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# Methodological advances, challenges and perspectives in field phenotyping and its application to forage crops

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## Abstract

Novel phenotyping techniques related to remote and proximal sensing hold great promise for landscape phenotyping, variety trials and to increase genetic gain in plant breeding. For their successful implementation into plant breeding, a lot of measures need to be considered that are related to timely, high-throughput data acquisition and processing. While data acquisition includes the identification of the regions of data interest, data processing includes a range of modelling steps to extract targeted traits from sensor data. Here, we will highlight these steps by providing examples from our work with sensors operated in a high-throughput manner from drones or from the field phenotyping platform (FIP) at ETH Zurich, respectively. We put these results into perspective with other ongoing research approaches and discuss how to use the developed concepts for monitoring of forage crops.

**Keywords:** remote sensing, phenotyping pipeline, data assembly, modelling, biomass estimation, stress resistance

## Introduction

Phenotyping, or more precisely high-throughput phenotyping, has become a new bandwagon in crop breeding research. Expectations are that this bandwagon will close ‘the genotype-phenotype gap’. Despite progress in genotyping, yields of many major crops, such as wheat, have stagnated in many countries (Brisson *et al.*, 2010), making it more urgent to invest into new breeding approaches. Today, DNA fingerprinting and sequencing has become routine in many breeding programmes and genomic selection holds great promise to increase the breeding progress. However, using measured yield or quality parameters to feed genomic prediction models suffers from the shortcoming that the processes contributing to the expression of these parameters are treated as a black box. Furthermore, yield particularly can only be assessed relatively late in the breeding process, when enough plant material is available. Thus, breeders measure traits or do eyeball ratings of traits positively correlated to yield and quality in early breeding cycles. These secondary traits are usually assessed throughout the growing season – starting with plant emergence and ending with yield components. This analytical approach follows an ideotype concept, i.e. the measurement of morphological and physiological traits conferring an adaptation to a particular environment, management or end use (Martre *et al.*, 2015).

The process of measuring secondary traits or plant features of interest can be summarized as ‘phenotyping’. The terms genotype (the genetic composition of an individual) and phenotype (the measurable effect including both genetic and environmental influences) were coined as early as 1911 by the geneticist Wilhelm Johannsen (1911), but phenotyping has more recently become an almost separate field of research (Furber and Tester, 2011; Walter *et al.*, 2015). The development of high-throughput phenotyping was driven by the availability of low-cost sensors, and sufficient computer power including software development to process and combine large images. It started off with large-scale, automated platforms in greenhouses and growth chambers aiming to handle the ever increasing sizes of genetic testing material (Granier and Vile, 2014; Hartmann *et al.*, 2011; Nagel *et al.*, 2012). Such material was and is generated in large-scale genetic transformation pipelines, mutation breeding approaches, or it consists of mapping populations, which were generated to map the genomic regions controlling

quantitative traits. The use of modern phenotyping methods by means of imaging sensors started under controlled conditions and later the field (Hund *et al.*, 2019). Initial steps to establish field phenotyping approaches consisted of the development of different carrier systems like the so-called phenomobiles, followed by drones and large, fully automated installations (see reviews by Cendrero-Mateo *et al.*, 2017; Hund *et al.*, 2019; Luis Araus *et al.*, 2018). The field phenotyping platform at ETH represents one of these fully automated installations (Kirchgessner *et al.*, 2015). A large array of active and passive sensors covering almost the entire electromagnetic spectrum are mounted on lots of these carriers to assess all kinds of plant features. In contrast to their indoor counterparts, many field phenotyping approaches were developed with the clear vision to directly assist the breeding progress.

However, the most crucial question is which traits are to be monitored to support genetic gain for the targeted traits yield and quality. This brings us back to the ideotype concept. It might be tempting to use the whole hyperspectral reflectance signal of a canopy and feed it into a machine learning pipeline to train a yield prediction model. This approach will likely fail, if it does not consider the basic physiological mechanism contributing to yield. Thus, most scientists target the traits which are thought to affect yield. The aim is to (1) replace breeders' ratings with new, high-throughput methods or (2) to measure additional features a breeder cannot assess. Such features are, for example, the canopy temperature (Deery *et al.*, 2016) but also the response of growth and development to changing meteorological conditions during the growing season (Grieder *et al.*, 2015).

