PROGRESS ON STEM RUST RESISTANCE GENETICS IN PERENNIAL RYEGRASS

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Introduction

As we reported in the 7th International Herbage Seed Conference Proceedings, we determined several years ago that the perennial ryegrass (*Lolium perenne*) cultivar 'Kingston' (PGG Wrightson Seeds, New Zealand) typically has a lower level of stem rust than other varieties we have tested under our OR production conditions and with our local populations of the pathogen (*Puccinia graminis* subsp. *graminicola*). To gain some insight into genetics of stem rust resistance, we created a mapping population by crossing two plants (resistant and susceptible) that we had selected from 'Kingston' after repeated stem rust testing under controlled conditions.

Mapping methods

Genetic maps were constructed for a population of 193 F1 progeny from this cross. We have published two genetic maps, one in 2011 and a revised version in 2013. The 2011 map (Pfender et al., 2011) was constructed with RAD (restriction-site associated DNA) (Baird et al., 2008) markers, plus tall fescue SSR (simple-sequence repeat) markers previously developed (Saha et al., 2006) by researchers at the Samuel Roberts Noble Foundation (Ardmore, OK, USA). Additional SSR markers, also run at the Noble Foundation, were originally developed for *Lolium* by other research groups (Gill et al., 2006). The 2013 map (Pfender and Slabaugh, 2013) supplements our 2011 map by having markers in common with several other *Lolium* species maps, including anchor markers from a consensus map published by other researchers (Studer et al., 2010). Our 2013 map therefore allows better comparison of our population and its stem rust phenotypes with various other *Lolium* populations that have been mapped by researchers elsewhere in the world.

Maps were assembled for each parent using JoinMap 4 software and CP (cross-pollinated) population type codes (Kyazma, Wageningen, Netherlands). We used the test for independence LOD (logarithm of the odds) score, which is not affected by segregation distortion, to group markers into seven linkage groups for each map.

Phenotyping methods

Disease phenotypes were determined in inoculation assays conducted in a greenhouse with bulk inoculum (field-collected, genetically mixed) for analysis of the 2011 map (Pfender et al., 2011). We used single-pustule isolates (genetically uniform) of the rust pathogen for the 2013 map (Pfender and Slabaugh, 2013). We had previously demonstrated pathotype specificity in stem rust of perennial ryegrass by purifying and increasing two different, single-pustule isolates of the pathogen (Pfender, 2009). Isolate 101 is avirulent on one of the mapping population parents, and resistance is inherited as a single dominant gene that is heterozygous in the resistant parent. Isolate 106 is virulent to some degree on both parents.

Phenotypes were scored as number of pustules per plant. There were three replicate (cloned) plants per F1 individual in each experiment, and each experiment was conducted at two different times. QTL (quantitative trait loci) analysis was conducted in MapQTL5 for the male and female parent maps. Kruskal-Wallace analysis and automatic cofactor selection were used to choose cofactors for use in MQM (multiple-QTL mapping) analysis.

Results

Three major QTL (i.e. locations on the *Lolium* chromosomes) for stem rust resistance were detected in these experiments (Fig. 1). One QTL, located on LG6 (linkage group 6) was associated with resistance to both stem rust pathotypes, and the other two were each associated with only one of the pathotypes (on LG1 for pathotype 106 and on LG7 for pathotype 101) (Pfender and Slabaugh 2013).

The QTL on LG6, designated qLpPg3, was detected on the male and female maps with both pathotypes. QTL qLpPg3 explains 7 to 10% of the phenotypic variance in the response to pathotype 101, and 9 to 11% in response to pathotype 106. This QTL is located between 60 and 68 cM on the female map, and between 59 and 63 cM on the male map (Fig. 1). In both maps the peak of qLpPg3 is located between

markers G01-002 and LP20. These markers have been placed on *Lolium* maps constructed by other research groups as well, but had not previously been associated with stem rust resistance.

Resistance response to pathotype 101 is associated also with a QTL on LG7, designated qLpPg1. QTL qLpPg1 is located in a 7-cM interval between markers G02-048 and NFFS275 (Fig. 1), markers which appear on other Lolium maps. It has a large phenotypic effect, explaining 50 to 58% of the phenotypic variance in response to pathotype 101. The response associated with the qLpPg1/pathotype 101 interaction is essentially all-or-none, as 92% of plants carrying the resistance-associated allele at the marker closest to the QTL are resistant, whereas only 5% without the "g" allele at the marker are resistant. Thus, this locus behaves genetically like a single dominant gene.

Resistance response to pathotype 106 is associated with a QTL (designated qLpPg2) on LG1. This QTL explained 17 to 30% of the phenotypic variance in these experiments. It is located between markers G01-031 and LpRa060 on the female and male maps (Fig. 1). QTL qLpPg2 on LG1 (unlike the QTL on LG7) is associated with a more quantitative response rather than acting as a single dominant gene.

QTL qLpPg3 and qLpPg1 together explained 60 to 65% of the phenotypic variance in response to pathotype 101, whereas qLpPg2 was not detected in response to this pathotype. qLpPg3 and qLpPg2 together explained 30 to 39% of the phenotypic variance in response to pathotype 106; qLpPg1 was not detected in response to pathotype 106. When the mapping population was inoculated with a mixed collection of stem rust spores from the field, all three QTL were activated (Pfender et al., 2011). It appears that this multiple-QTL response to mixed inoculum is due to independent activation of different QTL by specific pathotypes, as well as their activation of a common QTL.

