Theor Appl Genet (2005) 110: 244–251
DOI 10.1007/s00122-004-1819-1

ORIGINAL PAPER

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Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat

Received: 28 May 2004 / Accepted: 1 September 2004 / Published online: 1 December 2004

Abstract Slow rusting is considered a crucial component of durable resistance to wheat leaf rust caused by *Puccinia triticina* and is often expressed in the form of a prolonged latent period. Selection for a longer latent period is considered an effective approach to developing wheat cultivars with improved durable resistance to leaf rust. A recombinant inbred line (RIL) population derived from CI 13227 (long latent period) × Suwon 92 (short latent period) was phenotyped for latent period in two greenhouse experiments in separate years, and amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers were analyzed in the same population. Among the RILs, the frequency distribution for latent period was continuous, and latent period was highly correlated between years ($r = 0.94$, $P < 0.0001$). A quantitative trait locus (QTL) prolonging the latent period of *P. triticina*, designated as *QLrlp.osu-2DS*, explained 42.8% and 54.5% of the phenotypic and genetic variance in the two experiments, respectively. *QLrlp.osu-2DS* was mapped on the distal region of chromosome 2DS. Two other QTLs for latent period, *QLrlp.osu-2B* and *QLrlp.osu-7BL*, were localized on chromosome 2B and the long arm of chromosome 7B, respectively. Multiple regression analysis showed that these three QTLs collectively explained 58.0% and 73.8% of the phenotypic and genetic variance over two experiments, respectively. Fourteen RILs that carried all three alleles for long latent period at three AFLP loci flanking *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* had a mean latent period of 12.5 days, whereas 13 RILs without any long-latent-period alleles at the corresponding loci had a mean latent period of 7.4 days. Three SSR markers closely linked to these QTLs have potential to be applied in marker-assisted selection for prolonged latent period in wheat.

Communicated by G. Wenzel

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Introduction

Leaf rust, caused by *Puccinia triticina* (previously *P. recondita* Rob. Ex Desm), is one of the most widely distributed diseases of wheat worldwide. Yield losses caused by leaf rust mainly derive from premature senescence and poorly developed kernels. In many wheat-producing regions, breeding for leaf rust resistance too often leads to the wide deployment of race-specific resistance genes and subsequent rapid change of predominant races. The effectiveness of race-specific genes is usually short-lived in nature in the presence of pathogen races with matching virulence alleles. The slow leaf-rusting trait, which is not conferred by race-specific genes but quantitatively inherited, is gaining popularity in breeding programs due to its durability (Kuhn et al. 1978).

Slow-rusting cultivars display a compatible infection type, but the rate of disease progress is much slower than in susceptible cultivars (Bjarko et al. 1988a; Caldwell et al. 1970; Shaner et al. 1980, 1997). The slow-rusting trait consists of several components, including a longer latent period, reduced receptivity (a lower probability that a urediniospore will infect, thus reducing the number of uredinia per unit area of leaf for a given density of inoculum), smaller uredinia, and less spore

production (Milus et al. 1980; Ohm et al. 1976; Parlevliet et al.1979; Shaner et al. 1980). Among these, latent period has been identified as the most important component that explains most of the difference in slow-rusting resistance (Neervoort et al. 1978; Ohm and Shaner 1976; Parlevliet et al. 1975; Zadoks et al. 1971).

Prolonged latent period of leaf rust (*P. hordei*) was manifested in a comparison of two barley (*Hordeum vulgare* L.) cultivars, in which the resistant cultivar Vada had twice as much infection arrested in the substomatal vesicle phase than had the susceptible cultivar Midas (Clifford 1972). Vada developed fewer and smaller pustules, which ruptured 2–6 days later than pustules on Midas. Slow leaf-rusting resistance in wheat, which is conferred by *Lr*34, is associated with a reduced rate of haustorium formation during early infection and the result of less intercellular hyphal development rather than papilla formation in invaded host cells (Rubiales and Niks 1995). Heath (1977) noted an association between cell-wall appositions (papilla) and slow leaf-rusting resistance.

