Single-marker Assisted Introgression of Crown Rust Resistance in an Italian Ryegrass Breeding Program

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Single-marker assisted introgression of crown rust resistance in an Italian ryegrass breeding program

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Abstract Ecotype populations of Italian ryegrass (*Lolium multiflorum* Lam.) provide a valuable gene pool for further improvement of this important forage grass species. Although they are often characterized by high yield potential and persistence, ecotype populations usually suffer from severe susceptibility to leaf diseases such as crown rust, caused by *Puccinia coronata*. In this study we tested the potential of single-marker assisted selection to introgress a resistance gene from a biparental mapping population into ecotype-based breeding material. A single SSR marker linked to a major QTL for crown rust resistance on LG2 of *L. multiflorum* was used to generate three breeding populations with contrasting intensities of marker assisted selection (homozygous for the marker allele, heterozygous, absence of the marker allele). In the framework of our breeding program, these populations were continuously genotyped and characterized for crown rust resistance over five generations. Observations on individual plants of the F_3 generation showed significant linkage of the marker allele with crown rust resistance but there was no significant difference between individuals homozygous or heterozygous for the marker allele, respectively. In plot trials with Syn_2 progeny, crown rust scores ranged from 1.44 to 2.00 (1=resistant, 9=susceptible) for populations based on positive selection for the marker allele while populations based on pure phenotypic selection had crown rust scores ranging from 3.11 to 4.56. Thus, our results demonstrate that genotypic selection based on a single marker can complement phenotypic selection and facilitate the introgression of resistance traits in breeding material.

Keywords

Crown rust, Italian ryegrass, marker assisted selection, QTL

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is one of the most important forage grasses in temperature regions, particularly valued for its yield potential and its high forage quality. In Central Europe, ecotype populations of Italian ryegrass can be found in natural or semi-natural grassland. They are usually well adapted to their respective habitats and can show a high yield potential but often suffer from severe susceptibility to diseases such as crown rust, caused by *Puccinia coronata* f.sp. *lolii* (Boller et al. 2009).

Due to its economic importance, resistance to crown rust is a prime breeding target in any ryegrass improvement program. Although phenotypic recurrent selection has resulted in cultivars with considerable levels of crown rust resistance, further progress is often hampered by the complex composition of cultivars and rapidly evolving pathogen populations (Kimbeng 1999). Consequently, considerable effort has been taken to understand this complex host-pathogen interaction, to elucidate the genetic control of resistance and to develop tools and strategies for improved resistance breeding (reviewed in: Dracatos et al. 2010). Mainly based on bi-parental mapping populations, quantitative trait loci (QTL) explaining substantial proportions of phenotypic variance were discovered on most linkage groups of *L. perenne*. In *L. multiflorum*, broad spectrum QTL have been mainly identified on linkage groups (LG) 1 and 2 (Studer et al. 2007; Sim et al. 2007), while specific QTL conferring resistance to specific *P. coronata* isolates have been identified on LG 2, 4 and 7 (Dracatos et al. 2010).

Despite these advances, reports on utilization of markers linked to resistance genes and QTL in ryegrass breeding are scarce. The aim of this work was to test the suitability of single-marker assisted selection in a standard breeding program by (i) introgressing the QTL identified in a bi-parental mapping population into breeding lines and (ii) developing cultivar candidates with increased resistance to crown rust.

