QTL Mapping of Resistance to Gray Leaf Spot in Ryegrass: Consistency of QTL between Two Mapping Populations.

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ABSTRACT

Gray leaf spot (GLS) is a serious fungal disease caused by *Pyricularia oryzae Cavara, recently reported on the important turf and forage species, perennial ryegrass (Lolium perenneL.). This fungus also causes rice blast, which is usually controlled by host resistance, but durability of resistance is a problem. Few instances of GLS resistance have been reported in perennial ryegrass. However, two major QTL for GLS resistance have been detected on linkage groups 3 and 6 in an Italian x perennial ryegrass mapping population. To confirm that those QTL are still detectable in the next generation and can function in a different genetic background, a resistant segregant from this population has been crossed with an unrelated susceptible perennial clone, to form a new mapping population segregating for GLS resistance. QTL analysis has been* performed in the new population, using two different ryegrass field isolates and RAPD, RFLP, and SSR marker-based linkage maps for each parent. Results indicate the previously identified QTL on linkage group 3 is still significant in the new population, with LOD and percent of phenotypic variance explained ranging from 2.0 to 3.5 and 5% to 10%, respectively. Also two QTL were detected in the susceptible parent, with similar LOD and phenotypic variance explained. Although the linkage group 6 QTL was not detected, the major QTL on linkage group 3 appears to beconfirmed. These results will add to our understanding of the genetic architecture of GLS resistance in ryegrass, which will facilitate its use in perennial ryegrass breeding programs.

Abbreviations: GLS, gray leaf spot; LOD, logarithm of odds; QTL, quantitative trait loci

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Received : Apr. 6, 2008, Revised : Jun. 15, 2008, Accepted : Jun. 20, 2008

INTRODUCTION

Gray leaf spot (GLS) caused by the ascomycetous fungus *Pyricularia oryzae* Cavara [teleomorph *Magnaporthe oryzae* B. Couch, formerly known as *Magnaporthe grisea* (Hebert) Barr] is an important disease on perennial ryegrass (*Lolium perenne* L.), which is a widely used cool season grass for forage and golf course fairways and roughs. The disease has been reported in many areas of the eastern and central US, and has now spread to western states such as California and Nevada. Occurrences of GLS are difficult to predict, even though epidemiological studies have been conducted (Uddin et al., 2003), and once GLS occurs, severe damage to fairways and roughs can happen quickly (Landschoot and Hoyland, 1992). Fungicide application has become an important component of GLS management on golf courses and athletic fields, along with cultural practices. However, they can be costly, and strains of *P. oryzae* resistant to azoxystrobin, one of the most effective fungicides used to control GLS, have been reported (Vincelli and Dixon, 2002). All of these factors combine to make host resistance in perennial ryegrass an attractive strategy for controlling GLS, improving the utility of this versatile cool-season turf species, and reducing dependence on fungicide applications.

Until the recent release of a group of improved perennial ryegrass varieties with GLS resistance (Bonos, personal communication), there was a complete lack of resistant varieties in the US (Williams et al., 2001, Bonos et al., 2004), while some perennial ryegrass plant introductions seemed to show resistance, particularly from eastern European sources (Bonos et al., 2004; Hoffmann and Hamblin, 2001). However, it is not known how well the new varieties will retain their resistance in a field setting, especially after years of exposure to a potentially rapidly changing pathogen population. Breakdown of disease resistance has been a major problem for many crops, especially for blast disease on rice (Chauhan et al., 2002), which is caused by strains of the same fungus, *P. oryzae*, as GLS.

The addition of more sources of resistance to GLS to future cultivars may allow them to remain resistant against a variable pathogen population. The identification of additional sources of resistance in *Lolium*, followed by the characterization of genomic regions associated with resistance in those sources will be facilitated by quantitative trait locus (QTL) mapping. Then, development of DNA markers linked to QTL will allow the tracking and combining of genes from many sources of resistance into new cultivars. Also, more detailed

knowledge of the number and degree of effect of the genes involved will suggest the amount of breeding work required to transfer the resistance into perennial ryegrass cultivars.