Latent period in wheat is negatively associated with the area under disease progress curve (AUDPC), final rust severity, uredinium size, and receptivity (Das et al. 1993). Its heritability is moderately high and has varied from 0.46 to 0.90 (Bjarko 1988b; Das et al. 1992; Jacobs et al. 1989; Lee et al. 1985a). Three to five genes have been estimated to control latent period (Das et al. 1992; Shaner et al. 1997; VanderGaag and Jacobs 1997), based on observations of transgressive segregation (Broers et al. 1989; Lee et al. 1985b). Although improvement of the latent period is genetically feasible, labor-intensive and complicated phenotypic measurements render traditional breeding methods impractical.

Marker-assisted selection (MAS) provides an alternative for selecting complex traits such as slow-rusting resistance and its major component, long latent period. Several attempts have been made to identify molecular markers associated with quantitative or slow-rusting resistance. William et al. (1997) identified three random amplified polymorphic DNA (RAPD) markers associated with quantitative trait loci (QTLs) for slow-rusting resistance in cv. Parula, which is believed to contain *Lr*34, a widely used slow leaf-rusting resistance gene. Two of the cloned RAPD markers were located on chromosome 7BL and the third one hybridized to chromosomes 1BS and 1DS, respectively. Farris et al. (1999) reported that a chromosome region on 7BL was associated with reduced rust severity under natural infection. Using a population of recombinant inbred lines (RILs) derived from a cross between a synthetic wheat and cv. Opata, Nelson et al. (1997a) identified two loci for leaf rust resistance on chromosome 7DS, the expected position of *Lr*34, and chromosome 2BS. Messmer et al. (2000), using a RIL population derived from a cross between Forno, a Swiss winter wheat cultivar reported to carry *Lr*34 for slow rusting, and Oberkulmer, identified one major QTL on 7BL that explained 35% of the phenotypic variance for leaf rust resistance but were unable to detect the QTL on 7DS. Analyzing 240 $F_{5:7}$ RILs from an Arina × Forno population, Schnurbusch et al. (2004) detected eight QTLs for leaf rust resistance with two having major effects: one on 7DS and another on 1BS. QTLs for durable leaf rust resistance were detected on chromosomes 7DS and 1BL, which may be *Lr*34 and *Lr*46, in a doubled haploid population derived from a cross of Tukuho-Komugi × Oligoculm (Suenaga et al. 2003). William (2003) reported two amplified fragment length polymorphism (AFLP) markers associated with *Lr*46 and assigned them to the distal end of chromosome 1BL.

Although several chromosomal regions have been related to slow-rusting resistance in various studies, no specific gene for prolonged latent period has been identified, nor has molecular dissection of the trait been attempted. Hence, our objectives were to understand the molecular basis of the latent period of *P. triticina* in wheat, identify the QTLs conditioning longer latent period, and develop molecular markers that have the potential to be used in breeding wheat cultivars with prolonged latent period through MAS.

Materials and methods

Phenotypic evaluation

A mapping population of 98 RILs was developed by single-seed-descent from a cross between the leaf rust-resistant wheat line CI 13227 and the susceptible cultivar Suwon 92 (Shaner et al. 1997). CI 13227 has a high level of slow leaf-rusting resistance with a long latent period, and its pedigree is Wabash/American Banner//Klein Anniversario (Shaner et al. 1997). Suwon 92 is very susceptible to leaf rust with a short latent period, and it derives from a cross between Suwon 85 and Suwon 13 (Shaner et al. 1980, 1997). Neither parent is known to carry any race-specific leaf rust resistance gene.

Two greenhouse experiments were conducted at Purdue University, West Lafayette, IN, USA in the fall of 1988 and spring of 1989 using a completely randomized design with seven replications (Shaner et al. 1997). Each replication contained one plant from each F_7 family and five plants from each parent. Plants with flag leaves fully emerged were inoculated with urediniospores of *Puccinia triticina* culture 7434-1-1T. The latent period was measured as the mean number of days from inoculation to when a uredinium ruptured the epidermis (Shaner et al. 1997) and was used for analysis herein. This variable is designated as mean latent period (MLP).

