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Molecular mapping of quantitative trait loci determining resistance to septoria tritici blotch caused by *Mycosphaerella graminicola* in wheat

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Summary

A set of 65 recombinant inbred lines of the 'International Triticeae Mapping Initiative' mapping population ('W7984' × 'Opata 85') was analysed for resistance to septoria tritici blotch at the seedling and adult plant stages. The mapping population was inoculated with two Argentinean isolates (IPO 92067 and IPO 93014). At the seedling stage, three loci were discovered on the short arms of chromosomes 1D, 2D and 6B. All three loci were detected with both isolates. At the adult plant stage, two isolate-specific QTL were found. The loci specific for isolates IPO 92067 and IPO 93014 were mapped on the long arms of chromosomes 3D and 7B, respectively.

Introduction

Septoria tritici blotch caused by *Mycosphaerella graminicola* (Fuckel) Schroeter in Cohn (anamorph *Septoria tritici* Rob. ex Desm.) is an important disease in many wheat-producing areas of the world, and causes significant yield losses (Eyal, 1981; Eyal et al., 1987; Scharen, 1999). For the management of the disease, genetic resistance is the most cost-effective and environmentally appropriate technique. Because only a few currently available varieties have adequate levels of resistance, new sources of resistance are required.

Resistance to septoria tritici blotch conditioned by one or two genes was found in some materials (Narvaez & Caldwell, 1957; Rillo & Caldwell, 1966; Rosielle & Brown, 1979; Lee & Gough, 1984; Brading et al., 1999), whereas in some others at least three resistance genes were reported (Rosielle & Brown, 1979). Although most investigations have concentrated on the analysis of complete resistance, quantitative resistance has been found in different genotypes (Jlibene et al., 1994; Brown et al., 2001; Simón et al., 2001), and most commercial cultivars range from moderately resistant to susceptible, indicating that minor gene effects are also present. Whereas complete resistance is interesting because of the almost total absence of symptoms in the host, quantitative resistance is very important since it may be more durable (Parlevliet, 1983).

In quantitative analyses, additive gene effects contribute more to resistance than dominance effects. However, significant non-additive effects were often identified (van Ginkel & Scharen, 1987; Bruno & Nelson, 1990; Danon & Eyal, 1990; Jonsson, 1991; Jlibene et al., 1994; Simón & Cordo, 1997, 1998). While heritabilities tend to be only moderate (Simón et al., 1998), progress in breeding for resistance is possible.

Eight major genes (*Stb1* to *Stb8*) for resistance to *M. graminicola* have been described and mapped by using different types of molecular markers (Goodwin & Adhikari, 2003). The genes *Stb4* (Somasco et al., 1996), *Stb5* (Arraiano et al., 2001), *Stb6* (Brading et al., 2002), *Stb7* (McCartney, 2002) and *Stb8* (Adhikari et al., 2003) have been identified using single pathogen

isolates. Quantitative trait loci (QTL) were identified in some materials. Eriksen et al. (2001) found QTL on chromosomes 2D and 3A that originated from the resistant cultivar Senat.

Some synthetic hexaploid wheats, created by hybridising tetraploid wheats with *Aegilops tauschii*, have shown high resistance levels to *M. graminicola* (Gilchrist et al., 1999; Arraiano et al., 2001; Simón et al., 2001). Those high levels of resistance present in the synthetic hexaploids were successfully incorporated into susceptible bread wheat advanced lines (Gilchrist et al., 1999).

A mapping population obtained from the cross between a synthetic hexaploid wheat (*Aegilops tauschii* × *Triticum durum* variety 'Altar 84') with the spring wheat variety 'Opata 85' was used for mapping a gene conferring resistance to *M. graminicola* by Adhikari et al. (2003). The authors were able to map a major gene designated *Stb8* on chromosome 7B using a field isolate selected in Lafayette, U.S.A. In the present study, we investigated recombinant inbred lines (RILs) of the same mapping population, infected at the seedling and adult plant stages using two aggressive Argentinean isolates of *M. graminicola*.

