 | 1.355471482 | Ten DAP | 13.1.3.6.1.04 | amino acid metabolism |
| cassava4.1_007094m PA | AT4G31990 | 0.03861669 | 1.344153267 | Ten DAP | 13.1.1.2.01   | amino acid metabolism |
| cassava4.1_007524m PA | AT1G80600 | 0.01966984 | 1.692282687 | Ten DAP | 13.1.2.3.04   | amino acid metabolism |
| cassava4.1_006286m PA | AT4G24830 | 0.04182703 | 1.460609283 | Ten DAP | 13.1.2.3.022  | amino acid metabolism |
| cassava4.1_008771m PA | AT1G09795 | 0.02236232 | 1.348388951 | Ten DAP | 13.1.7.01     | amino acid metabolism |
| cassava4.1_019208m PA | AT2G43750 | 0.00588843 | 1.541881836 | Ten DAP | 13.1.5.3.01   | amino acid metabolism |
| cassava4.1_002871m PA | AT5G04590 | 0.04026437 | 1.20751879  | Ten DAP | 14.03         | S-assimilation        |
| cassava4.1_014697m PA | AT5G43830 | 0.01163371 | 1.499349065 | Ten DAP | 15            | metal handling        |
| cassava4.1_014185m PA | AT3G11050 | 0.03080794 | 2.039471953 | Ten DAP | 15.2          | metal handling        |
| cassava4.1_011289m PA | AT5G65550 | 0.04073648 | 1.380319881 | Ten DAP | 16.8.1.012    | secondary metabolism  |

|                       |           |            |             |         |              |                        |
|-----------------------|-----------|------------|-------------|---------|--------------|------------------------|
| cassava4.1_011460m PA | AT5G42800 | 0.02264061 | 1.520848984 | Ten DAP | 16.8.3.01    | secondary metabolism   |
| cassava4.1_010343m PA | AT1G17020 | 0.03374059 | 1.772202731 | Ten DAP | 16.8.4       | secondary metabolism   |
| cassava4.1_011708m PA | AT2G45400 | 0.02433786 | 1.423547767 | Ten DAP | 16.8.4.01    | secondary metabolism   |
| cassava4.1_029504m PA | AT4G39230 | 0.01731638 | 1.468246346 | Ten DAP | 16.8.5       | secondary metabolism   |
| cassava4.1_012203m PA | AT3G12070 | 0.04636544 | 1.254214436 | Ten DAP | 16.1.1       | secondary metabolism   |
| cassava4.1_010140m PA | AT4G37970 | 0.02272133 | 1.327226671 | Ten DAP | 16.2.1.010   | secondary metabolism   |
| cassava4.1_004359m PA | AT4G14210 | 0.02571433 | 1.388460969 | Ten DAP | 16.1.4.02    | secondary metabolism   |
| cassava4.1_015449m PA | AT1G28200 | 0.01043683 | 1.407374243 | Ten DAP | 17.1.3       | hormone metabolism     |
| cassava4.1_014697m PA | AT5G43830 | 0.01163371 | 1.499349065 | Ten DAP | 17.2.3       | hormone metabolism     |
| cassava4.1_013447m PA | AT1G52340 | 0.03886228 | 1.283231678 | Ten DAP | 17.1.1.1.011 | hormone metabolism     |
| cassava4.1_015980m PA | AT5G42650 | 0.04548834 | 1.540189318 | Ten DAP | 17.7.1.03    | hormone metabolism     |
| cassava4.1_001259m PA | AT1G67560 | 0.01325718 | 1.196296357 | Ten DAP | 17.7.1.02    | hormone metabolism     |
| cassava4.1_005844m PA | AT5G14220 | 0.02329841 | 1.544615719 | Ten DAP | 19.09        | tetrapyrrole synthesis |
| cassava4.1_018093m PA | AT1G53540 | 0.00534376 | 8.023552477 | Ten DAP | 20.2.1       | stress                 |
| cassava4.1_013205m PA | AT4G21320 | 0.01776866 | 2.769392266 | Ten DAP | 20.2.1       | stress                 |
| cassava4.1_008257m PA | AT2G38000 | 0.02219464 | 2.281747913 | Ten DAP | 20.2.1       | stress                 |
| cassava4.1_001300m PA | AT1G74310 | 0.01647251 | 2.158408629 | Ten DAP | 20.2.1       | stress                 |
| cassava4.1_001604m PA | AT3G50950 | 0.00758851 | 1.948000233 | Ten DAP | 20.1         | stress                 |
| cassava4.1_011775m PA | AT1G05260 | 0.01302614 | 1.752926694 | Ten DAP | 20.2.2       | stress                 |
| cassava4.1_015966m PA | AT3G62020 | 0.01599747 | 1.865450268 | Ten DAP | 20.2.99      | stress                 |
| cassava4.1_012839m PA | AT3G24170 | 0.00163086 | 1.41248156  | Ten DAP | 21.2.2       | redox                  |
| cassava4.1_006188m PA | AT3G24170 | 0.00282425 | 1.206469929 | Ten DAP | 21.2.2       | redox                  |
| cassava4.1_009693m PA | AT3G17880 | 0.03309878 | 1.637245385 | Ten DAP | 21.01        | redox                  |
| cassava4.1_007980m PA | AT3G52880 | 0.02382477 | 1.420397426 | Ten DAP | 21.2         | redox                  |