Analysis of AFLP

The CTAB (cetyltrimethyllammonium) method was used to isolate genomic DNA from 2-week-old wheat seedlings (Saghai-Maroof et al. 1984). The AFLP

analysis was according to the protocol of Vos et al. (1995) with respect to DNA digestion, adaptor ligation, and preamplification, with slight modifications (Bai et al. 1999). Selective amplification was carried out using *Pst*I and *Mse*I primers with three to four additional selective nucleotides. Infrared fluorescence dye-labeled *Pst*I primers were synthesized by Li-Cor (Lincoln, NE). AFLP products were separated on a 6.5% denaturing polyacrylamide gel running in 1× TBE buffer on a Li-Cor IR-4200 DNA sequencer (Li-Cor Inc., Lincoln, NE). The electrophoresis was conducted at 1,500 V, 40 W, 40 mA, and 45°C constant temperature for 3 h. The gel image file was collected and stored in a computer and the segregation patterns were scored by visual inspection.

Simple sequence repeat markers

For each SSR reaction, 20 ng of genomic DNA was used in a solution containing 200 μ*M* of each dNTP, 1× PCR buffer, 1 pmol of each primer with 2.5 m *M* $MgCl_2$ and 1 U *Taq* polymerase. A touch-down program was used for PCR amplification, in which the reaction mixture was denatured at 95°C for 5 min, followed by five cycles of 45 s at 95°C, 5 min of annealing at 68°C which decreased by 2°C in each subsequent cycle, and 1 min of extension at 72°C. For a second five-cycle series, the annealing temperature started at 58°C for 2 min with a decrease of 2°C for each subsequent cycle. PCR was then conducted for an additional 25 cycles of 45 s at 94°C, 2 min of annealing at 50°C, and 1 min of extension at 72°C, with a final extension at 72°C for 5 min. PCR products were separated on a 5% denaturing polyacrylamide gel and visualized by the silver staining method.

Bulked segregant analysis

A bulked segregant analysis (BSA) based on phenotypic data was used for the initial screening of informative AFLP primers (Michelmore et al. 1991). Among the 612 *Pst*I/*Mse*I primer pairs screened, 85 showed polymorphism between the two bulks and were used to characterize the population. A total of 459 AFLP markers were analyzed in the population. Three QTLs were identified in the initial analysis. To determine the tentative chromosome locations of these QTLs, we applied a revised BSA method to screen informative SSR primers. Three pairs of bulks contrasting in the presence or absence of an individual QTL for latent period were constructed based on AFLP markers flanking the target QTLs. For each pair, the long-latent-period bulk contained equal amounts of DNA from each of five RILs that had AFLP alleles flanking a QTL for long latent period, and the short-latent-period bulk contained equal amounts of DNA from each of five RILs that had alternative AFLP alleles. DNA from two parents and the six bulks were used to screen SSR primers. In total, 236 SSR primers were screened, and 28 SSR primers that detected polymorphism between at least one pair of bulks were used to characterize the entire population. Data from both SSR and AFLP analyses were combined for further QTL analysis.

Data analysis

One-way ANOVA was used to identify AFLP markers that were significantly associated with MLP ($P < 0.05$). Genetic linkage maps were constructed using MAPMAKER 3.0 (Lander et al. 1987). A threshold of LOD was set at 4.0 for the construction of linkage groups. Centimorgan (cM) values were calculated according to the Kosambi mapping function (Kosambi 1944). Single marker analysis and interval analysis from QGENE (Nelson 1997b) were used to characterize the effect of each marker and to map the long-latent-period QTLs. The SAS (SAS Institute, Raleigh, NC) procedures GCHART, REG, and MIXED, were used to generate histograms of phenotypic frequencies, perform multiple regression analysis, and partition the phenotypic variance, respectively.

Results and discussion

Latent period segregation in RILs

Across both experiments (1988 and 1989), the mean MLP of the slow-rusting parent CI 13227 was about 6 days longer than that of the susceptible parent Suwon 92. The RIL population showed a continuous distribution for MLP, varying from 7 to 15 days among lines (Fig. 1). The population mean MLP (9.2 days) was about 1 day less than that of the mid-parent (10.4 days). Transgressive segregation for MLP was found in both experiments (Fig. 1). The broad-sense heritability for MLP was 0.79 and consistent with previous estimates (Bjarko 1988b; Das et al. 1992; Jacobs et al. 1989; Lee et al. 1985a). The MLP values for each RIL were highly correlated between the two experiments ($r = 0.94$, $P < 0.0001$), so further analysis was conducted using RIL means across experiments.

