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ORIGINAL PAPER

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Microsatellite markers associated with two *Aegilops tauschii*-derived greenbug resistance loci in wheat

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Abstract A new source of greenbug (*Schizaphis graminum* Rondani) resistance derived from *Aegilops tauschii* (Coss.) Schmal was identified in W7984, a synthetic hexaploid wheat line and one parent of the International Triticeae Mapping Initiative (ITMI) mapping population. Segregation analysis of responses to greenbug feeding in a set of recombinant inbred lines (RILs) identified a single, dominant gene governing the greenbug resistance in W7984, which was placed in chromosome arm 7DL by linkage analysis with molecular markers in the ITMI population. Allelism tests based on the segregation of responses to greenbug feeding in F_2 and testcross plants revealed that the greenbug resistance in W7984 and Largo, another synthetic line carrying the greenbug resistance gene *Gb3*, was controlled by different but linked loci. Using the ITMI reference map and a target mapping strategy, we have constructed a microsatellite map of *Gb3* in a mapping population of 130 F_7 RILs from Largo × TAM 107 and identified one marker (*Xwmc634*) co-segregating with *Gb3* and four markers (*Xbarc76*, *Xgwm037*, *Xgwm428* and *Xwmc824*) closely linked with *Gb3*. Deletion mapping of selected microsatellite markers flanking the *Gb3* locus placed this resistance gene into the distal 18% region of 7DL. Comparative mapping in the ITMI and Largo × TAM 107 populations using the same set of microsatellite markers provided further evidence that greenbug resistance in W7984 and Largo is conditioned by two different loci. We suggest that the greenbug resistance gene in W7984 be designated *Gb7*. The microsatellite map of *Gb3* constructed from this study should be a valuable tool for marker-assisted selection of *Gb3*-conferred greenbug resistance in wheat breeding.

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Introduction

The greenbug, *Schizaphis graminum* (Rondani), is an economically important aphid pest of small grain crops in many parts of the world. In the southern Plains of the U.S., annual losses due to greenbug damages have been estimated to be over $100 million in wheat alone (Webster and Kenkel 1999). For many years, the major method of controlling the annual occurrence and frequent outbreaks of the greenbug in this region has been the application of pesticides, which adds environmental concerns to the already considerable financial impact caused by this pest.

The greenbug resistance gene *Gb3* in wheat cv. TAM 110 (Lazar et al. 1997) and several of its derivatives are currently in use as a means to confer resistance to prevailing biotypes (E, I and K) in the field. Greenbug biotypes are genetically distinct populations (Porter et al. 1997) and, over the years, there has been a shift of prevailing biotypes from C to E and E to I in the fields of the southern Plains (Berzonsky et al. 2002). New greenbug strains being able to damage all known host resistance of wheat have been found (D. Porter, personal communication), which indicates that the *Gb3*-conferred resistance could be potentially overcome by newly emerging virulent greenbug biotypes. In order to have a means for gaining effective and sustainable control of the greenbug, it is necessary to explore new sources of greenbug resistance and to study the genetic and molecular mechanisms of host resistance against this aphid pest, which are poorly understood at present.

Aegilops tauschii (Coss.) Schmal, the D-genome donor of hexaploid wheat has been a rich source of biotic and abiotic stress resistance genes for wheat breeding programs in the past. Of the six greenbug resistance

genes that have been identified to date, two (*Gb3* and *Gb4*) originated from *Ae. tauschii* (Joppa et al. 1980; Martin et al. 1982). While a number of hexaploid synthetic wheat lines carrying *Ae. tauschii*-derived resistance to different greenbug biotypes have been identified (Lage et al. 2003; Smith and Starkey 2003), very few have been characterized. The greenbug resistance gene *Gb3* in the germplasm line Largo was recently mapped onto chromosome arm 7DL and associated with microsatellite and amplified fragment length polymorphism (AFLP) markers (Weng and Lazar 2002). Zhu et al. (2004) located another greenbug resistance locus from *Ae. tasuchii* accession TA1695 to a similar position, but the allelic relationships between the two resistance loci are not clear.