Phenotyping of elite germplasm in a breeding nursery is not a piece of cake. The observation process needs to be performed precisely during occurrence of the critical stages at which the material segregates best and at which the secondary proxy trait shows the highest correlation with the targeted primary trait. In many cases multiple observations in time are required to measure the targeted traits, for example when aiming to determine the onset of shooting throughout time (Kronenberg *et al.*, 2017) or the response to temperature (Grieder *et al.*, 2015). Moreover, sensor and data fusion may be required to increase the predictive value of remote sensing features to a level relevant for assessing the targeted breeding traits (Maimaitijiang *et al.*, 2017). If traits that are routinely assessed in a breeding programme should be replaced by a new proxy measure, the relative efficiency of this indirect selection should be either higher than that of the proxy trait, or it should contribute to a significant reduction in the overall phenotyping costs.

## General considerations for setting up a phenotyping pipeline

### *Target traits and decisions on sensors and carriers*

The targeted traits drive the decision on the carrier and sensor combination. The very first decision to make, when aiming to include high-throughput field phenotyping (HTFP) techniques into the breeding programme, is which traits should be measured and to which level of precision. It requires previous knowledge about the importance of the trait in the breeding process and the feasibility to measure it using HTFP techniques. This information greatly drives the choice of the carrier system and the selection of the appropriate sensor. For breeders, the simple and robust application of both carrier and sensor including a timely data processing is thereby of major importance. Despite research progress and intense collaboration between breeders and the phenotyping research community, there is still a lot of room for improvement and for establishing applicable solutions. There is a wide range of literature introducing carrier systems and sensors for crop phenotyping (Cendrero-Mateo *et al.*, 2017; Deery *et al.*, 2016; Hund *et al.*, 2019; Luis Araus *et al.*, 2018; Tattaris *et al.*, 2016). Here we refer only to some systems to highlight the diversity of these approaches that are important steps towards generating applicable solutions.

If only the senescence behaviour is targeted, the widely used normalized difference vegetation index (NDVI) may be the right choice of proxy trait. It can be measured using active point sensors, such as Yara N-Sensor, Green Seeker or Crop Circle, which work reliably under varying illumination conditions. If the number of pots is in the range of a few hundred, the best carriers are probably the breeder's own feet. On the other extreme, high-end, active Light Detection And Ranging sensors (LIDAR) or multiple hyperspectral image sensors could deliver 3D spectral information of the canopy, potentially allowing the measurement of photosynthesis-related traits or the spread of diseases. Due to their weight, these devices are usually mounted on phenomobiles or integrated in dedicated phenotyping platforms. The analysis of images derived from these platforms often requires sophisticated image processing techniques.