Molecular markers linked to latent period

Molecular markers closely associated with MLP were identified by one-way ANOVA based on phenotypic data across the two experiments (Table 1). The determination coefficients (r^2) for 24 markers linked to MLP varied from 5.4% to 36.9% for any given year (Table 1). The additive effects of these markers varied from 0.4 days to 1.1 days. Linkage analysis assigned these markers to three chromosomes: 2DS, 2B, and 7BL. Three AFLP markers and three SSR markers on chromosome 2DS showed the highest r^2 values for MLP, indicating that these markers may be tightly linked to a major QTL for long latent period.

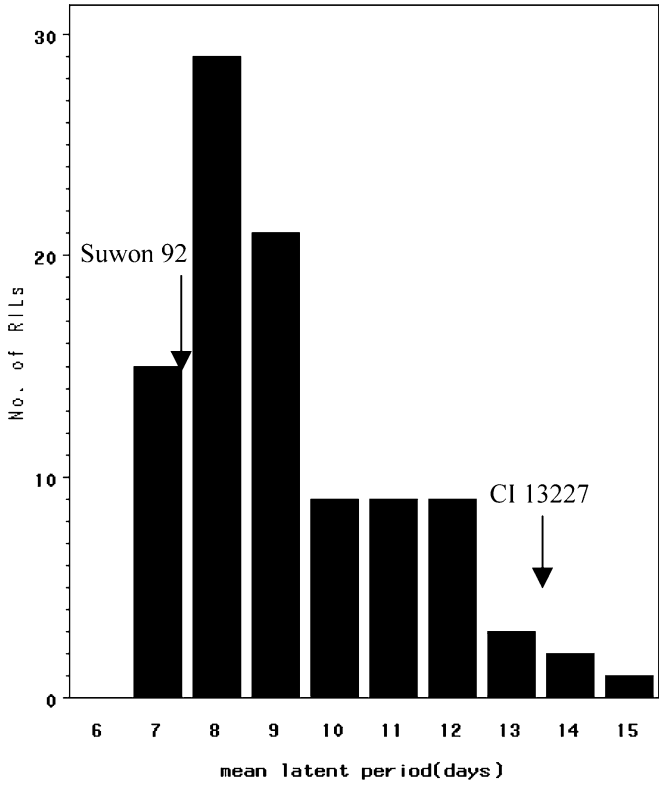

Fig. 1 Frequency distribution for mean latent period (MLP) measured across two experiments for CI 13227, Suwon 92, and RILs derived from CI 13227 × Suwon 92

Localization of QTL for latent period

Interval mapping located a putative QTL for long MLP between AFLP marker XACTG.GTG185 and SSR marker XBARC124 on the distal region of chromosome 2DS (Fig. 2). All four SSR markers linked to this QTL in this linkage group have been mapped previously on 2DS (Somers et al. 2004, http://www.scabusa.org/pdfs/BARC_maps_011106.pdf). The peak of this QTL, designated as *QLrlp.osu-2DS*, resides about 2.5 cM away from XACTG.GTG185 and 2.0 cM away from XBARC124 with an LOD value of 11.4 based on MLP means across years (Table 2). This QTL had a major effect on MLP and explained the highest proportion of the phenotypic variance among the QTLs identified in this population (Table 2). The phenotypic and genetic variances explained by this major QTL were 42.8% and 54.5%, respectively. The effect of this QTL on other manifestations of slow leaf rusting, such as AUDPC, final severity, and infection rate awaits further investigation in a field environment where these traits are more relevantly measured. We also suggest examining this QTL in multiple environments, especially in wheat-growing areas with different leaf rust infection patterns. The duration of rust infection may impact the effect of latent period on leaf rust resistance. In areas where the period for rust infection is relatively brief and hence where rust must build up quickly to cause serious damage, a longer latent period can substantially reduce spore accumulation, so that a rust epidemic may be minimized (Knott 1989). We expect that this QTL will have its greatest impact in regions where the leaf rust season is relatively short.

