Theor Appl Genet (2005) 111: 731–735
DOI 10.1007/s00122-005-2058-9

ORIGINAL PAPER

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Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat

Received: 16 February 2005 / Accepted: 26 April 2005 / Published online: 18 June 2005

Abstract The incorporation of effective and durable disease resistance is an important breeding objective for wheat improvement. The leaf rust resistance gene *Lr34* and stripe rust resistance gene *Yr18* are effective at the adult plant stage and have provided moderate levels of durable resistance to leaf rust caused by *Puccinia triticina* Eriks. and to stripe rust caused by *Puccinia striiformis* Westend. f. sp. *tritici*. These genes have not been separated by recombination and map to chromosome 7DS in wheat. In a population of 110 F_7 lines derived from a Thatcher × Thatcher isogenic line with *Lr34/Yr18*, field resistance to leaf rust conferred by *Lr34* and to stripe rust resistance conferred by *Yr18* cosegregated with adult plant resistance to powdery mildew caused by *Blumeria graminis* (DC) EO Speer f. sp. *tritici*. *Lr34* and *Yr18* were previously shown to be associated with enhanced stem rust resistance and tolerance to barley yellow dwarf virus infection. This chromosomal region in wheat has now been linked with resistance to five different pathogens. The *Lr34/Yr18* phenotypes and associated powdery mildew resistance were mapped to a single locus flanked by microsatellite loci *Xgwm1220* and *Xgwm295* on chromosome 7DS.

Communicated by B. Keller

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Introduction

Leaf rust, stripe rust and stem rust are common diseases of wheat throughout the world (Roelfs et al. 1992). Rust resistance genes offer a cost-effective strategy to reduce losses in wheat from attack by rust pathogens. Of the many rust resistance genes that have been identified in wheat, most interact with specific races of the pathogen to confer resistance in a gene-for-gene manner (Person 1959). Although race-specific genes have provided highly effective resistance, they have also selected rust races with the corresponding virulence, resulting in cultivars losing effective resistance within a short period of time. Wheat breeders are increasingly focusing on the identification and incorporation of race non-specific resistance genes that may provide only partial resistance but when used in combination with other genes can condition highly effective resistance. Race non-specific resistance is often characterised by its long-term effectiveness, partial resistance phenotype and optimal expression at the adult plant stage (McIntosh et al. 1995).

In wheat, genes *Lr34* and *Yr18* have provided durable resistance to leaf rust (caused by *Puccinia triticina*) and stripe rust (*Puccinia striiformis*), respectively (Dyck et al. 1966; Singh and Rajaram 1992; Ma and Singh 1996). Both genes contribute a partial level of rust resistance in many wheat cultivars grown across the world. Using monosomic analysis, Dyck (1987) mapped the *Lr34* gene to the short arm of chromosome 7D. This location was later confirmed by mapping either one or both of these genes as quantitative trait loci (QTLs) within a genomic region delineated by molecular markers (Singh et al. 2000; Boukhatem et al. 2002; Ramburan et al. 2004; Schnurbusch et al. 2004a). McIntosh (1992) and Singh (1992a) showed that *Lr34* and *Yr18* are tightly linked. Subsequent experiments confirmed that these genes are completely associated and, to date, recombinant lines with *Lr34* and *Yr18* individually have not been produced (Singh 1992a).

Lr34/*Yr18* have subsequently been associated with other traits and disease resistance in wheat. Singh (1992b) reported tight linkage of leaf-tip necrosis (*Ltn*) in flag leaves with *Lr34* in several different lines. Leaf-tip necrosis, however, is influenced by environmental effects and genetic background and can be too variable to be considered a reliable marker. The *Lr34*/*Yr18* gene combination is also associated with tolerance to barley yellow dwarf virus (*Bdv1*), which was described as a "slow yellowing" response in adult plants similar to the partial resistance observed with *Lr34* and *Yr18* (Singh 1993). In other studies, *Lr34* was shown to both enhance the effectiveness of other leaf rust genes (German and Kolmer 1992) and permit the expression of resistance to certain stem rust races normally inhibited by a suppressor gene, thereby resulting in enhanced stem rust resistance in a backcross-derived line of Thatcher (Dyck 1987; Kerber and Aung 1999). Joshi et al. (2004) suggested that the *Lr34*/*Yr18* region might also be associated with resistance to spot blotch disease (*Bipolaris sorokiniana* (Sacc.) Shoemaker) on the basis of resistance showing linkage with leaf-tip necrosis.

In the study reported here, a recombinant inbred population that segregated for *Lr34* and *Yr18* also segregated for powdery mildew resistance at the adult plant stage. We showed cosegregation of powdery mildew resistance with the durable leaf and stripe rust resistance conferred by *Lr34* and *Yr18*, respectively. This region in wheat has now been associated with resistance to at least five different pathogens.