| cassava4.1_006303m PA | AT4G35090 | 0.03955936 | 3.329616518 | Ten DAP | 21.6         | redox                  |
| cassava4.1_006302m PA | AT4G35090 | 0.00777775 | 3.291850587 | Ten DAP | 21.6         | redox                  |
| cassava4.1_006320m PA | AT4G35090 | 0.00042379 | 2.90455018  | Ten DAP | 21.6         | redox                  |
| cassava4.1_006367m PA | AT1G63940 | 0.03266988 | 1.746078082 | Ten DAP | 21.2.1       | redox                  |
| cassava4.1_006560m PA | AT5G27380 | 0.01339028 | 1.818096767 | Ten DAP | 21.2.2       | redox                  |
| cassava4.1_005081m PA | AT1G36280 | 0.00485676 | 1.772631821 | Ten DAP | 23.1.2.08    | nucleotide metabolism  |
| cassava4.1_014834m PA | AT5G63400 | 0.00959762 | 1.394046695 | Ten DAP | 23.4.01      | nucleotide metabolism  |
| cassava4.1_008175m PA | AT3G17810 | 0.02012165 | 1.346053171 | Ten DAP | 23.2         | nucleotide metabolism  |
| cassava4.1_029420m PA | AT5G42260 | 0.03324713 | 5.52076836  | Ten DAP | 26.03        | misc                   |
| cassava4.1_032853m PA | AT3G60140 | 0.01296309 | 3.99794269  | Ten DAP | 26.03        | misc                   |
| cassava4.1_027841m PA | AT4G19880 | 0.03792572 | 1.689813809 | Ten DAP | 26.09        | misc                   |
| cassava4.1_001233m PA | AT1G68560 | 0.03975045 | 1.79339563  | Ten DAP | 26.03        | misc                   |
| cassava4.1_005935m PA | AT3G18080 | 0.0413693  | 1.68803424  | Ten DAP | 26.03        | misc                   |
| cassava4.1_001244m PA | AT1G68560 | 0.04287359 | 1.296483626 | Ten DAP | 26.03        | misc                   |
| cassava4.1_011779m PA | AT5G05340 | 0.01235784 | 1.393003633 | Ten DAP | 26.12        | misc                   |

|                       |           |            |             |         |                |            |
|-----------------------|-----------|------------|-------------|---------|----------------|------------|
| cassava4.1_011574m PA | AT5G66390 | 0.02916163 | 1.388266508 | Ten DAP | 26.12          | misc       |
| cassava4.1_003404m PA | AT3G06510 | 0.0215416  | 2.038064428 | Ten DAP | 26.03          | misc       |
| cassava4.1_011655m PA | AT4G13010 | 0.00231783 | 2.695500686 | Ten DAP | 26.07          | misc       |
| cassava4.1_001602m PA | AT3G26720 | 0.00324419 | 1.615893902 | Ten DAP | 26.03          | misc       |
| cassava4.1_006167m PA | AT2G20710 | 0.04987384 | 1.601900133 | Ten DAP | 27.3.67        | RNA        |
| cassava4.1_029528m PA | AT5G10770 | 0.00716048 | 1.780161588 | Ten DAP | 27.3.99        | RNA        |
| cassava4.1_009485m PA | AT5G10770 | 0.00033151 | 1.54793695  | Ten DAP | 27.3.99        | RNA        |
| cassava4.1_010150m PA | AT4G17520 | 0.01107316 | 1.452526425 | Ten DAP | 27.4           | RNA        |
| cassava4.1_016819m PA | AT2G45820 | 0.00930541 | 1.662800685 | Ten DAP | 27.3.67        | RNA        |
| cassava4.1_015763m PA | AT5G63880 | 0.04002527 | 1.232033827 | Ten DAP | 27.3.71        | RNA        |
| cassava4.1_003845m PA | AT1G72160 | 0.00472349 | 1.345255193 | Ten DAP | 28.99          | DNA        |
| cassava4.1_007163m PA | AT4G38220 | 0.02599717 | 1.607520884 | Ten DAP | 29.5           | protein    |
| cassava4.1_007251m PA | AT4G38220 | 0.03538782 | 1.539019794 | Ten DAP | 29.5           | protein    |
| cassava4.1_002552m PA | AT1G50380 | 0.03976622 | 1.485492241 | Ten DAP | 29.5           | protein    |
| cassava4.1_009672m PA | AT3G54360 | 0.00386717 | 1.46021094  | Ten DAP | 29.5.11.04.02  | protein    |
| cassava4.1_006459m PA | AT1G06110 | 0.00178612 | 1.752486433 | Ten DAP | 29.5.11.4.3.02 | protein    |
| cassava4.1_013730m PA | AT3G27430 | 0.02456257 | 1.842292617 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_008212m PA | AT1G53750 | 0.03723627 | 1.524920926 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_008421m PA | AT5G58290 | 0.02399329 | 1.485892869 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_015645m PA | AT3G60820 | 0.02229892 | 1.17945286  | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_008374m PA | AT5G09900 | 0.02228975 | 1.165663759 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_022803m PA | AT3G13235 | 0.0051246  | 1.252930263 | Ten DAP | 29.5.11.01     | protein    |