Table 1 Determination coefficients (r^2) and estimates of additive effects for AFLP and SSR markers linked to MLP[a] averaged across 2 years

Marker	Chromosome	r^2 (%)	*P*	Allele mean MLP		Additive effect
				CI 13227	Suwon 92	
XACTG.GTG185	2DS	36.9	< 0.0001	10.5	8.3	1.1
XBARC124	2DS	36.1	< 0.0001	10.4	8.2	1.1
XBARC95	2DS	27.4	< 0.0001	10.3	8.4	1.0
XCAT.CGTA237	2DS	22.3	< 0.0001	10.1	8.4	0.9
XTGC.CTA208	2DS	16.5	< 0.0001	9.7	8.4	0.7
XGWM455	2DS	15.7	< 0.0001	10.1	8.6	0.7
XTGC.ACAG198	7BL	17.5	< 0.0001	10.1	8.6	0.8
XCAG.CGAT70	2B	15.5	0.0001	9.9	8.5	0.7
XBARC182	7BL	14.1	0.0002	9.9	8.5	0.7
XACA.CACG126	7BL	14.0	0.0003	9.9	8.5	0.7
XCATG.ATGC125	7BL	13.3	0.0003	9.9	8.5	0.7
XBARC18	2B	12.4	0.0009	9.8	8.5	0.7
XWMC344	2B	11.5	0.001	9.7	8.5	0.6
XCATG.ATGC60	2B	11.3	0.0012	9.9	8.6	0.6
XBARC167	2B	10.8	0.002	9.8	8.6	0.6
XCAT.CTA155	7BL	11.7	0.001	9.8	8.6	0.6
XAT.CGTA146	2B	7.5	0.0084	9.7	8.7	0.5
XBARC1073	7BL	7.3	0.0091	9.8	8.8	0.5
XCATG.CGTA152	2B	7.2	0.0113	9.6	8.6	0.5
XBARC50	7BL	8.4	0.0053	9.8	8.8	0.5
XCAT.CGTA150	2B	6.8	0.0139	9.7	8.7	0.5
XGWM261	2DS	6.6	0.0145	9.7	8.7	0.5
XGCTG.CGAT290	2B	6.0	0.0204	9.7	8.8	0.5
XACTG.ATGC165	2B	5.4	0.0379	9.5	8.7	0.4

[a]MLP, mean latent period (see Shaner et al. 1997)