Towards this end, Curley et al. (2005) used an Italian ryegrass (*Lolium multiflorum* Lam.) x perennial ryegrass interspecific mapping population to detect two major QTL for resistance to a ryegrass field isolate. These were located on linkage groups 3 and 6, and the Italian ryegrass grandparents of the population were strongly suggested to be the resistance source for both of these QTL. However, it is important to understand the consistency of QTL across different mapping populations, which is necessary for marker-assisted selection based on these QTL (Lubberstedt et al., 1998). Several studies have reported sensitivity of QTL to the environment, population size and genetic background, so that conclusions about these QTL may be erroneous if based on only one environment and genetic background (Brummer et al., 1997; Keim et al., 1990; Melchinger et al., 1998; Paterson et al., 1991). Therefore, it is also crucial to develop and map QTL in a second genetic background i.e. mapping population, in order to provide further evidence for the QTL and genetic conclusions we found from the Italian x perennial ryegrass population. This way, the utility of markers linked to QTL for GLS resistance may be confirmed prior to implementing them in a marker-assisted perennial ryegrass breeding program. Once transferability of this resistance source to new cultivars has been established, markers associated with these QTL can then be developed and used for marker-assisted breeding for GLS resistance in perennial ryegrass.

Thus, the objective of this study was to map QTL for GLS resistance in a second generation population derived from the original Italian x perennial ryegrass (MFA x MFB) population, in order to confirm that the QTL detected in the original population are still detectable in the next generation, and can function in a different genetic background.

Materials and Methods

Parental clones L4B-5 and MF-8 were originally obtained from Dr. R. Barker (USDA-ARS, Corvallis, OR, USA). Clone L4B-5 is a perennial ryegrass genotype resulting from a cross between a clonal genotype of the forage type perennial ryegrass cultivar 'Linn' and the turf type perennial ryegrass cultivar 'SR4400' (Fig. 1). Clone MF-8 is one of the 156 progeny individuals from the MFA x MFB

mapping population (Warnke et al., 2004), which was originally used for QTL mapping of GLS resistance (Curley et al., 2005). These clones were chosen as parents of the new mapping population based on their differential gray leaf spot reaction, with L4B-5 and MF-8 being susceptible and resistant to several isolates, respectively (Curley et al., 2005), as well as for their vigorous growth and ease of propagation.

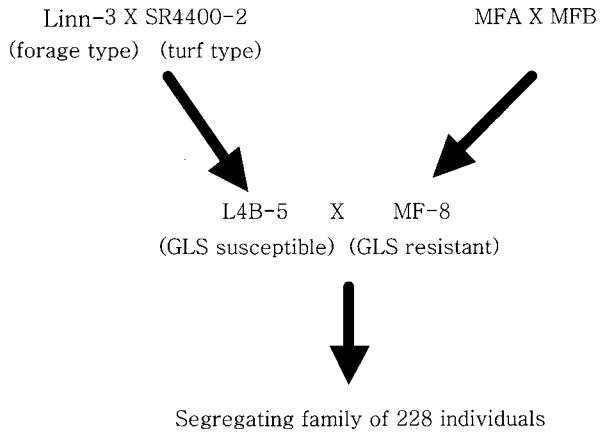

Fig. 1. Structure of the mapping population used in this study. The L4B-5 x MF-8 cross was performed in this laboratory by the first author, while the remaining crosses were performed at USDA-ARS, Corvallis, OR. All seeds used to create the population were collected from the L4B-5 parent.

Asexually prepared replicates of these two genotypes were artificially cold-treated in the greenhouse for approximately three months in an unheated room, and then flowering was induced using high intensity lights with an 18-hour photoperiod during spring. The flowering spikes of the two parents were bagged together before anthesis, and seeds were harvested from the L4B-5 parent after about two months. Seeds were stored at room temperature for about one month before being planted. Approximately 230 progeny individuals were produced from this cross.

Gray leaf spot inoculations were performed on 175 randomly selected mapping population individuals, as in Curley et al. (2005) except that the growth chamber method was used exclusively. Two *P. oryzae* isolates from diseased perennial ryegrass golf course fairways were used, GG9 which was collected in 1997 in Kentucky (Curley et al., 2005), and LexF2A which was collected in a ryegrass spaced-plant test plot at Lexington, KY in 2004. The parental genotypes, L4B-5

and MF-8, all four grandparents (Fig. 1), and perennial ryegrass clones Manh-1 and Manh-3 as well as Italian ryegrass clones Flor-2 and Flor-4 were included as checks. Disease severity ratings were made using the scale used in Curley et al. (2005), and the mean GLS rating over the four clonal replicates of each genotype was used as the phenotypic variable in QTL mapping.