| cassava4.1_007929m PA | AT1G51710 | 0.00018483 | 1.247724727 | Ten DAP | 29.5.11.05     | protein    |
| cassava4.1_008257m PA | AT2G38000 | 0.02219464 | 2.281747913 | Ten DAP | 29.6           | protein    |
| cassava4.1_015319m PA | AT2G18110 | 0.01445178 | 1.568825174 | Ten DAP | 29.2.4         | protein    |
| cassava4.1_007130m PA | AT1G04170 | 0.01277707 | 1.411027139 | Ten DAP | 29.2.3         | protein    |
| cassava4.1_009232m PA | AT1G53880 | 0.0491596  | 1.323855844 | Ten DAP | 29.2.3         | protein    |
| cassava4.1_011934m PA | AT2G40010 | 0.01360298 | 1.172392775 | Ten DAP | 29.2.2         | protein    |
| cassava4.1_024858m PA | AT5G07090 | 0.0453126  | 1.360660378 | Ten DAP | 29.2.2         | protein    |
| cassava4.1_003839m PA | AT3G03060 | 0.01806783 | 1.337480979 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_009061m PA | AT1G45000 | 0.03165599 | 1.202475005 | Ten DAP | 29.5.11.20     | protein    |
| cassava4.1_006415m PA | AT1G63500 | 0.01579717 | 1.310031239 | Ten DAP | 29.4           | protein    |
| cassava4.1_004238m PA | AT3G25800 | 0.04452667 | 1.170059726 | Ten DAP | 29.4           | protein    |
| cassava4.1_006148m PA | AT4G35230 | 0.00299608 | 1.945108137 | Ten DAP | 29.4.1.52      | protein    |
| cassava4.1_022125m PA | AT4G20360 | 0.04550581 | 2.152845168 | Ten DAP | 29.2.4         | protein    |
| cassava4.1_003427m PA | AT5G57580 | 0.03973165 | 2.126304302 | Ten DAP | 30.3           | signalling |
| cassava4.1_012932m PA | AT5G39790 | 0.04500051 | 1.769509262 | Ten DAP | 30.1           | signalling |
| cassava4.1_008933m PA | AT2G43790 | 0.02218486 | 1.476349168 | Ten DAP | 30.6           | signalling |
| cassava4.1_017520m PA | AT3G24540 | 0.03343178 | 1.416408678 | Ten DAP | 30.2.22        | signalling |

|                       |           |            |             |         |         |              |
|-----------------------|-----------|------------|-------------|---------|---------|--------------|
| cassava4.1_009469m PA | AT5G62390 | 0.03536776 | 1.561448059 | Ten DAP | 30.3    | signalling   |
| cassava4.1_009237m PA | AT1G52290 | 0.00906804 | 1.582418007 | Ten DAP | 30.2.22 | signalling   |
| cassava4.1_009779m PA | AT5G09810 | 0.03402981 | 1.185250403 | Ten DAP | 31.1    | cell         |
| cassava4.1_012276m PA | AT2G38750 | 0.01158737 | 1.127961248 | Ten DAP | 31.1    | cell         |
| cassava4.1_013229m PA | AT3G23400 | 0.03021156 | 1.774555917 | Ten DAP | 31.1    | cell         |
| cassava4.1_021183m PA | AT1G35720 | 0.01170947 | 1.169080202 | Ten DAP | 31.1    | cell         |
| cassava4.1_012423m PA | AT2G28680 | 0.02682253 | 3.966860939 | Ten DAP | 33.1    | development  |
| cassava4.1_010513m PA | AT2G28680 | 0.02426298 | 3.009674099 | Ten DAP | 33.1    | development  |
| cassava4.1_010570m PA | AT1G07750 | 0.00165494 | 1.904043819 | Ten DAP | 33.1    | development  |
| cassava4.1_008799m PA | AT2G26560 | 0.00748142 | 1.641450843 | Ten DAP | 33.1    | development  |
| cassava4.1_013350m PA | AT3G45600 | 0.00174226 | 1.687943839 | Ten DAP | 33.99   | development  |
| cassava4.1_009614m PA | AT4G32400 | 0.0418598  | 1.204114911 | Ten DAP | 34.8    | transport    |
| cassava4.1_013267m PA | AT3G54820 | 0.00177835 | 1.936787885 | Ten DAP | 34.19.1 | transport    |
| cassava4.1_013231m PA | AT4G00430 | 0.04499177 | 1.724545394 | Ten DAP | 34.19.1 | transport    |
| cassava4.1_013284m PA | AT5G60660 | 0.01643505 | 1.4258252   | Ten DAP | 34.19.1 | transport    |
| cassava4.1_003845m PA | AT1G72160 | 0.00472349 | 1.345255193 | Ten DAP | 34.99   | transport    |
| cassava4.1_009839m PA | AT1G12840 | 0.00419574 | 1.388372798 | Ten DAP | 34.1    | transport    |
| cassava4.1_023189m PA | AT3G42050 | 0.02032189 | 1.4106215   | Ten DAP | 34.1.01 | transport    |