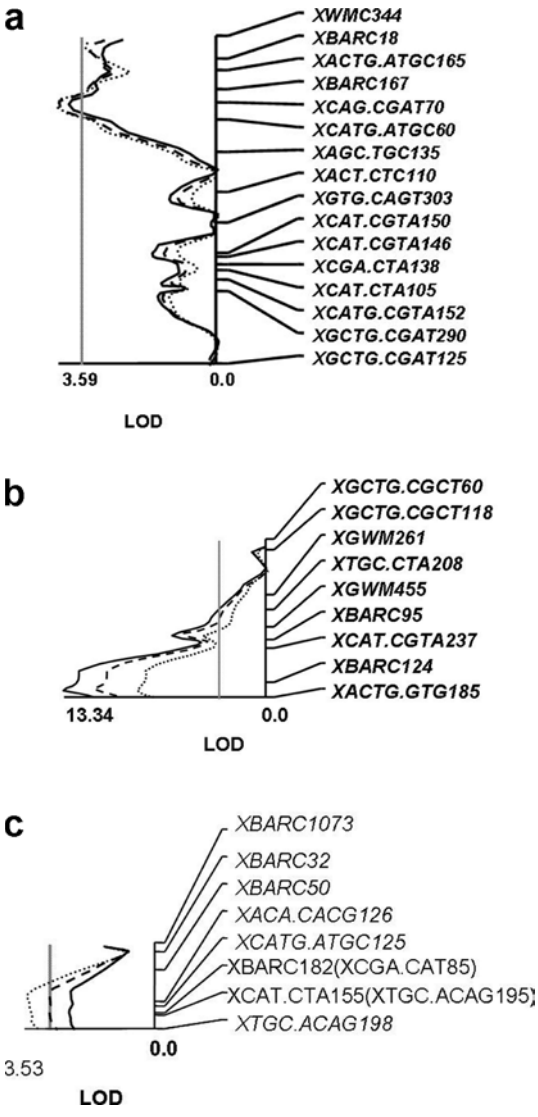

Fig. 2 Likelihood plots of three QTLs for latent period—*QLrlp.osu-2B* (**a**) *QLrlp.osu-2DS* (**b**) and *QLrlp.osu-7BL* (**c**). *Solid and dot curves* represent results from experiments conducted in 1988 and 1989, respectively, *slash curves* are based on data averaged across two experiments. The *vertical lines* represent the threshold LOD value of 3.0. Markers XCGA.CAT85 and XTGC.ACAG195 were mapped on the same locus as for XBARC182 and XCAT.CTA155, respectively

A second QTL was detected on chromosome 2B and designated as *QLrlp.osu-2B* (Fig. 2). This QTL appeared between AFLP XCAG.CGAT70 and XCATG.ATGC60 with a LOD value of 3.6 across 2 years. The peak LOD value was 1.0 cM away from XCAG.CGAT70 and 5.5 cM away from XCATG.ATGC60. This QTL was quite stable and explained 16.2% and 20.6% of the phenotypic and genetic variances, respectively, for MLP across 2 years. This QTL appears to be close to the centromere because the linked SSR markers, XBARC167, XBARC18, and XWMC344, have all been previously mapped on the proximal end of 2BS (Somers 2004).

The third QTL for latent period was localized on chromosome 7BL and designated as *QLr.osu-7BL* (Fig. 2) based on locations of four linked SSR markers which have all been previously mapped on 7BL; however, one of them, XBARC32, has also been mapped on 5BL (http://www.scabusa.org/pdfs/BARC_maps_011106.pdf). The peak of this QTL was placed between AFLP marker XCATG.ATGC125 and SSR marker XBARC182, with a LOD value of 2.4 for the phenotypic data collected in 1988. However, the peak of this QTL appeared between AFLP markers XACA.CACG126 and XCATG.ATGC125 with a LOD value of 3.6 for the 1989 data. Averaged across years, this QTL was mapped between AFLP marker XACA.CACG126 and SSR marker XBARC182 and explained 13.8% and 17.6% of the total phenotypic and genetic variance, respectively for MLP.

Three markers that flank *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* with the highest r^2 values in each linkage group were selected for multiple regression analysis (Table 3). A multi-collinearity test did not show significant correlations among them ($P = 0.17$–0.58). Thus, they were assumed to be independent. The respective partial r^2 values of *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* were 36.2%, 12.8%, and 9.0%. They collectively explained 58.0% and 73.8% of the total phenotypic and genetic variance, respectively.

Depending on the genetic materials evaluated, previous investigators have estimated that there are between two and five genes for long MLP (Bjarko et al. 1988b; Broers et al. 1989; Das et al. 1992; Lee et al. 1985a). Lee et al. (1985a) reported that three recessive genes conferred a long latent period of *P. triticina* in CI 13227, the

Table 2 Chromosome locations, marker intervals, QTL peak positions to the closest marker (QTL peaks), determination coefficients (r^2) and LOD values for *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* in CI 13227

QTL name	Chromosome	Year	Interval	QTL peak (cM)	r^2 (%)	LOD
QLrlp.osu-2DS	2DS	1988	XACTG.GTG185/XBARC124	2.0	48.2	13.4
		1989	XACTG.GTG185/XBARC124	2.0	34.6	8.7
		Mean	XACTG.GTG185/XBARC124	2.0	42.8	11.4
QLrlp.osu-2B	2B	1988	XCAG.CGAT70/XCATG.ATGC60	0.7	15.1	3.3
		1989	XCAG.CGAT70/XCATG.ATGC60	1.3	16.2	3.6
		Mean	XCAG.CGAT70/XCATG.ATGC60	1.0	16.2	3.6
QLrlp.osu-7BL	7BL	1988	XBARC182/XCATG.ATGC125	0.8	11.1	2.4
		1989	XACA.CACG126/XCATG.ATGC125	0.3	16.0	3.6
		Mean	XBARC182/XCATG.ATGC125	0.2	13.8	3.0