Statistical analysis of GLS reaction, such as ANOVA and correlation analysis, were performed using JMP and SAS software. Broad sense heritability based on means of clonal replicates was estimated using the formula $Hc = \sigma^2_g/(\sigma^2_g + \sigma^2_{gy}/y + \sigma^2_{gl}/l + \sigma^2_{gr(l)}/rl + \sigma^2_{gyl}/ly + \sigma^2_e/rly)$ (Bonos et al., 2003). This formula is typically used for field experiments evaluated in multiple locations over multiple years, but in this study, data from all four inoculations were used, with the two isolates treated as locations, and both experiments using each isolate treated as years. Thus, the letters in the denominator refer to the number of replicates, which was 4, locations representing isolates, which was 2, and years representing experiments, which was 2. The broad sense heritability estimate was used in this case to provide a more thorough statistical analysis of the data, as well as to demonstrate the reproducibility of the growth chamber assay.

Two linkage maps were constructed separately for each parent, L4B-5 and MF-8, using the DH or double haploid population type option available in Joinmap 3.0 (Van Ooijen and Voorrips, 2001), similar to the procedure used in Curley et al. (2005). The maps were constructed separately mainly due to unequal rates of recombination between male and female parents previously reported in ryegrass (Warnke et al., 2004). The maps were constructed using RFLP, SSR, and RAPD markers, using 90 progeny individuals for RFLPs, and 94 for SSRs and RAPDs. For selected RAPD markers, an additional 96 individuals were genotyped, for a total sample size of 190. All of the RAPD primers used in the MFA x MFB population (Warnke et al., 2004) have been used, to facilitate map integration between the resistant parent MF-8, and the original MFA and MFB maps. Additional primers were used to generate markers, for increased map coverage and density. Heterologous cereal RFLP markers labeled 'CDO' (Sim et al., 2005) and bentgrass cDNA markers labeled 'Ast' (Chakraborty et al., 2005) were added, to assist in confirming the original QTLs from the MFA x MFB population as well as allow creation of an integrated map based on both parents of the current population. Genomic and EST-derived SSR markers, originally developed in ryegrass and tall fescue, were generated using the following PCR profile: 3 minutes at 94°C, followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 60°C, 45 seconds at 72°C, with a final extension of 90 seconds at

72°C. Amplified bands were detected using ethidium bromide-containing Metaphor agarose gels at a concentration of 3% in 0.5x Tris-borate-EDTA buffer.

QTL analyses were performed as in Curley et al. (2005), using MapQTL software (Van Ooijen et al., 2002), using the mean GLS rating over the four clonal replicates of each genotype as the phenotypic variable. Since there were four separate experiments and two separate linkage maps, a total of eight analyses were performed. Three analysis methods were used: Kruskal-Wallis analysis, interval mapping, and multiple QTL mapping. Interval mapping was used to estimate the map location and phenotypic effect of potential QTL, in terms of both the percentage of phenotypic variance explained, and multiple QTL mapping was used to refine the estimate of QTL position obtained from interval mapping. The nonparametric, single marker-based Kruskal-Wallis analysis was performed to check that markers linked to QTL detected by interval mapping were significant when examined individually. Finally, significance of QTL was decided using two methods. The permutation test of MapQTL was used, with the 5% genome-wide significance threshold LOD score determined using 1000 iterations. Additionally, markers significant in the Kruskal-Wallis analysis after correcting for the total number of markers in the separate parental maps were considered to be closely linked to significant QTL.

Results

Frequency distributions for four growth chamber inoculations using two different ryegrass field isolates, GG9 and LexF2A, are given in Fig. 2. As was observed previously (Curley et al., 2005), none of the progeny showed score 0, and score 1 did not occur separately from score 2. For both isolates, a higher proportion of resistant individuals was observed in this population compared to the MFA x MFB population, as all four graphs appeared skewed towards resistance (Fig. 2), and the mean reaction of the population ranged from 2.58 to 2.66. Additionally, only very slight transgressive segregation was observed, and only in the direction of susceptibility. Specifically, some progeny had larger susceptible lesions than the susceptible parent (data not shown), but the resistant progeny showed equal resistance as the resistant parent. Further, both isolates showed a similar reaction on the progeny, and correlations between the four combinations of isolate and experiment were generally strong and significant, with coefficients between 0.5 and 0.75 and all p-values less than 0.0001.