| cassava4.1_015379m PA | AT4G11150 | 0.00229837 | 1.346622159 | Ten DAP | 34.1.01 | transport    |
| cassava4.1_008188m PA | AT3G48530 | 0.01710694 | 11.3332787  | Ten DAP | 35.1    | not assigned |
| cassava4.1_032921m PA | AT3G48690 | 0.00682039 | 6.305755438 | Ten DAP | 35.1    | not assigned |
| cassava4.1_008547m PA | AT1G49820 | 0.0011878  | 2.376973953 | Ten DAP | 35.1    | not assigned |
| cassava4.1_012172m PA | AT4G24340 | 0.02779684 | 2.232144911 | Ten DAP | 35.1    | not assigned |
| cassava4.1_015242m PA | AT5G63620 | 0.01593325 | 2.085665939 | Ten DAP | 35.1    | not assigned |
| cassava4.1_006656m PA | AT5G57655 | 0.00166793 | 1.989665603 | Ten DAP | 35.1    | not assigned |
| cassava4.1_009991m PA | AT4G17370 | 0.0350487  | 1.72928579  | Ten DAP | 35.1    | not assigned |
| cassava4.1_008528m PA | AT1G49820 | 0.03764094 | 1.716446498 | Ten DAP | 35.1    | not assigned |
| cassava4.1_004506m PA | AT1G60420 | 0.02391674 | 1.678195678 | Ten DAP | 35.1    | not assigned |
| cassava4.1_034124m PA | AT2G38610 | 0.03189873 | 1.463435978 | Ten DAP | 35.1    | not assigned |
| cassava4.1_013823m PA | AT1G19580 | 0.02560833 | 1.192395482 | Ten DAP | 35.1    | not assigned |
| cassava4.1_001079m PA | AT3G08840 | 0.02814544 | 1.125596694 | Ten DAP | 35.1    | not assigned |
| cassava4.1_033294m PA | AT5G44640 | 0.00609917 | 1.123323949 | Ten DAP | 35.1    | not assigned |
| cassava4.1_001530m PA | AT5G06350 | 0.01826866 | 1.962559571 | Ten DAP | 35.2    | not assigned |
| cassava4.1_010559m PA | AT5G25770 | 0.02037935 | 1.271911282 | Ten DAP | 35.2    | not assigned |
| cassava4.1_003090m PA | AT3G10740 | 0.01364819 | 1.783904242 | Ten DAP | 35.1    | not assigned |
| cassava4.1_001514m PA | AT5G12950 | 0.04047566 | 1.331413898 | Ten DAP | 35.2    | not assigned |
| cassava4.1_011670m PA | AT4G27585 | 0.00365175 | 1.356094422 | Ten DAP | 35.1    | not assigned |
| cassava4.1_013108m PA | AT4G28510 | 0.04786737 | 1.095548426 | Ten DAP | 35.1    | not assigned |
| cassava4.1_011051m PA | AT5G22330 | 0.01721033 | 1.579219012 | Ten DAP | 35.1    | not assigned |



|                       |                          |            |             |          |           |                                                  |
|-----------------------|--------------------------|------------|-------------|----------|-----------|--------------------------------------------------|
| cassava4.1_011202m PA | AT1G50510                | 0.0206008  | 1.938611586 | Ten DAP  | 35.1      | not assigned                                     |
| cassava4.1_016410m PA | AT4G20260                | 0.04832071 | 1.827661574 | Ten DAP  | 35.1      | not assigned                                     |
| cassava4.1_006689m PA | AT1G64760                | 0.01403815 | 1.233673092 | Ten DAP  | 35.1      | not assigned                                     |
| cassava4.1_001385m PA | AT1G47550                | 0.00319409 | 1.328324206 | Ten DAP  | 35.2      | not assigned                                     |
| cassava4.1_007931m PA | AT1G53280                | 0.0355971  | 1.584043363 | Ten DAP  | 35.1      | not assigned                                     |
| cassava4.1_000890m PA | AT1G22610                | 0.00492897 | 2.923968357 | Ten DAP  | 35.1.19   | not assigned                                     |
| cassava4.1_006938m PA | AT3G06960                | 0.0269218  | 1.810813641 | Ten DAP  | 35.2      | not assigned                                     |
| cassava4.1_010825m PA | AT5G08540                | 0.0192857  | 1.541150533 | Ten DAP  | 35.2      | not assigned                                     |
| cassava4.1_009976m PA | AT1G74640                | 0.02576811 | 1.406039938 | Ten DAP  | 35.2      | not assigned                                     |
| cassava4.1_021615m PA | AT3G55260                | 0.02328896 | 1.648389028 | Ten DAP  | 35.1      | not assigned                                     |
| cassava4.1_006146m PA | AT5G24318                | 0.0490241  | 1.605479466 | Ten DAP  |           |                                                  |