We believe that the typical breeding applications fall between the two extremes outlined above. While large phenotyping platforms and traditional breeder's rating will deliver the necessary tools and trait calibrations, flexible carrier systems will bring these tools into the breeding nursery. The most flexible and cost-efficient carrier system today are drones or simple phenomobiles. Drones may be of first choice, when large areas have to be covered in a short time period and mainly when general canopy characteristics are in focus. Phenomobiles come into play when more detailed information is required and the timing of the measurement is less critical. Furthermore, in canopies of tall or dense crops, such as maize, sorghum or rapeseed, phenomobiles may be restricted to early developmental stages. At minimum, the carriers should be equipped with a high-resolution consumer-grade RGB camera. In the example of cereals, RGB imaging can be used to measure a wide range of potential features. While this potential is huge, feature extraction is not yet widely used in routine breeding. The reasons for this are related to the complex computation process, which most of the time requires trait and crop-specific image-processing algorithms. Nevertheless, proof-of-concept studies show that RGB information can be used directly to assess a wide range of traits: to count seedlings (Jin *et al.*, 2017; Liu *et al.*, 2017, 2016), detect ears and determine the heading stage (Fernandez-Gallego *et al.*, 2018; Sadeghi-Tehran *et al.*, 2017; Zhu *et al.*, 2016), follow the early canopy cover (Kipp *et al.*, 2014; Liu *et al.*, 2017) or to measure leaf chlorophyll content (Baresel *et al.*, 2017). Yet, RGB imaging can be also used to measure height from multiple images of a scene, applying 'structure from motion' algorithms (Aasen *et al.*, 2015; Bareth *et al.*, 2016; Holman *et al.*, 2016; Madec *et al.*, 2017) or to measure the response of canopy growth to changes in temperature (Grieder *et al.*, 2015) and likely also responses to other changes in meteorological conditions. When only global characteristics of closed canopies are required, hyperspectral and thermal image sensors are suitable. Drones carrying lightweight thermal and multispectral sensors offer the opportunity to monitor hyperspectral and thermal characteristics of large areas in a short time. Compared to the somewhat slower observation with phenomobiles or fixed installations, this minimizes the risk of changes in the illumination conditions during imaging. Such changes influence the signal reflected by the canopy and considerably reduce the heritability of the trait.

In addition to the above-mentioned passive 2D sensors, active sensing devices can be used to scan the environment with potentially higher precision. The most relevant examples in this context are LIDAR sensors that use laser light. LIDAR systems are widely used for crop phenotyping and the lightweight versions can also be carried by unmanned aerial vehicles (UAVs). LIDARs can be used to generate surface models, to measure canopy height (Friedli *et al.*, 2016) or canopy structure (Jimenez-Berni *et al.*, 2018).

Yet, the appropriate choice of sensors, carrier systems and an appropriate experimental design (not discussed here) to generate relevant imaging data is only the prelude to a successful phenotyping process chain. Relevant field phenotyping approaches require a range of different steps that can be grouped into 'data acquisition' and 'data modelling'. Data acquisition comprises three steps: (1) mission planning; (2) the mission itself; and (3) data assembly and identification of the regions of interest. In the following, we

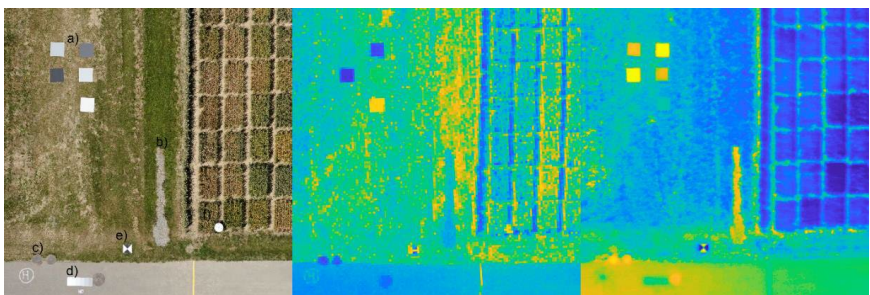
elaborate aspects of the data acquisition pipeline for the case example of drone-based phenotyping, since this carrier system is currently of most relevance for applications of high throughput field phenotyping.

### Mission planning

For data acquired with drones, photogrammetric software, such as Pix4D (Pix4D, 2016) and Agisoft PhotoScan (LLC, 2016) offer the first step to construct orthomosaic images and digital surface models. To ensure that orthomosaics and digital surface models are assembled at sufficient quality, ground control points (GCPs) are required. These GCPs are the implicit standard to obtain information of the orientation and position of each image to be located. Moreover, GCPs are needed to align images taken at different times during crop development. At least one visible GCP per image is required to achieve high vertical precision, while a lower number may be sufficient if only a high horizontal precision is required (Harwin *et al.*, 2015). The PhenoFly Planning Tool enables an appropriate placement of GCPs given the attained ground sampling distance as a function of flight height, sensor size of the camera and focal length of the lens (Roth *et al.*, 2018).