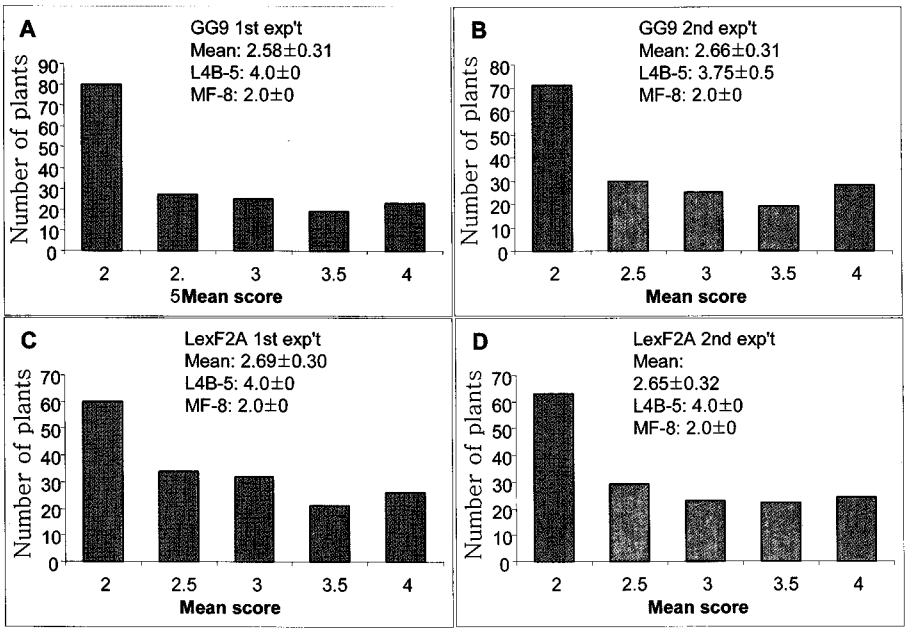

Fig. 2. Frequency distributions of GLS phenotypic data from four greenhouse inoculations, based on the mean score over four clonal replicates of each progeny genotype. For each experiment, the population mean and standard deviation of GLS reaction, as well as the mean and standard deviation of GLS reaction for each of the two mapping parents, L4B-5 and MF-8, are given. A: GG9, first growth chamber experiment. B:GG9, second growth chamber experiment. C: LexF2A, first growth chamber experiment. D: LexF2A, second growth chamber experiment

ANOVA tests for all four experiments (Table 1) showed highly significant genotype effects. Also significant were isolate effect and genotype x isolate

Table 1. General statistics for the two experiments using two P. oryzae isolates

Source	df	Type III SS	MS	F value	Pr > F	σ^2
Isolate	1	1.76	1.76	5.71	0.0171	
Rep(Isolate)	6	7.74	1.29	4.18	0.0004	
Expt	1	0.48	0.48	1.57	0.2108	
Isolate*Expt	1	0.89	0.89	2.90	0.0892	
Expt*Rep(Isolate)	6	6.92	1.15	3.74	0.0011	
Genotype	173	960.76	5.55	18.02	<.0001	0.363
Genotype*Isolate	172	120.29	0.70	2.27	<.0001	0.058
Genotype*Rep(Isolate)	1004	292.88	0.29	0.95	0.7971	0.0
Genotype*Expt	170	55.07	0.32	1.05	0.3284	0.0023
Genotype*Isolate*Expt	156	48.24	0.31	1.00	0.4790	0.0003
Error	818	252.16	0.31			

interaction, which is surprising given the relatively strong correlations between the four experiments, although the mean squares were much lower than for the genotype effect. The broad-sense heritability, estimated using all four inoculations (two isolates with two experiments each), was 0.88. All variance components used in the calculation are given in Table 1. Taken together, these results suggest that the phenotypic assay is reproducible, and that there is a high degree of genetically controlled variation in GLS reaction in this population.