Research is in progress, using crosses of plants from this mapping population with other plants, to further test and select genetic markers that co-segregate with the stem rust resistance QTL. Such markers could be useful in a marker assisted selection strategy for genetic improvement of perennial ryegrass. We expect to release germplasm with stem rust resistance, and information on markers linked with that resistance, as the products of this research.

References

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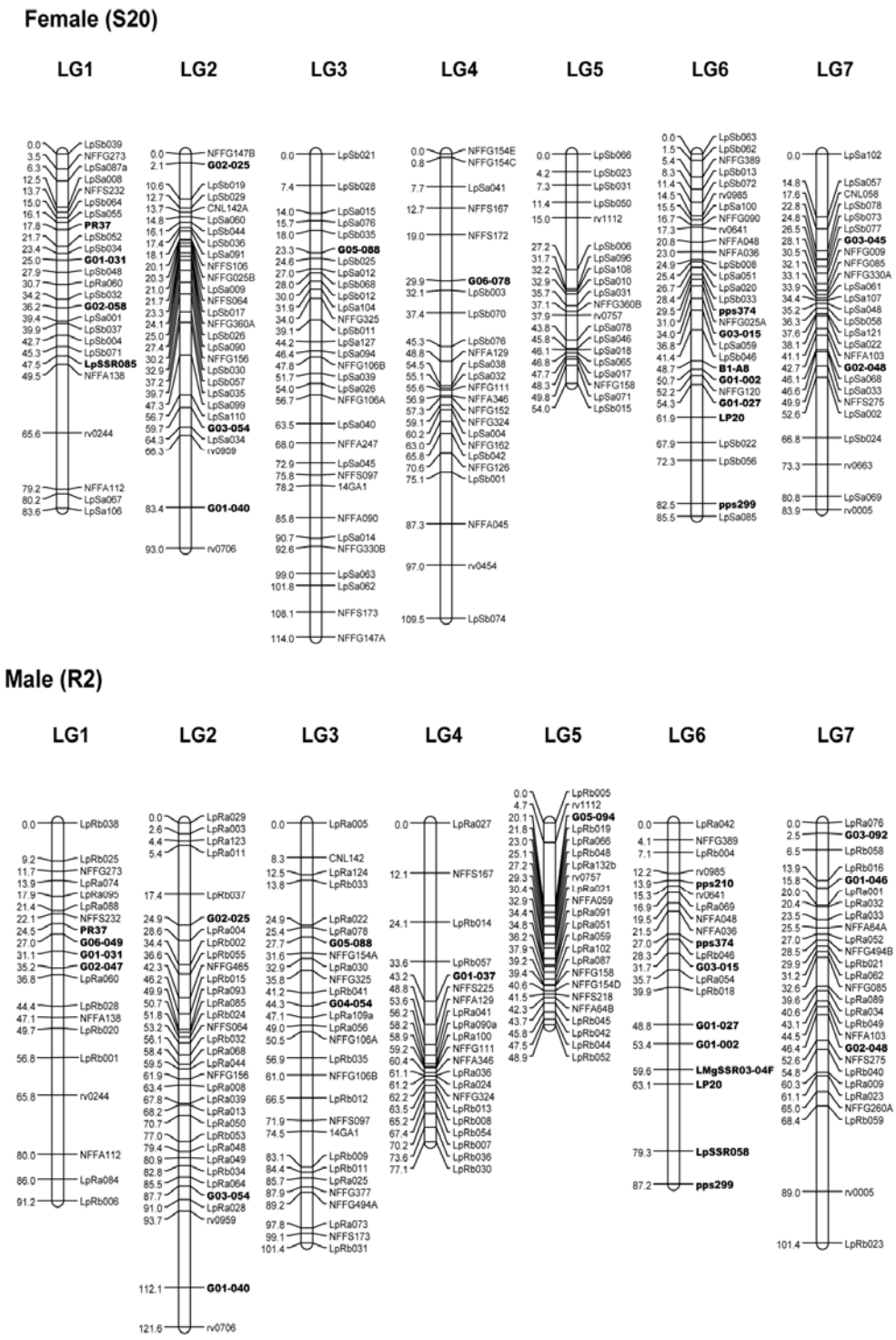

Fig. 1. Linkage maps of parents (S20 rust-susceptible female, R2 rust-resistant male) of *Lolium perenne* F1 population used to detect QTL activated by inoculation with pathotypes of the stem rust pathogen, *Puccinia graminis* subsp. *graminicola*. QTL (2-LOD interval) are indicated by shaded sections of chromosomes. Two QTL, qLpPg1 (LG7) and qLpPg3 (LG6), were detected when plants were inoculated with pathotype 101. The QTL qLpPg2 (LG1) and qLpPg3 (LG6) were detected when plants were inoculated with pathotype 106. The star, within qLpPg1 on male LG7, indicates map location of binary phenotype (resistant vs. susceptible) for plants inoculated with pathotype 101. Markers in bold font, selected from those used on other *Lolium* maps, were added to the previously-published map (Pfender et al., 2011) to create this map (Pfender and Slabaugh, 2013).