Materials and methods

Plant material

The resistant line RL6058 is a backcross-derived line of Thatcher (Thatcher*6/PI58548) and has genes for resistance to leaf rust (*Lr34*) and stripe rust (*Yr18*). The original donor line PI58548, held in the USDA Wheat Collection, was from China and was used by Dyck (1977) to develop RL6058. We generated 110 F_6 lines from the cross Thatcher/RL6058 (male) by single seed descent (SSD). F_7 rows together with parental lines Thatcher and RL6058 were grown in the field to evaluate disease reactions to leaf rust, stripe rust and powdery mildew. JupatecoR and JupatecoS are near-isogenic lines that differ for the presence and absence of the *Lr34*/*Yr18* genes, respectively (Singh 1992a).

Leaf rust and stripe rust pathotypes

The two experimental sites at Cobbitty near Sydney (Australia) are approximately 800 m apart. *Puccinia striiformis* f. sp. *tritici* pathotype 134 E16A+ and *P. triticina* pathotypes 104-1,2,3,6,7,11,13 and 104-1,2,3,6,7,9,11 were released in 2004 as the predominant pathotypes at both sites. At one site at Cobbitty, powdery mildew infections caused by *Blumeria graminisf*. sp. *tritici* developed fortuitously as this disease is a common occurrence in the irrigated field sites at Cobbitty. The mildew pathogen population was not characterised for virulence, but RL6058 and Thatcher showed clear, qualitative differences in mildew response at the late jointing/early booting stages when the rust responses were first scored.

Field scoring

The Thatcher/RL6058 population was grown at the two Cobbitty sites in 2004. At the late stem elongation/early booting stage, the plants at one site were infected primarily by stripe rust with some pustules of leaf rust near the leaf base. The subsequent level of stripe rust infection on Thatcher was clearly higher than that on RL6058 (trace resistant). Powdery mildew reactions were scored at the same time, and extensive mildew growth was found on the lower stem segments of Thatcher, while infections were reduced to small colonies or were absent entirely in RL6058. F_7 lines were rated according to the disease severity and response of the parental lines and were scored as either resistant or susceptible, with the exception of two lines that were scored as segregating for both stripe rust and mildew resistance. At the second site, leaf rust infections were heavy, and Thatcher was scored as 90S, with 90% its leaf area covered by large, susceptible-type leaf rust pustules, and RL6058 as trace resistant, with only a few small leaf rust pustules. Each of the F_7 lines was unambiguously classified and rated as one of the two parental responses. Differences in disease reactions to stripe rust, leaf rust and powdery mildew were qualitative between the parental lines and progeny, resulting in bi-modal distributions of resistant and susceptible lines. JupatecoR and JupatecoS lines were grown in 2003 at one site only as 1-m rows that became infected with powdery mildew during the early booting stage.

Genetic mapping

DNA extracted from the parental lines and 110 progeny was tested with three microsatellite markers (*Xgwm130*, *Xgwm295* and *Xgwm1220*) that previously showed linkage to *Lr34*/*Yr18* (Suenaga et al. 2003; Ramburan et al. 2004; Schnurbusch et al. 2004a). These markers were polymorphic between Thatcher and RL6058 and were mapped relative to leaf and stripe rust resistance, and resistance to powdery mildew. Recombination frequency was directly converted into genetic distance estimates without the use of a mapping function. Primer sequences and PCR protocols were as previously published for GWM130 and GWM295 (Röder et al. 1998), and those for GWM1220 were kindly provided by Dr. M. Ganal, TraitGenetics Germany.

Results and discussion

Disease assessment

Recombinant inbred lines from Thatcher/RL6058 were scored for leaf rust and stripe rust severity and responses at the adult plant stage in the two field plots at Cobbitty. Thatcher was susceptible to leaf rust and stripe rust, while RL6058 had very low leaf and stripe rust severity due to the presence of *Lr34/Yr18*. The F_7 rows at two sites were separately classified for responses to both leaf and stripe rust. The near-isogenic background of the parents allowed for the unambiguous classification of the F_7 lines as either homozygous resistant or homozygous susceptible to both rust diseases and, as such, quantitative evaluation using area under disease progress curve was not required. Two lines were scored as segregating for resistance and susceptibility to both leaf and stripe rust. Resistance to leaf rust failed to recombine with resistance to stripe rust in this population of 110 F_7 lines, a result which confirmed those of earlier studies reporting tight linkage between *Lr34* and *Yr18* (Singh 1992a). Leaf-tip necrosis (*Ltn*), which was previously shown to be associated with the presence of *Lr34/Yr18*, was also observed in this population (Singh 1992b) (Fig. 1). However, the variable expression prevented us from reliably scoring *Ltn*.