Table 3 Multiple regression of three QTLs on MLP in a RIL population derived from CI 13227 × Suwon 92 ($n = 98$ lines)

QTL	Marker	Mean			1988		1989	
		Chromosome	Partial r^2	Model r^2	Partial r^2	Model r^2	Partial r^2	Model r^2
QLrlp.osu-2DS	XACTG.GTG185	2DS	36.2	36.2	40.6	40.6	29.5	29.5
QLrlp.osu-2B	XCAG.CGAT70	2B	12.8	49.0	11.8	52.4	13.9	43.4
QLrlp.osu-7BL	XACA.CACG126	7BL	9.0	58.0	7.1	59.5	9.8	53.2

slow-rusting parent used in this study. Based on early-generation analysis of a cross between CI 13227 and Suwon 92, Shaner et al. (1997) estimated that two to five genes were responsible for long latent period. They further analyzed latent period segregation among RILs derived from the same cross and concluded that four loci with epistatic effects conferred long MLP and that one of the loci exerted a major effect (Shaner et al. 1997). Our study confirmed the major QTL segregating in the same population, which we believe to be *QLrlp.osu-2DS*. Though the three QTLs for MLP identified in this study explained a major portion of genetic variation for this trait, about one-fourth of the genetic variation still remains unexplained. Other minor QTLs or modifiers for long MLP may also be involved in this population. A larger population size may be needed to detect them. Therefore, long latent period in CI 13227 may be conditioned by at least three QTLs with one having a major effect.

Future MAS for long latent period

Slow leaf-rusting resistance represents a race-nonspecific form of resistance that can reduce disease losses by retarding the onset of disease epidemics. Slow rusting consists of several major components, among which latent period is regarded as a more stable parameter when plants are inoculated under controlled greenhouse conditions (Shaner et al. 1997). Long latent period may effectively retard the disease development (Knott 1989). Therefore, introgressing the QTLs identified in this study into commercial cultivars or pyramiding them with other known slow leaf-rusting resistance genes, such as *Lr*34 and *Lr*46, has agronomic importance. However, measuring the latent period in large breeding populations is a daunting task. MAS for long latent period may simplify the selection process and improve the selection efficiency. In this study, we identified molecular markers closely linked to *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL*, which provide new tools for screening progenies with long latent period in breeding programs. With these molecular markers, the genotype of each plant can be easily determined without time-consuming inoculation and complicated measurements. The 14 RILs containing all longer latent period alleles at each marker locus closely linked to *QLrlp.osu-2DS*, *QLrlp.osu-2B* and *QLrlp.osu-7BL* (XACTG.GTG185, XCAG.CGAT70, and XACA.CACG126) had an average MLP of 12.5 days (Table 4), which is similar to that of CI 13227 (13.4 days). In contrast, the 13 RILs lacking a marker allele for long MLP at the same loci had an average MLP of 7.4 days, the same as that of Suwon 92 (7.4 days). These results again suggest that the three QTLs are responsible for long MLP in CI 13227 and explain most of the genetic variation for latent period. Therefore, selection for long latent period based on these markers should be effective.

Direct application of AFLP markers in MAS still presents procedural barriers. More recently, AFLPs have been transformed into sequence-tagged-site (STS) markers which can be directly used in MAS (Shan et al. 1999). We recommend transforming these closely linked AFLPs into STS markers for MAS. An alternative is to use SSR markers linked to *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* as selectable markers for the corresponding long MLP QTLs. For example, marker

Table 4 MLP with minimum and maximum values for genotypes with different allelic combinations of QTLs in a RIL population derived from CI 13227 × Suwon 92