Materials and methods

Plant materials

A selection of 65 RILs from the ITMI (International Triticeae Mapping Initiative) mapping population created by crossing the synthetic hexaploid wheat 'W7984' with the spring wheat cultivar 'Opata 85' was examined. 'W7984' was generated via a cross of the *Triticum tauschii* accession 'CIGM86.940' (DD) with the tetraploid wheat 'Altar 84' (AABB).

Seedling stage examination

The experiment was performed at the Facultad de Ciencias Agrarias y Forestales, La Plata, Argentina. Seeds were germinated in petri dishes and vernalised for 4 weeks at 4–8 °C. On 8 July, 2002 the seedlings were planted outdoors in 10 l pots in a randomised block design with three replications for each of the two isolates. Eight to ten seedlings were grown in each pot. Pots were fertilised with 50 kg ha^{-1} N as urea and 50 kg ha^{-1} P as ammonium diphosphate at sowing and with 50 kg ha^{-1} N as urea at tillering.

Plants were inoculated with two Argentinean isolates designated IPO 92067 and IPO 93014 (named by the former IPO-DLO, The Netherlands). The isolates were selected according to a previous screening of parents in the seedling stage, which showed high levels of resistance in 'W7984' and moderate susceptibility in 'Opata 85'. The isolates were grown on petri dishes of potato dextrose agar and transferred to malt extract agar. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending the conidia in deionized water. The conidial suspension was adjusted to 3×10^6 spores ml^{-1}. Plants were inoculated at the 1-leaf stage. After inoculation, the experiment was covered with transparent plastic to maintain wet conditions for 48 h. After that plants were grown at a mean temperature of 9.6 °C and a mean relative humidity of 89%. The plants were irrigated regularly. Pycnidial coverage (%) on the first leaf of each plant was recorded 26 days after inoculation.

Adult plant stage examination

The plant material was inoculated with the same isolates at the tillering stage (GS 21, Zadoks et al., 1974). After inoculation the conditions were similar to the seedling stage experiments for the first 48 h. From inoculation to evaluations the mean temperature was 12.2 °C and the mean relative humidity 91%. As for the seedling stage, the plant was irrigated regularly. Pycnidial coverage (%) on the three upper leaves of each plant was scored, when each line reached the growth stages of flowering (GS 65), early milk stage (GS 73) and dough stage (GS 83). Averages of the three leaves were calculated for each growth stage. The area under the disease progress curve (AUDPC) was also calculated using the data of the three observations in the adult stage. Plant height (cm) and days to flowering were recorded.

Because of the different flowering dates and plant heights of the RILs, a multiple linear regression analysis of days to heading and height as independent variables on pycnidial coverage as dependent variable for each growth stage and for the AUDPC was performed to correct data, according to these models. Neither the regression models nor any of the independent variables were significant, thus, the real values were analysed.

QTL mapping

The RFLP map was created by C. Nelson, Cornell University, Ithaca, U.S.A. QTL analysis was performed using the programme QGENE (Nelson, 1997). Single marker regression mapping was applied.

Results

Analysing the disease scores, no clear cut deviations allowing a qualitative approach were detected either at the seedling or adult plant stage for the two isolates used. The distributions together with the parental means obtained for the race IPO 92067 are given in Figure 1.

In the mapping analysis, QTL with LOD scores $\geq$3.0, and with LOD $\geq$ 2.0 but <3.0 were considered. In regions where such loci were detected, we also searched for QTL with LOD $\geq$ 1.5 but <2.0.