Fig. 1 Flag leaves from RL6058 (*three leaves on left*) showing resistance reaction to leaf rust (*Puccinia triticina*) and leaf-tip necrosis, and flag leaves from Thatcher (*three leaves on right*) showing susceptible reaction to leaf rust and limited expression of leaf-tip necrosis

At the time of rust scoring, the F_7 population was also infected with powdery mildew. There was extensive mildew development on the lower stem segments of Thatcher, whereas little or no mildew was observed on RL6058. High levels of mildew infection were restricted to leaf- and stripe rust-susceptible F_7 lines. The two lines that segregated for leaf rust and stripe rust resistance also segregated for resistance to powdery mildew. The qualitative differences in disease reaction enabled us to group each F_7 line into either the homozygous resistant (HR), homozygous susceptible (HS) or heterozygous category. The segregation of leaf rust, stripe rust and powdery mildew resistance fit the expected ratio for one locus (observed: 57 HR:2 Seg:51 HS; experimental: 53:4:53; $\chi^2 = 1.38$, $df = 2$, $P > 0.5$) with a maximum genetic distance of 1.4 cM between loci (Hanson 1959). This result confirmed previously observed links between resistance to powdery mildew and rust resistance conferred by *Lr34/Yr18* (Singh et al. 2000). When near-isogenic lines JupatecoR (*Lr34/Yr18*) and JupatecoS were scored for stripe rust resistance in the field at Cobbitty, the line JupatecoR was resistant to stripe rust and powdery mildew, and JupatecoS was susceptible to both diseases. Singh et al. (2000) also reported that quantitative resistance to leaf rust and stripe rust conferred by *Lr34/Yr18* was associated with powdery mildew resistance in the ITMI Synthetic/Opata recombinant inbred lines. It remains to be shown whether this powdery mildew resistance also confers broad-spectrum resistance to *B. graminis tritici* populations.

Mapping of disease resistance genes

Both genes *Lr34* and *Yr18* were previously mapped to chromosome 7DS and positioned within confidence intervals delineated by molecular markers (Singh et al. 2000; Suenaga et al. 2003; Schnurbusch et al. 2004b). In the present study, *Lr34* and *Yr18* mapped to a single locus flanked by microsatellite markers *Xgwm295* and *Xgwm1220* on chromosome 7DS. Both genes were separated by two recombinants on the distal side from *Xgwm1220* (0.9 cM) and by six recombinants on the proximal side from *Xgwm295* (2.7 cM) (Fig. 2). Adult plant resistance to powdery mildew cosegregated with both rust resistance genes. The single locus for *Lr34/Yr18* is coincident with previously reported confidence intervals for QTLs associated with quantitative resistance to leaf rust and stripe rust (Fig. 2).

Results obtained from several studies indicate that the genomic region linked to microsatellite markers *Xgwm1220* and *Xgwm295* on chromosome 7DS confers:

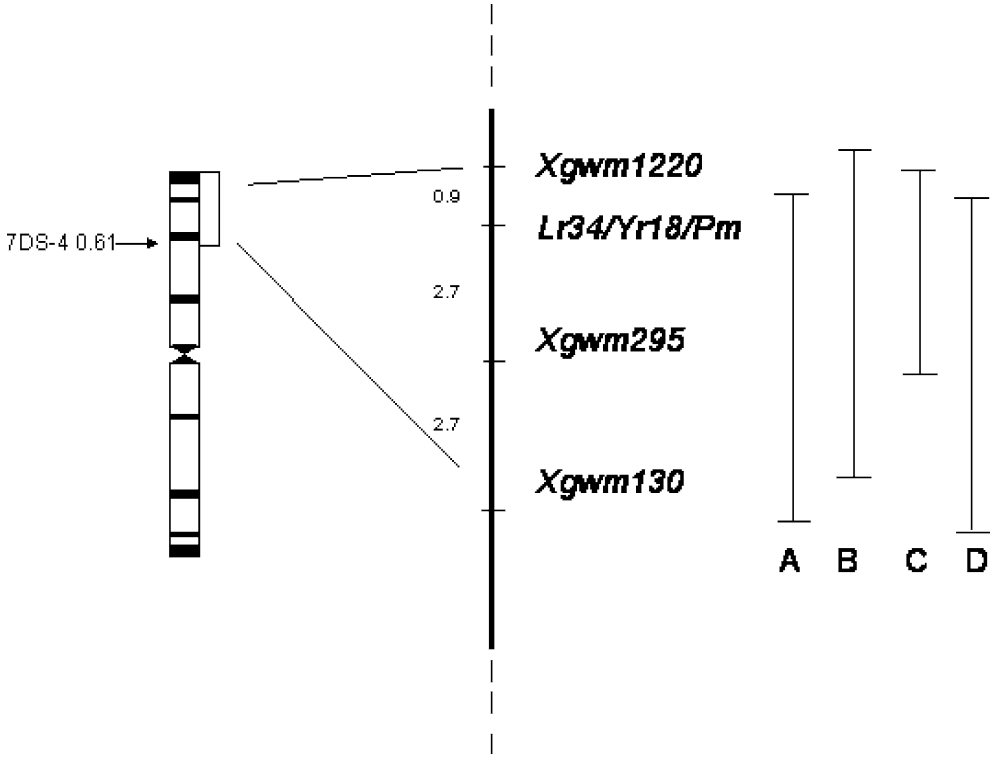

Fig. 2 Physical map of chromosome 7D in wheat showing markers that are flanking *Lr34/Yr18* and contained within the deletion bin 7DS-4 which consists of approximately 39% of the short arm of chromosome 7D (*on the left*). The genetic map of the *Lr34/Yr18* region shows microsatellite markers that flank the resistance genes. *Numbers* on the *left hand side* denote genetic distance in centiMorgans. Resistance to powdery mildew (*Pm*) cosegregated with leaf rust and stripe rust resistance. *A–D* denote confidence intervals for QTLs for rust resistance previously assigned to this region: *A* QTL for leaf rust and stripe rust identified by Suenaga et al. (2003), *B* QTL for leaf rust *QLr.str-7DS* described by Schnurbusch et al. (2004), *C* QTL for leaf rust *QLrP.sfr-7DS* identified by Schnurbusch et al. (2004), *D* QTL for stripe rust *QYr.sgi-7D* identified by Ramburan et al. (2004)

– durable, adult plant resistance to leaf rust (*Lr34*) (Singh and Gupta 1991);
– durable, adult plant resistance to stripe rust (*Yr18*) (McIntosh 1992; Singh 1992a);
– adult plant resistance to powdery mildew (this study);
– tolerance to barley yellow dwarf virus (*Bdv1*) (Singh 1993);
– enhanced expression of stem rust resistance (Dyck 1987);
– leaf-tip necrosis of flag leaves (*Ltn*) (Singh 1992b).

With the exception of *Bdv1*, all of the above traits were evaluated using line RL6058, thereby avoiding possible complications of different alleles being assessed in different wheat backgrounds. The association of broad-spectrum rust resistance with leaf-tip necrosis appears analogous to that of necrotic lesions and accelerated leaf senescence with broad-spectrum resistance to powdery mildew (*B. graminis* (DC) E.O. Speer f. sp. *tritici*) mediated by the *mlo* gene in barley (Jørgensen 1992). In barley, these undesirable pleiotropic effects can reduce grain yield. Leaf-tip necrosis is also associated with reduced grain yield in wheat (Singh and Huerta-Espino 1997). The severity of necrosis seen in the Thatcher background suggests that *Ltn* can reduce the leaf area significantly (Fig. 1). All resistance specificities located within this 7DS region are expressed during the adult plant stage, while at least some are associated with a slow increase of disease infections. Rust resistance genes *Lr34* and *Yr18* and possibly the gene for barley yellow dwarf virus tolerance (*Bdv1*) have played an important role in providing durable disease resistance in a wide range of CIMMYT-generated spring wheats and also in many wheats in the USA and Canada (Kolmer 1996). Quantitative leaf rust resistance has also been mapped in European winter wheat to the *Lr34/Yr18* region on chromosome 7DS, indicating that these resistance genes may also be prevalent in the European winter wheat gene pool (Messmer et al. 2000; Schnurbusch et al. 2004a). The present study links the genomic region of *Lr34/Yr18* with adult plant resistance to powdery mildew further adding to the value of this region as a source of valuable and durable disease resistance in wheat (Powdery mildew resistance has not yet been shown to be durable). Although *Lr34* and *Yr18* are present in wheats grown worldwide, a reliable PCR-based marker has not been developed for marker-assisted selection. It is still not known whether some or all of these traits are controlled by a single gene or by several tightly linked resistance genes. We are in the process of developing a diagnostic PCR assay for these genes and addressing the question of single versus multiple genes by isolating genes conferring disease

resistance from this region. Characterisation of near isogenic lines, which has enabled the easy classification of progeny into either resistant or susceptible classes and, hence, the mapping of resistance genes to a single locus, will facilitate the isolation of candidate genes from this important genomic region in wheat.

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