Two linkage maps have been constructed for this population using RAPD, SSR, and RFLP markers, one for each parent (Figs. 3A and 3B). For the L4B-5 susceptible parent, there were 81 RAPDs, 8 SSRs, and 3 RFLPs mapped, covering 515 cM on the expected number of 7 linkage groups, with 22 unmapped markers. Linkage groups 2, 3, and 6 of this map have been aligned with Triticeae maps as well as previous ryegrass maps using the RFLP markers mapped on these groups. Linkage groups 4, 5, and 7 were aligned with ryegrass maps using SSR markers, thus the remaining group of markers was assigned to linkage group 1. For the MF-8 resistant parent, there were 97 RAPDs, 10 SSRs, and 5 RFLPs mapped, covering 558 cM on 7 linkage groups, with 29 unmapped markers. In this map, the linkage groups are aligned with Triticeae chromosomes, via RAPD markers in common with the MFA x MFB population as well as RFLP and SSR markers.

Results of QTL mapping using Kruskal-Wallis, interval mapping, and MQM analysis are presented in Table 2. QTL mapping has revealed 4 potential QTLs, 2 from the resistant parent, and 2 from the susceptible parent (Table 2), and results were generally consistent over the isolates and experiments. For the resistant parent, a QTL on linkage group 3 linked to RAPD marker M.T17.680 showed the most consistency and a maximum LOD score of 4.2 and percent of phenotypic variance explained of 10.8%. Another QTL, on linkage group 7, was less significant and consistent, as it was only detected in experiment 1 using GG9, and experiment 2 using LexF2A. For the susceptible parent, two QTL of approximately equal consistency and significance were detected, one on linkage group 4 and the other on linkage group 5. The first, on linkage group 4 was linked to RAPD marker L.A13.820 and had maximum LOD and percent of phenotypic variance explained of 3.9 and 9.8%, respectively. The other, on linkage group 5, was linked to RAPD marker L.E8.1500 and had maximum LOD and percent of phenotypic variance explained of 3.2 and 9.5%, respectively. Permutation analysis performed for all 8 combinations of experiment and parental map showed 5% genome-wide significance threshold LOD scores ranging from 2.5

to 2.7, with 2.5 occurring most frequently (Table 2), and that most QTLs had LOD scores exceeding these thresholds.