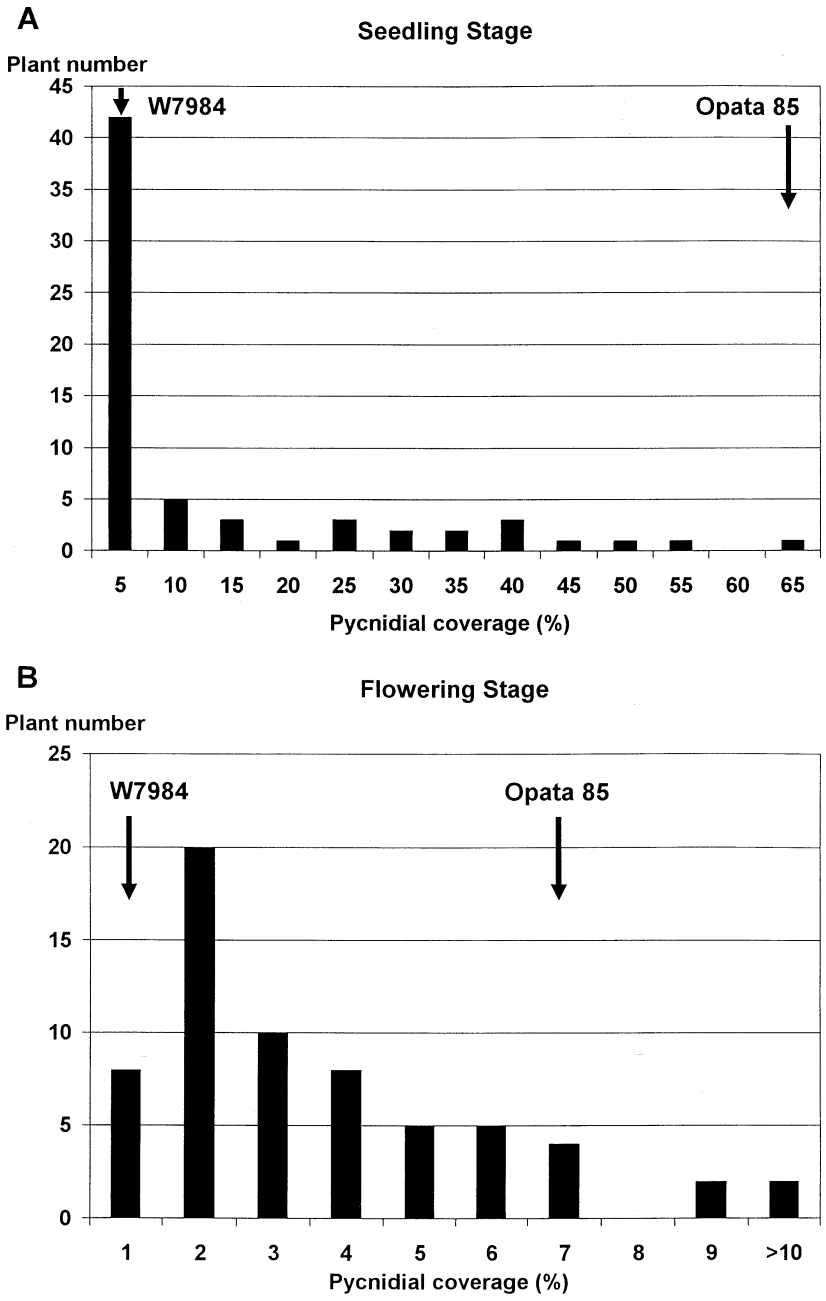

Figure 1. Phenotypic distribution of RILs innoculated with isolate IPO 92067 for the traits pycnidial coverage at (A) seedling stage, (B) flowering stage, (C) early milk stage and the character (D) area under the disease progress curve (AUDPC). The parental means are marked by arrows.
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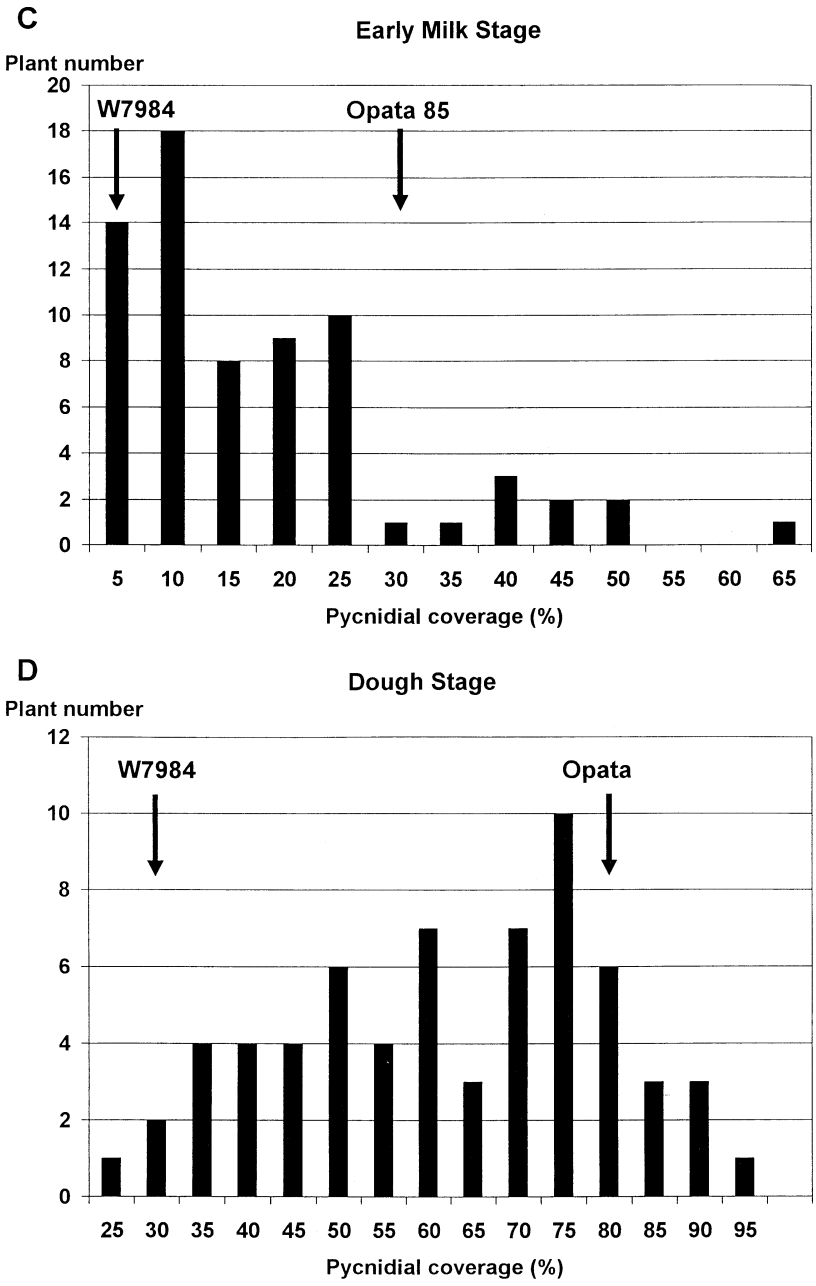

Figure 1. (*Continued*)

Investigating the RILs at the seedling stage, three loci were discovered on the short arms of chromosomes 1D, 2D and 6B, respectively (Figure 2A). Identical loci were detected with the two isolates, although IPO 92067 gave QTL with higher LOD scores. The QTL accounted for between 18% and 25% (IPO 93014) and between 10% and 16% (IPO 92067) of the variation in response, respectively. Resistance was contributed by the synthetic wheat 'W7984' (chromosomes 1D and 6B) and also by 'Opata 85' (chromosome 2D).

At the adult plant stage two loci were found on the long arms of chromosomes 3D and 7B, respectively (Figure 2B). The resistance at both loci originated from 'W7984'. Both loci were isolate specific. The IPO 92067-specific locus *QStb.ipk-3D* was detected by scoring the RILs at the early milk stage, at