The ground sampling distance covered by a sensor pixel (GSD) determines which feature can be resolved. For a given sensor size and sensor resolution, the GSD depends on the distance of the camera to the object and on the focal length of the lens. The maximum GSD to detect a feature in an image should be smaller than 1/5 of the feature size (O'Connor *et al.*, 2017). This means that a feature, like a plot, plant, leaf or a flower head, should at least be covered by five pixels in its smallest object dimension. Due to the requirement of achieving a high GSD, a high sensor resolution is the first choice when aiming for high-quality images of 2D sensors. The number of recorded pixels of a sensor range from 1,024×768 for today's thermal cameras over 2,048×1,088 for multispectral sensors to 6,000×4,000 for visual (RGB) sensors (e.g. Aasen *et al.*, 2018). Thus, generally RGB imaging is at the higher end of sensor resolution while hyperspectral and thermal imaging mark the lower end. An example for the results of different sensors is given in Figure 1.

Given a certain sensor resolution, the GSD can be adjusted by the combination of the lens system and the carrier system determining distance between canopy and sensor. Phenomobiles can bring cameras

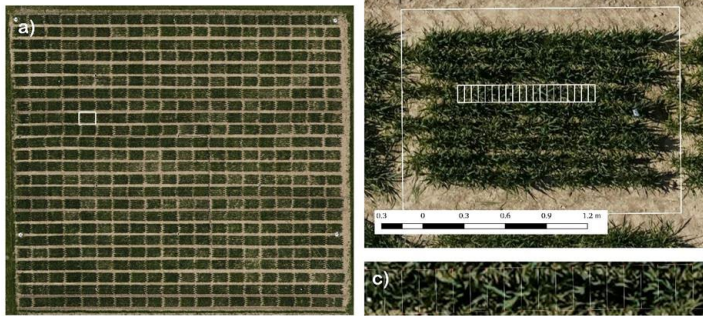


Sensor	RGB	NIR 800 nm band	Thermal
Model	Sony, alpha 9	Imec, SNm5x5model	FLIR, A65
Res. (px)	4,000×6,000	409×216 (per channel)	640×480
Alt. (m)	80	60	60
GSD (mm)	6.5	65	42

Figure 1. Field scene taken with three different types of sensors (Model, sensor resolution (Res.), flying altitude (Alt.) and ground sampling distance (GSD) are given). The central and right panels show the reflectance in false-colour. The following features are indexed: spectral calibration targets (a), field path with drainage strip of gravel (b), duct covers (c), grey-scale calibration target (d), thermal ground control point (e), and machine-readable ground control point (f).



## 2D information



## 3D information

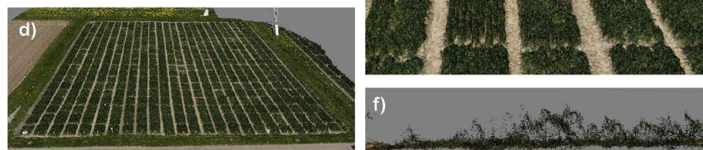


Figure 3. Two-dimensional othomosaic of wheat lot 3 of the field phenotyping platform FIP (Kirchgessner *et al.*, 2015) derived from 588 images of a copter flight at 28 m height with a ground sampling distance of 3 mm (A), individual plot (B), and subplot of one metre length dissected into 20 subsections (C). Three-dimensional image generated by structure from motion of the same lot (D); individual plots in 3D view (E); vertical cross section through the point cloud of the subplot in C showing the vertical position and colour of each respective point (F). Data were acquired in the framework of the TraitSpotting project.

information in the canopy. This information can be derived from point sensors (1D), images (2D; e.g. Figure 3D-F), or point clouds derived from laser scanners or photogrammetry (3D; e.g. Figure 3B). An additional dimension is the distinct spectral reflectance information for each point, pixel or point cloud (Figure 3F) and yet another dimensions is offered by following the change of this information through time. Meteorological data, derived from weather stations or soil sensors, delivers additional covariates to the time dimension. The most prominent use of these covariates is the expression of the temporal dimension as thermal rather than ordinal time.