Fig. 3A

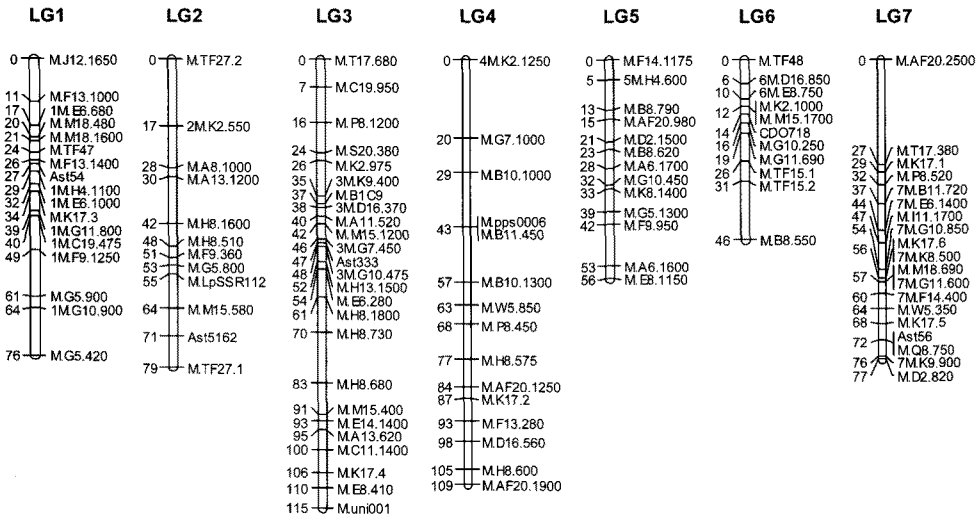

Fig. 3B

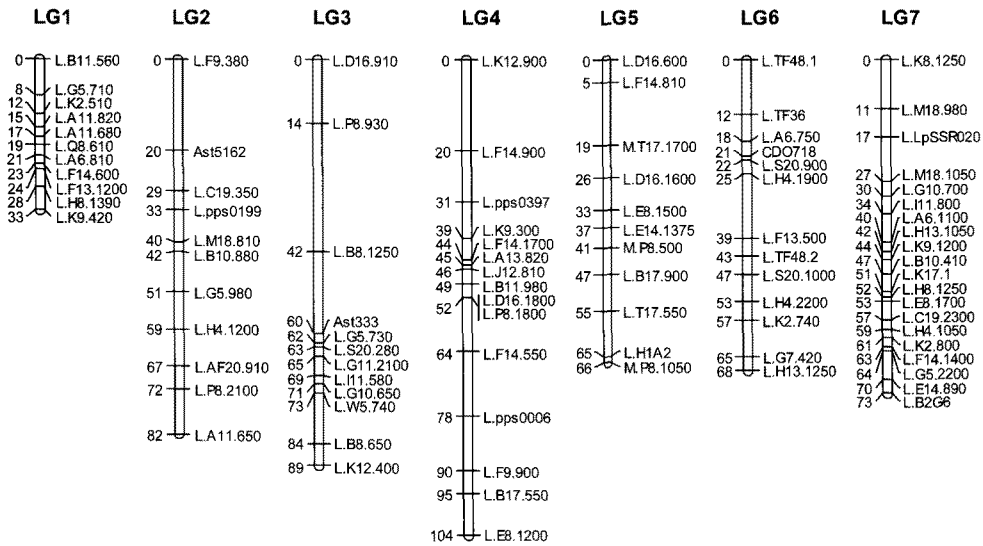

Fig. 3. Linkage maps of the L4B-5 x MF-8 population used for the confirmation of gray leaf spot resistance QTL previously detected in the MFA x MFB interspecific ryegrass population (Curley et al. 2005), constructed separately using RFLP, RAPD, and SSR marker data. Linkage groups for both the MF-8 and L4B-5 maps are numbered according to Warnke et al. (2004). A: MF-8 parent map. B: L4B-5 parent map

Table 2. Summary of the QTL for resistance to two different *P. oryzae isolates in the L4B-5 x MF-8 mapping population*

P. oryzae isolate: exp't[†]	Ryegrass LG[‡]	Marker[§]	KW significance[¶]			IM[#]	IM	MQM[††]	MQM	$\alpha g^{‡‡}$
			P-value	Allele means		LOD	% variance	LOD	% variance	
				a	b					
GG9 Exp't 1	M3	M.T17.680	0.005	2.43	2.75	2.0	5.1	2.3	5.5	2.5
GG9 Exp't 2	M3	M.T17.680	0.0005	2.45	2.88	3.5	8.8	3.8	9.3	2.6
LexF2A Exp't 1	M3	M.T17.680	0.0005	2.50	2.91	3.4	8.6	3.4	8.6	2.7
LexF2A Exp't 2	M3	M.T17.680	0.0001	2.45	2.91	3.8	10.4	4.2	10.8	2.6
GG9 Exp't 1	M7	M.M18.690	0.01	2.27	2.69	2.7	8.9	2.9	9.3	2.5
LexF2A Exp't 2	M7	M.M18.690	0.005	2.29	2.89	2.0	7.2	2.4	7.6	2.6
GG9 Exp't 1	L4	L.A13.820	0.0001	2.88	2.42	3.9	9.8	3.5	8.2	2.5
GG9 Exp't 2	L4	L.A13.820	0.0005	2.94	2.49	3.2	8.1	3.2	8.1	2.6
LexF2A Exp't 1	L4	L.A13.820	0.001	2.92	2.56	2.3	5.9	2.0	4.9	2.5
LexF2A Exp't 2	L4	L.A13.820	0.005	2.91	2.53	2.3	6.5	1.9	4.7	2.5
GG9 Exp't 1	L5	L.E8.1500	0.05	2.91	2.47	2.5	7.6	2.2	6.0	2.5
GG9 Exp't 2	L5	L.E8.1500	NS	2.90	2.66	1.2	3.7	1.0	2.7	2.6
LexF2A Exp't 1	L5	L.E8.1500	0.05	2.95	2.61	1.8	5.3	1.5	4.3	2.5
LexF2A Exp't 2	L5	L.D16.1600	0.001	2.88	2.36	3.2	9.5	2.8	7.8	2.5