Materials and Methods

Plant material and selection scheme

As a source for crown rust resistance, progeny of a bi-parental mapping population segregating for crown rust resistance were used (FALXtg03; Studer et al. 2007). In this population, a major QTL explaining up to 55.5 % of the phenotypic variance was identified on LG 1 in field experiments at different locations as well

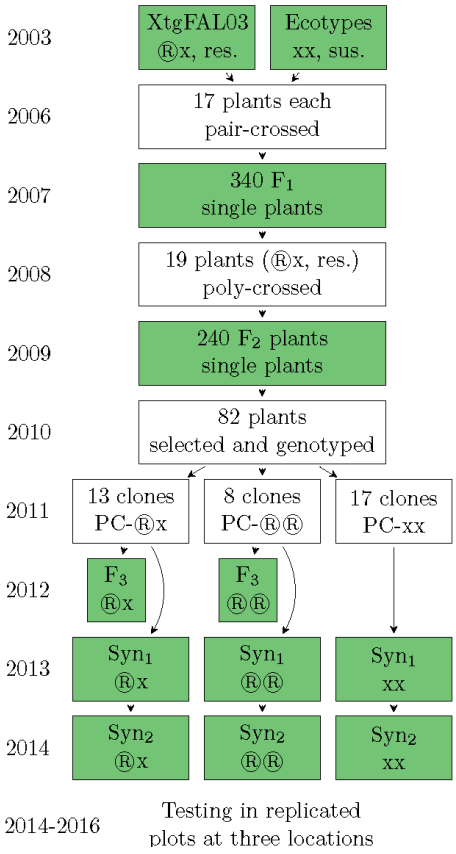

Fig.1. Starting material and breeding scheme used for the development of cultivar candidates for *L. multiflorum* based on marker assisted and phenotypic selection (RR, Rx) or pure phenotypic selection (xx). Green boxes indicate the different generations produced and analysed. Plants were genotyped using a single SSR linked to a crown rust resistance QTL (R-allele) and phenotypically characterized for crown rust resistance using a visual score from 1 (resistant) to 9 (susceptible).

as in glasshouse evaluations and using a leaf segment test. As novel germplasm, *L. multiflorum* ecotypes from a diverse collection which were characterised by high agronomic performance but poor crown rust resistance were selected (Peter-Schmid et al. 2008). Seventeen plants from the mapping population and 17 from the ecotype collection were selected for 17 pair-crosses (Fig. 1). From the resulting 340 F1 progeny, 19 resistant plants with the marker allele Rx were selected and poly-crossed. From the resulting 240 F2 plants 82 plants not showing rust symptoms in the breeding nursery were genotyped for the marker allele and phenotypic characteristics

such as vigor and persistence. Thirty-eight plants were selected for three polycrosses with the parental marker allele composition Rx, RR and xx (Fig. 1). Single F3 plants were phenotyped and genotyped and simultaneously, Syn1 and Syn2 populations were developed. In parallel, a similar scheme was used to develop breeding lines based on a different source of resistance and solely based on phenotypic characterisation (Table 1).

Phenotypic and genotypic characterization

At all stages, resistance to crown rust was determined based on a visual scoring using a scale from 1 (resistant) to 9 (susceptible). Individual plants (up to F_3) were scored in the breeding nurseries at Agroscope, Zurich. Resistance of the second generation synthetic populations (Syn_2) was assessed in replicated field trials at three locations.
The SSR marker NFFA012 (Saha et al. 2004), closely linked to the QTL in the mapping population, was used for genotyping of individual plants. Protocols for DNA extraction and marker genotyping were as described by Studer et al. (2007). Polycross parents homozygous for the resistance allele (RR), heterozygous for the resistance allele (Rx) and not carrying the resistance allele (xx) were selected to give rise to the respective synthetic progenies (Fig. 1).

Statistical analysis

Linkage of the marker allele with crown rust resistance was tested using a χ^2 test. Differences in rust resistance among the different populations were tested using analysis of variance and Duncan's multiple range test.

Results and Discussion

Characterisation of the resistance allele

In order to characterize the occurrence of the allele of SSR marker NFFA012 which was linked to resistance in the mapping population FALXtg03 (R-allele), 48 vigorous and persistent individuals from seven ecotype populations cultivated as spaced plants as described by Peter-Schmid et al. (2008) were genotyped and characterized for crown rust resistance. Twenty individuals carried a marker allele of the same length as the R-allele, but only two of these were resistant to crown rust, while three of the 28 individuals not carrying the marker allele were also resistant. Thus, in

ecotype populations no linkage was observed between the marker allele and crown rust resistance ($\chi^2 = 0.0064$, $p = 0.93$).