Generally, the complexity of this multidimensional space is reduced stepwise: first by extracting features from the spatially distributed spectral information and second by summarizing the change of these features over time. There is an additional step in between, as suggested by Eeuwijk *et al.* (2018): correcting for the influence of nuisance factors in the field by means of design factors and spatial trends. Eeuwijk *et al.* (2018) classify the cascade of modelling steps from the original sensor-derived raw data to the prediction of the primary target traits yield and quality in five steps: feature extraction, correction of nuisance factors in the field, dynamical modelling, model dependence on environmental gradients and target trait prediction (Figure 4). Thereby, the size of the input data decreases from one step to the next, whereas the dependency on the previous model output increases. This means that there are many different ways to integrate the sensor-derived raw information into breeding decisions. At the far end of this pipeline, modelers develop crop models for yield and quality prediction. Today many of the steps described above are just in the exploration phase. Implementing them in robust and generic software solutions to generate the desired output within days rather than weeks will be necessary to deliver these tools to breeders.

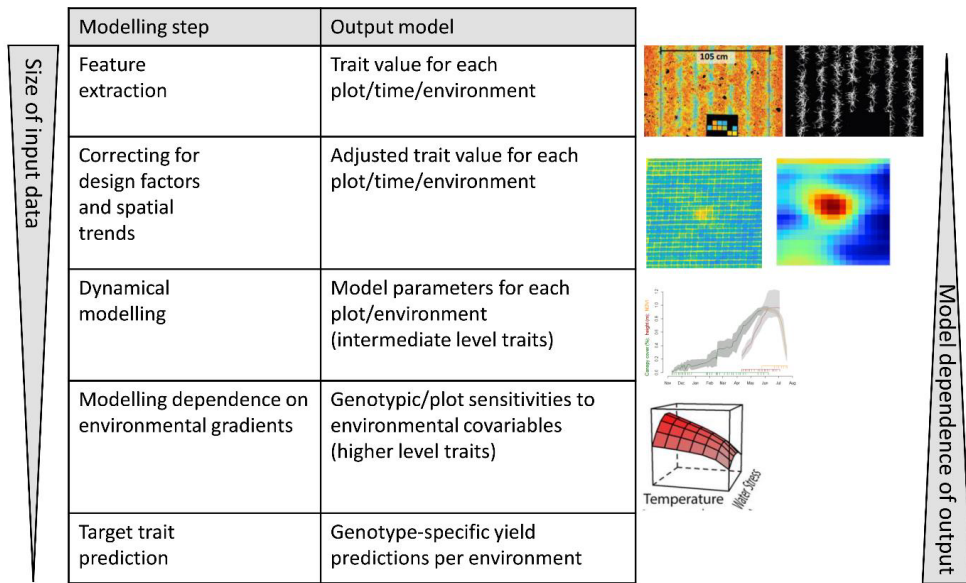


Figure 4. Modelling process to convert sensor-derived raw data into yield and quality prediction. As this of the input data is decreasing at each modelling step, the model dependency is increasing. Modified according to Eeuwijk *et al.* (2018).

## Implementing field phenotyping for forage crop breeding: short and long-term aims

### *Biomass estimation*

Unlike grain crops, forage crops have the advantage that the harvest product can be more directly estimated by means of remote sensing techniques. Yet, given small differences in elite germplasm, a robust biomass estimate will likely involve different traits measured at the appropriate time. However, one-trait measurements may work well for single plants or in special cases. For example, image-derived canopy cover of clover (*Trifolium*) showed a high genetic correlation with dry matter production ( $r_g=0.88$ ,  $P<0.001$ ) with a broad-sense heritability ( $H^2$ ) value of 0.56 (Inostroza *et al.*, 2016). Fresh matter yield may also be estimated using simple indices based on RGB images, such as the green/red index as shown in the example of alfalfa (*Medicago sativa*) (Figure 5). For robust estimates from year-to-year, additional information may be required.