| cassava4.1_018274m PA | cassava4.1_018274m PF024 | 0.0412543  | 2.271361357 | Ten DAP  |           |                                                  |
| cassava4.1_029754m PA | At:cassava4.1_029754m    | 0.04823543 | 2.005286887 | Ten DAP  |           |                                                  |
| cassava4.1_011584m PA | AT3G59480                | 0.01329758 | 1.475956124 | Zero DAP | 2.2.1.01  | major CHO metabolism                             |
| cassava4.1_005409m PA | AT4G39210                | 0.01135198 | 2.088975338 | Zero DAP | 2.1.2.01  | major CHO metabolism                             |
| cassava4.1_011197m PA | AT1G43670                | 0.03943856 | 1.217314961 | Zero DAP | 2.1.1.03  | major CHO metabolism                             |
| cassava4.1_000497m PA | AT5G26570                | 0.02678393 | 1.321020787 | Zero DAP | 2.2.2.03  | major CHO metabolism                             |
| cassava4.1_005410m PA | AT5G53850                | 0.04145164 | 1.357691971 | Zero DAP | 3.5       | minor CHO metabolism                             |
| cassava4.1_006818m PA | AT2G29560                | 0.01307646 | 1.559280338 | Zero DAP | 4.012     | glycolysis                                       |
| cassava4.1_005990m PA | AT5G56350                | 0.00065179 | 1.598008389 | Zero DAP | 4.013     | glycolysis                                       |
| cassava4.1_007678m PA | AT2G36530                | 0.01738317 | 1.184387774 | Zero DAP | 4.012     | glycolysis                                       |
| cassava4.1_004579m PA | AT3G16950                | 0.01441446 | 1.119370463 | Zero DAP | 8.1.01.03 | TCA / org                                        |
| cassava4.1_011800m PA | AT4G24570                | 0.03003869 | 12.83297076 | Zero DAP | 9.8       | mitochondrial electron transport / ATP synthesis |
| cassava4.1_006215m PA | AT3G61490                | 0.03045097 | 6.021070584 | Zero DAP | 10.6.3    | cell wall                                        |
| cassava4.1_023284m PA | AT3G51160                | 0.03260657 | 1.612490451 | Zero DAP | 10.1.07   | cell wall                                        |
| cassava4.1_003070m PA | AT1G78570                | 0.02489405 | 1.939766287 | Zero DAP | 10.1.010  | cell wall                                        |
| cassava4.1_007645m PA | AT3G62830                | 0.04688203 | 2.474521947 | Zero DAP | 10.1.05   | cell wall                                        |
| cassava4.1_013011m PA | AT3G23730                | 0.03490783 | 7.078208205 | Zero DAP | 10.7      | cell wall                                        |
| cassava4.1_013014m PA | AT5G13870                | 0.00236123 | 2.053823582 | Zero DAP | 10.7      | cell wall                                        |
| cassava4.1_026770m PA | AT5G07720                | 0.01590742 | 7.792853011 | Zero DAP | 10.3      | cell wall                                        |
| cassava4.1_003705m PA | AT5G49720                | 0.00509153 | 4.673208839 | Zero DAP | 10.2      | cell wall                                        |
| cassava4.1_005517m PA | AT1G75680                | 0.01531539 | 2.472428331 | Zero DAP | 10.6.1    | cell wall                                        |
| cassava4.1_006282m PA | AT1G31070                | 0.00964423 | 1.901909375 | Zero DAP | 10.1      | cell wall                                        |
| cassava4.1_012622m PA | AT1G63000                | 0.02775869 | 3.058469716 | Zero DAP | 10.1.011  | cell wall                                        |
| cassava4.1_012617m PA | AT1G63000                | 0.01729863 | 2.510786326 | Zero DAP | 10.1.011  | cell wall                                        |
| cassava4.1_004720m PA | AT2G26260                | 0.00487059 | 1.953040311 | Zero DAP | 11.8.04   | lipid metabolism                                 |
| cassava4.1_006550m PA | AT4G36480                | 0.0441364  | 1.299408497 | Zero DAP | 11.8.1    | lipid metabolism                                 |