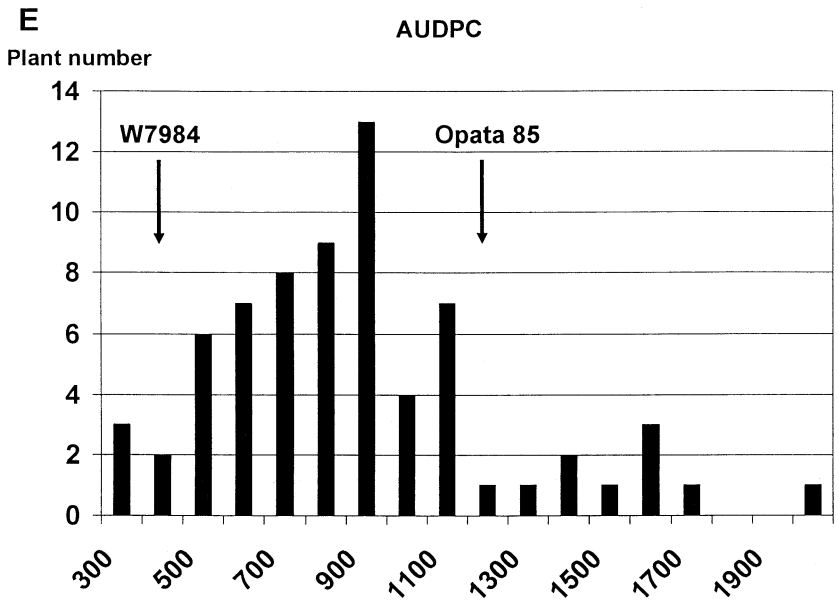

Figure 1. (*Continued*)

the dough stage, and by analysing the AUDPC values. *QStb.ipk-7B* was found by analysing the scores of all three growth stages and AUDPC. The loci accounted for 16–21% of the variation in response.

Discussion

Information on genetic analysis of resistance to *Mycosphaerella graminicola* is scarce and in several experiments natural infection instead of specific isolates was used. The use of single isolates enables breeders to distinguish isolate-specific and non-specific resistance in the host (Parlevliet, 1983). In addition, the identity of resistance genes can be confirmed when specific isolates are used. Eight genes for resistance have been identified and mapped on seven different chromosomes including 5BL (*Stb1*), 3BS (*Stb2*), 6DS (*Stb3*), 7DS (*Stb4, Stb5*), 3AS (*Stb6*), 4AL (*Stb7*) and 7BL (*Stb8*) as summarised by Goodwin & Adhikari (2003). Eriksen et al. (2001) described two QTL on chromosomes 2D and 3A and a major gene for resistance on 3A in cultivar 'Senat'.

It has been shown that markers for QTL can be used to increase the effectiveness of selection for quantitative traits over that of phenotypic selection (Charmet et al., 1999). Molecular markers can increase selection efficiency and allow selection for resistance in years with unfavourable conditions for disease development, avoiding the need for inoculations.

It is not clear if the same QTL are expressed at all stages of plant development. Cultivars with good quantitative resistance in the adult stage and susceptibility in seedlings or vice versa have been found (Arama, 1996; Kema & van Silfhout, 1997; Kema et al., 1999; Simón & Cordo, 1999). Our results showed that resistance in seedling and adult stages in the ITMI cross was determined by different QTL. However, the same QTL were detected at different adult growth stages and with the AUDPC.

Quantitative resistance is often considered synonymous with horizontal, or race non-specific, resistance (van der Plank, 1968). Nevertheless, for some pathosystems there are reports of race-specific quantitative resistance (Leonards-Schippers et al., 1994; Caranta et al., 1997). In the present study, identical QTL for seedling resistance were found for both isolates. More isolates should be used to test, whether race non-specific seedling resistance is present in these materials. At the adult stage different QTL were detected for both isolates, indicating race-specific resistance.

The presence of resistance in synthetic wheats is interesting. Synthetics are relatively easy to cross with common wheats and their resistance can be introgressed into agronomically acceptable genotypes and combined with other resistances. Various accessions of *Aegilops tauschii,* the donor of the D genome in synthetic wheats, carry genes for resistance to leaf rust, powdery mildew, greenbug, Russian wheat aphid,