[†] Trait: combination of isolate and experiment
[‡] Linkage group and parental map (L = L4B-5, M = MF-8) in which each QTL was detected
[§] Name of the marker most closely linked to the QTL in question.
[¶] For Kruskal-Wallis analysis, the P-value at the indicated marker is presented, along with mean phenotypic score of progeny carrying the 'a' or 'b' allele of each marker
[#] For interval mapping (IM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given
[††] For multiple QTL mapping (MQM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given. Also, the named markers were used as cofactors, after being selected for use as cofactors in the automatic cofactor selection test ($P<0.02$)
[‡‡] LOD thresholds derived from permutation analysis, for each combination of trait and parent linkage map, are given. The αg indicates the 5% LOD threshold for all seven linkage groups of the parental map in question

Discussion

In this study QTL mapping was performed using a mapping population derived from the MFA x MFB population, which had previously been used for QTL mapping for GLS resistance (Curley et al., 2005), with the main purpose of confirming that the original QTL are still detected in the next generation and in a population with a different genetic background.

In the original MFA x MFB population, QTL had been observed on linkage groups 3 and 6, with the linkage group 3 QTL showing higher significance and effect, as well as a syntenous relationship with several reported rice blast

resistance genes and QTL (Curley et al., 2005). The detection of a QTL on linkage group 3 in the current study provides evidence that this QTL is still functioning in the current population. On linkage group 6, though, no QTL were detected in the current population. One possible reason is that since the map length of linkage group 6 is only 46 cM compared with 100 cM for the original MFA linkage group 6, portions of this linkage group may still be unmapped, or have no segregating markers at all. Thus, portions of the genome containing QTL intervals with higher significance levels may remain undetected. Additionally, the linkage group 6 QTL had a much lower significance level in the MFA x MFB population than did the linkage group 3 QTL. Finally, the RAPD marker that was linked to the MFA linkage group 6 QTL, C19.390, was not segregating in this population, since for this marker the absence of the band was associated with reduced lesion score. Since the MF-8 resistant parent of this population lacked that band, it was not possible to use this marker for QTL confirmation.

Further, the significance of the linkage group 3 QTL, in terms of the LOD score and percentage of the phenotypic variation explained by the QTL, was much lower in the L4B-5 x MF-8 population than in the MFA x MFB population. Possible reasons for this difference include low numbers of susceptible individuals in the population resulting from resistance QTL being present in both parents, possibly lowering the power of QTL detection. Another possible explanation is that since the marker linked to the linkage group 3 QTL with the highest LOD score is the most distal marker, the actual QTL may be located more distal than this marker, thus allowing for recombination between the marker and QTL and lowering its apparent significance.

These results open up several possible avenues for further research. For example, several additional steps can be implemented to provide further confirmation of the QTL for GLS resistance. For one, synteny with rice can be utilized to select additional markers for use in lengthening the linkage group 3 and linkage group 6 maps of the resistant parent, MF-8. For linkage group 3, this would be useful in obtaining markers located more distally than the RAPD marker M.T17.680, which might be more closely linked to the major QTL and thus show higher LOD and percent of phenotypic variation explained. For linkage group 6, this would be useful in lengthening the map to allow a better comparison between the two populations. Another possible test of the linkage group 6 QTL may be converting the RAPD marker C19.390, which was linked to the QTL in the MFA x MFB population, into a SCAR marker in order to specifically test this QTL in the new population.

The QTL on linkage group 7 of the current population appears to be a new QTL, although reexamination of data from the MFA x MFB population showed weak associations observed on linkage group 7 of the MFA parent linkage map as well (p=0.05). This is interesting since the QTL with stronger effect on linkage group 3 was detected on the MFB map, with only the weaker effect QTL on linkage group 6 of the MFA map being detected. Since both of these clones showed the same intermediate reaction, it seemed that there must be more QTLs segregating in MFA than what had been detected.

Finally, two potential QTL were detected from the susceptible parent, L4B-5. This was not expected at the start, but it does provide an explanation for an observed slight transgression in susceptibility, in that some progeny showed larger type 4 lesions than had been observed in the susceptible parent. Further, since transgressive segregation in the direction of resistance was not observed in this population (Fig. 2), it was suggested that there are no additional QTL for resistance derived from the resistant parent, MF-8, relative to the first population. This is likely because QTL alleles conferring resistance, which were likely inherited from MFA and MFB, have accumulated in the resistant parent and are now segregating in the population. The QTL alleles from the L4B-5 parent did not appear to produce a detectably more resistant phenotype than observed in the MF-8 parent, however they may well account for the higher than expected frequency of resistant individuals in the population.