| cassava4.1_000041m PA | AT1G36160                | 0.02212595 | 4.840405433 | Zero DAP | 11.1.01   | lipid metabolism                                 |
| cassava4.1_000033m PA | AT1G36160                | 0.00031027 | 2.372967306 | Zero DAP | 11.1.01   | lipid metabolism                                 |

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|-----------------------|-----------|------------|-------------|----------|--------------|-----------------------|
| cassava4.1_004314m PA | AT3G22960 | 0.01550972 | 2.069258196 | Zero DAP | 11.1.030     | lipid metabolism      |
| cassava4.1_004230m PA | AT3G22960 | 0.03100602 | 1.581515844 | Zero DAP | 11.1.030     | lipid metabolism      |
| cassava4.1_004405m PA | AT5G52920 | 0.02926856 | 1.460937398 | Zero DAP | 11.1.030     | lipid metabolism      |
| cassava4.1_009356m PA | AT2G36880 | 0.04276679 | 3.634983363 | Zero DAP | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_010021m PA | AT3G61440 | 0.04257288 | 3.602058257 | Zero DAP | 13.1.5.3.01  | amino acid metabolism |
| cassava4.1_009789m PA | AT3G61440 | 0.01001605 | 2.966724969 | Zero DAP | 13.1.5.3.01  | amino acid metabolism |
| cassava4.1_009260m PA | AT4G01850 | 0.028676   | 7.173982227 | Zero DAP | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_009245m PA | AT4G01850 | 0.01161816 | 4.153689723 | Zero DAP | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_009247m PA | AT4G01850 | 0.00779596 | 3.966421553 | Zero DAP | 13.1.3.4.011 | amino acid metabolism |
| cassava4.1_011785m PA | AT4G14880 | 0.01686101 | 1.470820095 | Zero DAP | 13.1.5.3.01  | amino acid metabolism |
| cassava4.1_012023m PA | AT5G65780 | 0.01547336 | 1.914311188 | Zero DAP | 13.1.4.1     | amino acid metabolism |
| cassava4.1_008023m PA | AT1G17745 | 0.0235088  | 1.721439695 | Zero DAP | 13.1.5.1.01  | amino acid metabolism |
| cassava4.1_009356m PA | AT2G36880 | 0.04276679 | 3.634983363 | Zero DAP | 15.2         | metal handling        |
| cassava4.1_005974m PA | AT5G07990 | 0.03427019 | 4.336721255 | Zero DAP | 16.8.3.03    | secondary metabolism  |
| cassava4.1_009295m PA | AT5G13930 | 0.02547052 | 8.825291154 | Zero DAP | 16.8.2.01    | secondary metabolism  |
| cassava4.1_009402m PA | AT5G13930 | 0.02789483 | 2.648803168 | Zero DAP | 16.8.2.01    | secondary metabolism  |
| cassava4.1_005134m PA | AT4G32810 | 0.04483787 | 9.38927503  | Zero DAP | 17.1.1       | hormone metabolism    |
| cassava4.1_005510m PA | AT2G26170 | 0.04271988 | 2.30123054  | Zero DAP | 17.2.2       | hormone metabolism    |
| cassava4.1_006458m PA | AT1G11680 | 0.03635065 | 1.461856026 | Zero DAP | 17.3.1.2.03  | hormone metabolism    |
| cassava4.1_003527m PA | AT5G63120 | 0.00266744 | 2.073351598 | Zero DAP | 17.5.3       | hormone metabolism    |
| cassava4.1_000306m PA | AT2G36910 | 0.04024011 | 2.828121082 | Zero DAP | 17.2.2       | hormone metabolism    |
| cassava4.1_023409m PA | AT1G79460 | 0.0040696  | 42.83336756 | Zero DAP | 17.6.1.02    | hormone metabolism    |
| cassava4.1_003857m PA | AT4G18030 | 0.01467908 | 3.663598697 | Zero DAP | 20.2.3       | stress                |
| cassava4.1_003765m PA | AT1G26850 | 0.02665556 | 3.301021418 | Zero DAP | 20.2.3       | stress                |
| cassava4.1_004106m PA | AT4G00740 | 0.002492   | 2.374777322 | Zero DAP | 20.2.3       | stress                |
| cassava4.1_001780m PA | AT2G34300 | 0.04541922 | 2.306619469 | Zero DAP | 20.2.3       | stress                |
| cassava4.1_011715m PA | AT5G61240 | 0.04474006 | 3.867016975 | Zero DAP | 20.1         | stress                |
| cassava4.1_007339m PA | AT2G17840 | 0.03736536 | 1.769091637 | Zero DAP | 20.2.3       | stress                |
| cassava4.1_012699m PA | AT5G26667 | 0.02229545 | 1.289052438 | Zero DAP | 23.4.3       | nucleotide metabolism |
| cassava4.1_000264m PA | AT1G74260 | 0.04694149 | 1.157447776 | Zero DAP | 23.1.2.04    | nucleotide metabolism |
| cassava4.1_005818m PA | AT5G58860 | 0.00178994 | Infinity    | Zero DAP | 26.10        | misc                  |