Genotype[a]	Year	Mean (*d*)	Minimum-maximum
Q1Q1Q2Q2Q3Q3	1988	12.7 ± 0.8	10.8–15.2
	1989	12.2 ± 0.7	10.6–14.3
	Mean	12.5 ± 0.7	10.7–14.8
Q1Q1Q2Q2q3q3	1988	10.1 ± 0.8	9.1–11.3
	1989	9.7 ± 1.1	8.5–11.3
	Mean	9.9 ± 0.9	8.8–11.3
Q1Q1q2q2Q3Q3	1988	11.5 ± 1.4	10.7–12.4
	1989	11.2 ± 1.4	9.9–11.8
	Mean	11.3 ± 1.3	10.3–12.0
Q1Q1q2q2q3q3	1988	9.5 ± 0.6	8.3–11.3
	1989	9.0 ± 0.6	8.0–11.1
	Mean	9.2 ± 0.6	8.4–11.2
q1q1Q2Q2Q3Q3	1988	8.7 ± 0.8	7.2–12.4
	1989	8.9 ± 1.0	7.4–13.2
	Mean	8.8 ± 0.9	7.3–12.8
q1q1Q2Q2q3q3	1988	8.7 ± 1.1	7.6–12.6
	1989	8.5 ± 0.8	7.5–10.7
	Mean	8.6 ± 0.9	7.6–11.6
q1q1q2q2Q3Q3	1988	8.0 ± 0.4	7.2–9.0
	1989	8.0 ± 0.4	7.2–8.7
	Mean	8.0 ± 0.3	7.5–8.7
q1q1q2q2q3q3	1988	7.3 ± 0.2	6.8–8.1
	1989	7.4 ± 0.3	6.8–8.8
	Mean	7.4 ± 0.2	6.9–8.3

[a]Q1Q1, Q2Q2, Q3Q3 represent the CI 13227 allele at the XACTG.GTG185, XCAG.CGAT70, XACA.CACG126 locus, respectively; q1q1, q2q2, q3q3 represent the Suwon 92 allele at the XACTG.GTG185, XCAG.CGAT70, XACA.CACG126 locus, respectively

XBARC124 was 2.0 cM away from *QLrlp.osu-2DS* and explained 36.1% of the total variance of MLP. This marker can be used for MAS for this major QTL. In addition, XBARC18 and XBARC182 had r^2 values similar to those of XCAG.CGAT70 and XACA.-CACG126 for the other two QTLs (Table 2). Therefore, these three SSR markers can be used for MAS of long latent period from CI 13227.

Molecular markers associated with *Lr*34 and *Lr*46 have been identified (Nelson et al. 1997a; Schnurbusch et al. 2004; Suenaga et al. 2003; Williiam et al. 2003). Some of these are SSR markers that can be directly used in MAS for slow leaf-rusting resistance (Schnurbusch et al. 2004; Suenaga et al. 2003), and the others have the potential to be converted into PCR-based markers. These markers, together with those identified herein, can be used to develop durable leaf rust-resistant cultivars by pyramiding the QTLs for long latent period, *Lr*34, and *Lr*46.

Conclusions

Latent period is a reliable component of predicting slow leaf-rusting resistance under controlled conditions in the greenhouse (Shaner et al. 1997). We identified a major QTL on the distal region of chromosome 2DS, *QLrlp.osu-2DS*, which conferred long latent period and explained 54.4% of the genetic variance. Two other QTLs for long latent period (*QLrlp.osu-2B* and *QLrlp.osu-7B*) were also detected but had smaller effects. *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLrlp.osu-7BL* collectively explained 58% and 74% of the phenotypic and genetic variance, respectively. The introgression of *QLrlp.osu-2DS*, *QLrlp.osu-2B*, and *QLr.osu-7BL* into commercial cultivars bears considerable agronomic importance and with MAS appears feasible. The identified SSR markers closely linked to these QTLs—XBARC124, XBARC18 and XBARC182—can be directly used in MAS for developing cultivars with long latent period.

Acknowledgements We thank Perry Cregan and Qijian Song (Soybean Genomics and Improvement Laboratory, USDA, ARS, Beltsville Agricultural Research Center, Beltsville, Md., USA) for providing the BARC SSR primer sequences, Richard Ward (Department of Crop and Soil Science, Michigan State University, East Lansing, Mich., USA) and Bikram S. Gill (Department of Pathology, Kansas State University, Manhattan, Kan., USA) for providing mapping information for the BARC SSR markers, and Daryl Somers for providing primer and mapping information for the WMC SSR markers.

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