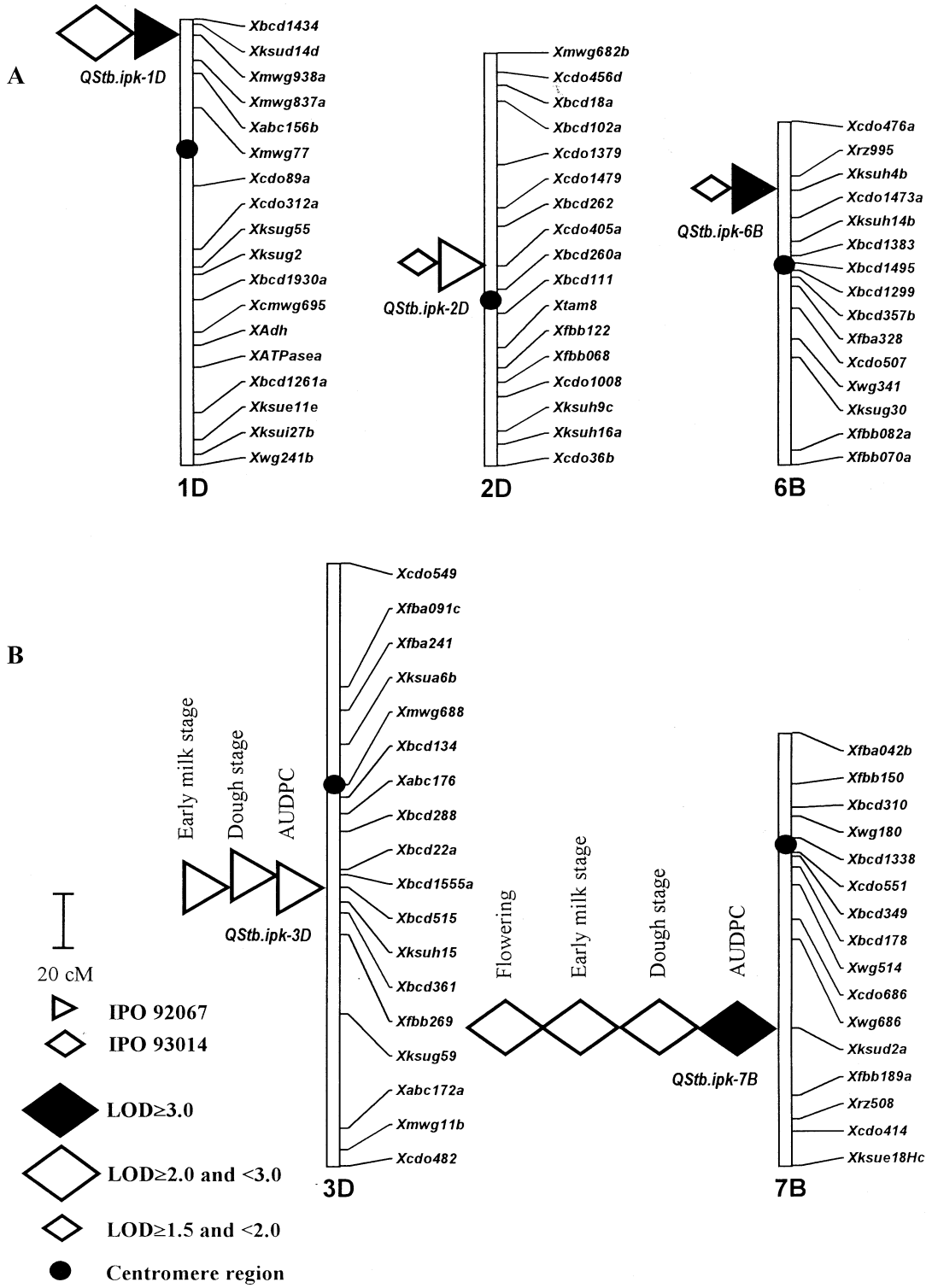

Figure 2. Linkage maps of chromosomes 1D, 2D, 6B, 3D and 7B showing the location of QTL determining resistance to septoria tritici blotch at (A) seedling stage and (B) adult plant stage.

Hessian fly, soil borne mosaic virus or stagonospora nodorum blotch (Kerber & Dyck, 1969; Gill et al., 1986; Cox et al., 1992; Murphy et al., 2000; Smith et al., 2000; May & Lagudah, 1992). The synthetic wheat 'W7984' used in our study was shown not only to carry QTL for mildew resistance (Börner et al., 2002) but also a major gene (*Stb8*) conferring resistance against septoria tritici blotch (Adhikari et al., 2003). The map position of *Stb8*, detected after inoculating plants at the adult stage, is comparable with that found for *QStb.ipk-7B*, discovered in the present study by a different isolate. Both are located on chromosome 7BL and may represent the same locus. However, it is demonstrated that 'W7894' carries further resistance genes at loci that have not been reported.

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