Interestingly, an isolate effect and genotype x isolate interaction were noted (Table 1). Although the associated mean squares were weak compared to the mean squares for the genotype effect, this seems at odds with previous results (Curley et al., 2005) which suggested that different isolates produce similar reaction patterns. One possible explanation is the two isolates, although both collected near Lexington, KY, were collected at different dates (1997 for GG9 and 2004 for LexF2A). Thus, they may be genetically and pathogenically different, as the LexF2A isolate appeared to be slightly more virulent than GG9 (data not shown). Field evaluations of gray leaf spot reaction using both artificial and natural inoculation may be very useful in estimating the response to a more varied pathogen population. Additionally, data on field reaction will be very useful in demonstrating the agreement of results from growth chamber studies with data from an environment that is much closer to what an eventual ryegrass cultivar would be exposed.

In conclusion, the results of this study have important implications for breeding perennial ryegrass for resistance to GLS. Based on the detection of QTL for GLS

resistance on linkage group 3 of the current population, the most significant of the QTL detected in the MFA x MFB population appears to be confirmed. This result is very important for using this Italian ryegrass-derived source of resistance in a GLS resistance breeding program, as well as for development of markers tightly linked to the QTL for use in marker-assisted selection, as was accomplished in Fjellstrom et al. (2004). Also, the mean reaction score of the current population was approximately 2.66, markedly less than the mean of the MFA x MFB population, 3.20. This provides further evidence that the frequency of resistance alleles in the current population has increased, and similar to the findings of Bonos et al. (2004) suggests a rapid response to selection in one generation, and control of GLS resistance by a low number of genes. It is likely that other GLS-resistant Lolium genotypes, such as those found in Miura et al. (2005) and Hoffman and Hamblin (2001) will share similar high heritability and low numbers of genomic regions. Combined with high heritabilities observed in this study as well as others (Curley et al., 2005; Han et al., 2006), this transferability to new cultivars will be very important for all breeding programs aiming to improve GLS resistance.

Acknowledgement

We gratefully acknowledge financial support from the United States Golf Association.

국문 요약

Gray leaf spot (GLS)은 *Pyricularia oryzae* Cavara에 의해 발병하는 중요한 곰팡이 병으로 최근 주요 잔디류 및 목초류에 해당하는 퍼레니얼 라이그래스 (Perennial ryegrass; *Lolium perenne L.*)에서 발생되는 것으로 보고되었다. 또한 이 곰팡이는 벼의 도열병을 일으키는데, 이는 기주 저항성에 의해 방제될 수 있지만 이 저항성의 지속기간에 문제가 있는 것으로 알려져 있다. 지금까지 퍼레니얼 라이그래스에서는 GLS 저항성에 관한 내용이 거의 보고되지 않았다. 그러나 이탈리안 라이그래스 x 퍼레니얼 라이그래스 mapping population에서 GLS 저항성에 관한 주요 양적형질 유전자좌 (QTL)가 연관군 (linkage group) 3과 6 상에서 각각 발견되었다. 이 두 가지 양적형질 유전자좌가 다음 세대에서도 여전히 나타나고, 이들이 다른 유전적 배경 하에서도 기능할 수 있다는 사실을 확인하기 위해 기존의 mapping population으로부터 나온 저항성 개체를 저항성을 갖고 있지 않은 다른 퍼레니얼 클론과 교잡시켜 새로운 mapping population을 만들었다. 이 새로운 mapping population에서RAPD, RFLP 및 SSR 마

커를 이용하여 QTL 분석을 실시하였다. 이 결과, 비록 연관군 6 상에서는 양적형질 유전자좌가 확인되지 않았지만 연관군 3 상의 양적형질 유전자좌는 새로운 mapping population에서도 여전히 나타나고 있음이 확인되었다. 또한 두 개의 새로운 양적형질 유전자좌가 저항성을 갖고 있지 않았던 부모개체에서도 발견되었다. 본 실험 결과는 라이그래스에 있어서 GLS 저항성의 유전적 구조를 이해하는데 도움을 줄 뿐만 아니라 퍼레니얼 라이그래스 육종 프로그램에 사용상 편의성을 제고시킬 것이다.

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