Table 1 Mean rust scores of cultivar candidates based on different parental populations and selection strategies. MAS RR, Rx, xx indicate genotypic selection for the resistance allele R combined with phenotypic selection, phenotypic indicates phenotypic selection based on visual scores only. Syn_2 progeny were sown in replicated plot trials at three locations and rust scores were determined repeatedly during 2014 using a score from 1 (resistant) to 9 (susceptible).

Syn2 Population	Selection strategy	Crown rust score
Ecotypes[1] x XtgFAL03[2]	MAS: RR	1.44 a[4]
Ecotypes x XtgFAL03	MAS: Rx	2.00 a
Ecotypes x XtgFAL03	MAS: xx	3.67 bc
Ecotypes x 'Tigris'[3]	Phenotypic	3.56 b
Ecotypes x 'Tigris'	Phenotypic	3.78 bc
Ecotypes x 'Tigris'	Phenotypic	3.11 b
Ecotypes	Phenotypic	4.56 c
Standard (Tigris)	Phenotypic	3.89 bc

[1] ecotypes from diverse Swiss ecotype collection (Peter-Schmid et al. 2008)
[2] progeny of a mapping population segregating for crown rust resistance (Studer et al. 2007)
[3] breeding material derived from the Tigris (Agroscope, Switzerland) genepool
[4] means followed by the same letter are not significantly different (Duncan's multiple range test)

On the other hand, significant linkage between the R-allele and crown rust resistance was observed after intercrossing individuals from ecotype populations and FALXtg03 ($\chi^2 = 20.76$, $p < 0.0001$). In single plants of the F_3 generation which were the progeny of polycrosses between parental plants selected for crown rust resistance and the presence of the R-allele (Fig. 1), individuals homozygous or heterozygous for the R-allele showed a rust score of 1.60 or 1.76, respectively, while individuals from the Rx – polycross progeny (Fig. 1) not carrying any R-allele were significantly more susceptible with a rust score of 5.62 ($R^2 = 0.52$, $p < 0.001$).

Introgression of resistance allele into cultivar candidates

The ultimate aim of the study was to investigate, whether single-marker assisted selection can be used to enhance crown rust resistance in *L. multiflorum* candidate cultivars. Therefore, Syn_2 populations of several candidate cultivars were grown in a replicated field plot trial at three locations and scored for crown rust resistance in 2014. Although disease pressure in the testing period was not very severe, distinct differences among the individual populations were observed (Table 1).

The synthetics created by marker assisted selection in addition to phenotypic selection showed significantly improved resistance when compared to populations based on phenotypic selection only (Table 1). No significant difference was observed for the population based on parents homozygous for the resistant allele when compared to the population based on heterozygous parents. The XtgFAL03 population based on parents where the R-allele was absent showed a similar level of resistance as the populations based on "Tigris" related germplasm indicating a similar value of both sources of resistance. On the other hand, ecotypes which were continuously selected for crown rust resistance but not intercrossed with a resistant plant showed significantly higher susceptibility (Table 1). The initial ecotype populations were evaluated in different trials and multi-annual data analysis was applied to be able to compare the scores of different trials. Compared to the values shown in Table 1, unimproved ecotype populations showed a rust score of 6.96. Thus, pure phenotypic selection resulted in a progress of 2.4 rust scores, while MAS resulted in a progress of 5.52.

Conclusions

In this study, single-marker assisted selection proved useful to introgress a novel source of resistance efficiently into breeding germplasm, and to fix it in a candidate variety by selecting homozygous polycross parents. The synthetics based on marker assisted and phenotypic selection were clearly superior regarding crown rust resistance when compared to synthetics based on phenotypic selection only.

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