| cassava4.1_005079m PA | AT4G39950 | 0.01089354 | 19.48809178 | Zero DAP | 26.10        | misc                  |
| cassava4.1_005817m PA | AT5G57260 | 0.00433796 | 8.255161118 | Zero DAP | 26.10        | misc                  |
| cassava4.1_003746m PA | AT1G14830 | 0.04394445 | 3.043660566 | Zero DAP | 26.17        | misc                  |
| cassava4.1_021147m PA | AT1G22380 | 0.02619422 | 3.670478589 | Zero DAP | 26.02        | misc                  |
| cassava4.1_005904m PA | AT2G43820 | 0.01048592 | 1.811946214 | Zero DAP | 26.02        | misc                  |
| cassava4.1_026770m PA | AT5G07720 | 0.01590742 | 7.792853011 | Zero DAP | 26.02        | misc                  |
| cassava4.1_003858m PA | AT5G42080 | 0.04414592 | 1.979692128 | Zero DAP | 26.17        | misc                  |
| cassava4.1_005517m PA | AT1G75680 | 0.01531539 | 2.472428331 | Zero DAP | 26.03        | misc                  |

|                       |           |            |             |          |                |             |
|-----------------------|-----------|------------|-------------|----------|----------------|-------------|
| cassava4.1_003527m PA | AT5G63120 | 0.00266744 | 2.073351598 | Zero DAP | 27.1           | RNA         |
| cassava4.1_002126m PA | AT4G10070 | 0.02132054 | 1.638279498 | Zero DAP | 27.2           | RNA         |
| cassava4.1_003389m PA | AT5G27120 | 0.00480916 | 3.165424654 | Zero DAP | 27.3.67        | RNA         |
| cassava4.1_000656m PA | AT1G09620 | 0.0418764  | 5.828654907 | Zero DAP | 29.1.04        | protein     |
| cassava4.1_032535m PA | AT4G10320 | 0.03877881 | 1.554065116 | Zero DAP | 29.1.05        | protein     |
| cassava4.1_007409m PA | AT1G14570 | 0.01370051 | 1.67872442  | Zero DAP | 29.5           | protein     |
| cassava4.1_009231m PA | AT2G38860 | 0.00100595 | 1.360936044 | Zero DAP | 29.5           | protein     |
| cassava4.1_001804m PA | AT4G30020 | 0.0333283  | 2.780422867 | Zero DAP | 29.5.01        | protein     |
| cassava4.1_000634m PA | AT5G06460 | 0.01233332 | 1.512632939 | Zero DAP | 29.5.11.02     | protein     |
| cassava4.1_018147m PA | AT5G42190 | 0.02892897 | 1.633379408 | Zero DAP | 29.5.11.4.3.01 | protein     |
| cassava4.1_000599m PA | AT5G06600 | 0.02722764 | 1.88056626  | Zero DAP | 29.5.11.05     | protein     |
| cassava4.1_005302m PA | AT3G18190 | 0.01171907 | 1.595956909 | Zero DAP | 29.6           | protein     |
| cassava4.1_005057m PA | AT3G03960 | 0.01397332 | 1.244498262 | Zero DAP | 29.6           | protein     |
| cassava4.1_001585m PA | AT3G07100 | 0.04597656 | 2.204258055 | Zero DAP | 29.3.4.2       | protein     |
| cassava4.1_000150m PA | AT1G71220 | 0.01414175 | 1.661860023 | Zero DAP | 29.7           | protein     |
| cassava4.1_032325m PA | AT5G67360 | 0.04500085 | 5.267634152 | Zero DAP | 29.5.01        | protein     |
| cassava4.1_004221m PA | AT4G34980 | 0.009117   | 2.534486168 | Zero DAP | 29.5.01        | protein     |
| cassava4.1_006951m PA | AT2G03640 | 0.0437768  | 2.356099306 | Zero DAP | 29.3.1         | protein     |
| cassava4.1_008506m PA | AT3G50000 | 0.00118861 | 2.277335938 | Zero DAP | 29.4           | protein     |
| cassava4.1_004241m PA | AT4G26300 | 0.00095321 | 1.243089333 | Zero DAP | 29.1.019       | protein     |
| cassava4.1_000688m PA | AT4G20850 | 0.01658751 | 1.6375074   | Zero DAP | 29.5.01        | protein     |
| cassava4.1_000103m PA | AT3G43300 | 0.03177999 | 1.986283503 | Zero DAP | 30.5           | signalling  |
| cassava4.1_003858m PA | AT5G42080 | 0.04414592 | 1.979692128 | Zero DAP | 30.5           | signalling  |
| cassava4.1_000607m PA | AT4G32640 | 0.04264856 | 2.028837774 | Zero DAP | 31.4           | cell        |
| cassava4.1_007617m PA | AT5G23860 | 0.03076012 | 2.338573269 | Zero DAP | 31.1           | cell        |
| cassava4.1_007632m PA | AT5G23860 | 0.02360334 | 1.820090119 | Zero DAP | 31.1           | cell        |
| cassava4.1_007713m PA | AT5G23860 | 0.0352653  | 1.496295869 | Zero DAP | 31.1           | cell        |
| cassava4.1_000191m PA | AT5G20490 | 0.01560708 | 1.468547668 | Zero DAP | 31.1           | cell        |