### *Flowering time and number and size of flowers*

There are many efforts to determine flowering time and to count the number of ears in grain crops (Fernandez-Gallego *et al.*, 2018; Sadeghi-Tehran *et al.*, 2017) which may be utilized for the same purpose in forage crops. At the moment the most promising approaches are machine-learning techniques to detect flowers under many contrasting conditions. These techniques will require large sets of images taken under a wide range of conditions for training in order to be robust enough for routine application.

### *Species detection in mixtures*

Forage crop breeding also involves the analysis of species mixtures. Phenotyping may be used to evaluate the change in the relative abundance of the different species. Some limited functionality is available in standard software using different colour spaces given clear colour contrasts among the species. However,



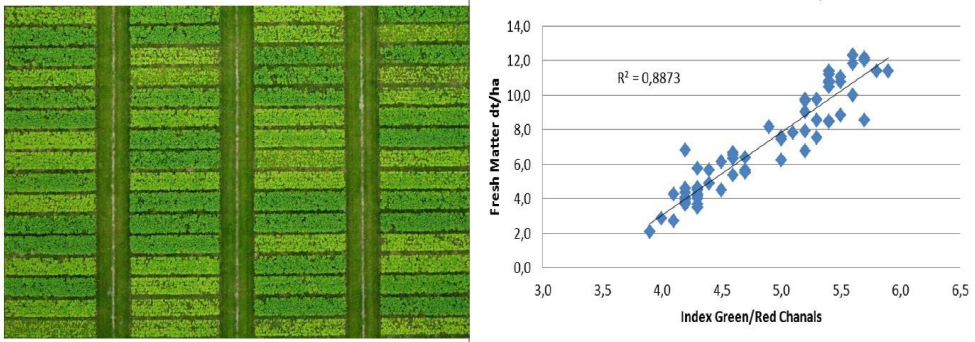


Figure 5. Experiment of 16 alfalfa entries planted in four replications at the test site of the Deutsche Saatveredelung (DSV), Asendorf, Germany (left); Fresh dry matter yield as related to the green/red index derived from RGB images using a multicopter as carrier.

often this requires manual thresholding for each measuring campaign. Again, artificial intelligence approaches are the first choice for classifying different crop species in images.

#### *Canopy development after successive harvests*

Not only the temporal dynamics of height growth after successive cuts, but also the response of this growth dynamics to changes in meteorological conditions could be evaluated. Height of experimental plots can be measured efficiently using either terrestrial laser scanners or structure from motion data derived from drone campaigns as explained above. For single plants, i.e. early in the breeding cycle, laser scans can be used to measure the increase in height and canopy volume as a proxy for biomass accumulation (Figure 6).