| cassava4.1_007650m PA | AT5G12250 | 0.00480469 | 2.491595981 | Zero DAP | 31.1           | cell        |
| cassava4.1_004971m PA | AT5G55230 | 0.00282443 | 1.661939589 | Zero DAP | 31.1           | cell        |
| cassava4.1_000134m PA | AT3G11130 | 0.03473158 | 1.336794767 | Zero DAP | 31.4           | cell        |
| cassava4.1_009783m PA | AT2G37620 | 0.04260394 | 1.252216472 | Zero DAP | 31.1           | cell        |
| cassava4.1_001824m PA | AT3G13870 | 0.02502362 | 1.969382217 | Zero DAP | 33.99          | development |
| cassava4.1_007339m PA | AT2G17840 | 0.03736536 | 1.769091637 | Zero DAP | 33.99          | development |
| cassava4.1_001902m PA | AT1G16780 | 0.04429749 | 4.250299323 | Zero DAP | 34.30          | transport   |
| cassava4.1_000716m PA | AT1G07670 | 0.04293513 | 2.185440264 | Zero DAP | 34.21          | transport   |
| cassava4.1_000306m PA | AT2G36910 | 0.04024011 | 2.828121082 | Zero DAP | 34.16          | transport   |
| cassava4.1_004081m PA | AT5G60790 | 0.00809003 | 2.762754986 | Zero DAP | 34.16          | transport   |
| cassava4.1_001104m PA | AT2G24520 | 0.02515531 | 1.971669953 | Zero DAP | 34.1           | transport   |

|                        |           |            |             |          |         |              |
|------------------------|-----------|------------|-------------|----------|---------|--------------|
| cassava4.1_026874m PA  | AT5G04420 | 0.02636905 | 2.744156117 | Zero DAP | 35.1    | not assigned |
| cassava4.1_016634m PA  | AT5G04420 | 0.04276579 | 2.610155898 | Zero DAP | 35.1    | not assigned |
| cassava4.1_004109m PA  | AT2G01970 | 0.04926956 | 2.583957311 | Zero DAP | 35.1    | not assigned |
| cassava4.1_000239m PA  | AT2G20190 | 0.00456591 | 2.152447807 | Zero DAP | 35.1    | not assigned |
| cassava4.1_005840m PA  | AT2G03820 | 0.01861132 | 1.931357425 | Zero DAP | 35.1    | not assigned |
| cassava4.1_000375m PA  | AT5G37830 | 0.0454964  | 1.906025628 | Zero DAP | 35.1    | not assigned |
| cassava4.1_008317m PA  | AT3G14100 | 0.03734577 | 1.732291775 | Zero DAP | 35.1    | not assigned |
| cassava4.1_008088m PA  | AT5G41040 | 0.00687466 | 1.63843743  | Zero DAP | 35.1    | not assigned |
| cassava4.1_001698m PA  | AT1G04080 | 0.00824663 | 1.675107876 | Zero DAP | 35.1.41 | not assigned |
| cassava4.1_003400m PA  | AT5G10840 | 0.00552129 | 5.328285484 | Zero DAP | 35.2    | not assigned |
| cassava4.1_014182m PA  | AT3G49720 | 0.0475328  | 4.068148662 | Zero DAP | 35.2    | not assigned |
| cassava4.1_033822m PA  | AT3G48770 | 0.04769923 | 3.658330681 | Zero DAP | 35.2    | not assigned |
| cassava4.1_000195m PA  | AT2G38770 | 0.03369909 | 2.771771461 | Zero DAP | 35.2    | not assigned |
| cassava4.1_033676m PA  | AT5G54440 | 0.0281984  | 2.50853873  | Zero DAP | 35.2    | not assigned |
| cassava4.1_000458m PA  | AT3G62360 | 0.01451146 | 1.964032548 | Zero DAP | 35.2    | not assigned |
| cassava4.1_012402m PA  | AT5G01410 | 0.01077575 | 4.622868947 | Zero DAP | 35.1    | not assigned |
| cassava4.1_008405m PA  | AT1G51630 | 0.03584193 | 2.036739081 | Zero DAP | 35.2    | not assigned |
| cassava4.1_017972m PA  | AT5G24710 | 0.03680977 | 3.734045149 | Zero DAP | 35.1    | not assigned |
| cassava4.1_014594m PA  | AT2G38740 | 0.01039082 | 3.204474615 | Zero DAP | 35.1    | not assigned |
| cassava4.1_003361m PA  | AT2G07360 | 0.03843676 | 2.255211256 | Zero DAP | 35.1    | not assigned |
| cassava4.1_004018m PA  | AT1G70770 | 0.03630797 | 2.065843638 | Zero DAP | 35.2    | not assigned |
| cassava4.1_021312m PA  | AT5G49830 | 0.00128175 | 1.449046934 | Zero DAP | 35.2    | not assigned |
| cassava4.1_001142m PA  | AT2G23520 | 0.02021699 | 1.376081175 | Zero DAP | 35.2    | not assigned |
| cassava4.1_009169m PA  | AT5G12470 | 0.0424654  | 1.544540869 | Zero DAP | 35.2    | not assigned |
| cassava4.1_014777m PA  | AT3G07030 | 0.00744154 | 1.497351383 | Zero DAP | 35.2    | not assigned |
| gi 169794080 ref YP_00 | -         | 0.02212956 | 3.452999046 | Zero DAP | -       | -            |