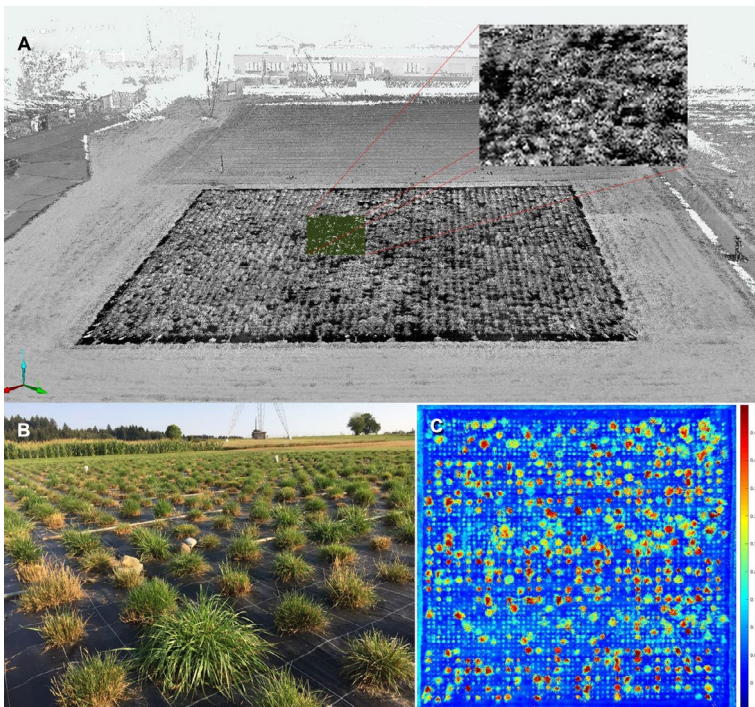


Figure 6. Terrestrial laser scan of a diverse panel of >1000 perennial ryegrass (*Lolium perenne* L.) genotypes comprising both turf and forage types (A), Faro Focus 3D, 4 scans registering, and visualization done with Faro Scene software) to retrieve traits like height and canopy volume (B). The false-colour height map shown in panel (C) was developed with a custom MatLab script. The experiment was planted as completely randomized block design, arranged in three replicates

## Diseases and abiotic stresses

Diseases and resistances to diseases are at the top of the list in most breeding programmes. While early disease development is difficult, if not impossible, to assess by means of remote sensing, we may be able to quantify later phases. In cereal crops, the major challenge is to separate senescence-driven changes in canopy reflectance from disease-driven changes. In perennial forage crops this may be less of a problem, and thus disease detection may be more straightforward. Some diseases like crown rust (*Puccinia coronata*) in *Lolium* are easy to detect by eye due to the yellow colour of the diseased canopy of the susceptible genotypes. The disease is a good candidate for drone-based phenotyping, which may even be measured using simple indices derived from RGB images (Figure 7). For more complex situations, we expect that the assessment of disease development rather than a snapshot in time will be necessary to quantify the disease and distinguish among genotypes. Abiotic stress factors of major focus are overwintering ability, measurable by means of the greenness of the canopy after winter, or drought tolerance, measurable by means of thermography.

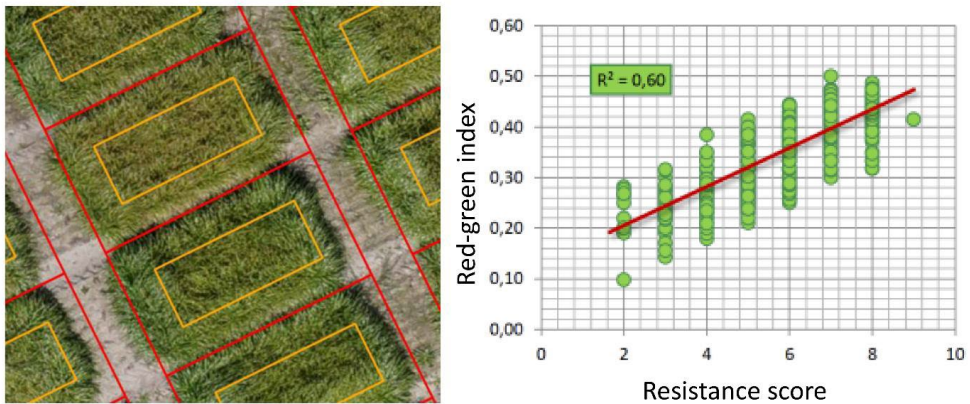


Figure 7. *Lolium perenne* plots at the test site of the Deutsche Saatveredelung (DSV), Asendorf, Germany (left) showing the plot (red) and measuring area (yellow with 30 cm distance to the plot border). Red-green index derived from RGB images with a ground sampling distance of 2 cm as related to the general rust resistance score (1 = susceptible to 9 = resistant) of the plots.

## Conclusion

Intensive research and education in field phenotyping will deliver proof-of-concepts and know-how to implement and improve standard protocols for crop breeding. These protocols need to take into account a wide range of aspects from choice of sensors to the application of appropriate statistical models to extract relevant features. In order to improve their applicability, the phenotyping protocols need to be fine-tuned for each trait and each crop, thereby integrating with first-hand information of breeders. Importantly, it is not the technology but the targeted trait and its contribution to yield (and quality) that must be the focus of our efforts.

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