In previous germplasm screening tests, wheat synthetic line W7984 exhibited a high resistance to the prevailing greenbug biotypes. W7984 was one parent of the widely used International Triticeae Mapping Initiative (ITMI) mapping population (the other parent was Opata 85). Over 1,000 molecular markers have been mapped to the wheat genome using this ITMI population [all of the mapping data are available in the public domain (the GrainsGene database at http://wheat.pw.usda.gov/)], and genes or quantitative trait loci (QTLs) for several agronomically important traits identified in either W7984 or Opata 85 have also been mapped using these mapping data (for example, Nelson et al. 1995; Sourdille et al. 1996; Li et al. 1999; Singh et al. 2000). The ITMI map with high-density markers also serves as an important reference for mapping studies in other Triticeae populations, but it is not amenable to high-throughput uses in molecular breeding because it is primarily made up of restriction fragment length polymorphism (RFLP) markers (Somers et al. 2004).

One class of molecular marker—microsatellite or simple sequence repeats (SSRs)—has recently received much attention in genetic mapping and marker assisted-selection studies in wheat. The PCR-based, co-dominant microsatellite markers are highly polymorphic, often chromosome-specific (Röder et al. 1998), and preferentially associated with non-repetitive DNA in plant genomes (Morgante et al. 2002). These features make microsatellites the marker system of choice for gene tagging and marker-assisted molecular breeding in wheat. To date, more than 1,200 microsatellite markers have been developed and genetically mapped in the wheat genome (Somers et al. 2004), while more are being developed by data mining of the large numbers of expressed sequence tags (ESTs) of wheat (Holton et al. 2002; Gao et al. 2004).

There is a clear disparity in the wheat genome between the distribution of genes on the genetic map and that on the physical maps (Akhunov et al. 2003). Each chromosome arm can be divided into gene- or recombination-rich or -poor regions (Weng et al. 2000; Erayman et al. 2004). Genes residing in recombination-poor regions are not thought to be amenable to map-based cloning (Qi and Gill 2001). In wheat, the sub-arm location of a gene of interest can be determined by deletion mapping molecular markers flanking the target gene (Werner et al. 1992). In the investigation reported here we used the newly identified greenbug-resistant line W7984 and the well-characterized germplasm line Largo (Weng and Lazar 2002; Weng et al. 2004) to: (1) characterize greenbug resistance in W7984; (2) identify microsatellite markers closely linked with *Gb3* in Largo; (3) clarify the allelic relationship of the greenbug resistance loci in Largo and W7984; (4) locate *Gb3* to a deletion bin.

Materials and methods

Plant materials

All of the materials used in this investigation (greenbug screening tests, crosses, linkage analyses and deletion mapping) are listed in Table 1. Linkage analysis of the greenbug resistance loci using molecular markers was carried out in two mapping populations: (1) the ITMI population comprising 112 F_8 recombinant inbred lines (RILs) (lines 1–116, with the exception of line 61, which was not used, and lines 108, 109, and 115, which were not available); (2) 130 F_7 RILs derived from the Largo × TAM 107 cross, which was developed by the Texas Agricultural Experiment Station at Bushland, Texas, USA.

To determine the physical locations of microsatellite loci, we used one ditelosomic (DT7DS) and six nullitetrasomic (N7AT7B, N7AT7D, N7BT7A, N7BT7D, N7DT7A and N7DT7B) stocks of Chinese Spring for chromosome-arm assignment. Six 7DL deletion lines (7DL-1–6) (Endo and Gill 1996; Qi et al. 2002) were used to locate these markers on the sub-arm regions.

Allelism test

To examine the allelic relationships of the greenbug resistance loci in W7984 (*Gbx*) and Largo (*Gb3*), we developed an F_2 population from the cross W7984 × Largo. Three-way testcross (TC_1) populations were also developed using (W7984 × Largo) F_1s as the pollen donor and four greenbug biotype E-susceptible wheat cultivars, TAM 111, TAM 400, Cutter and Chinese Spring, as the female parents. Responses of the F_2 and TC_1 plants to biotype E greenbug feeding were examined. Since Chi square (χ^2) tests indicated that the two resistance loci in W7984 and Largo were linked, the genetic distance (in centiMorgans) of the two loci was estimated. Assuming that the recombination rate between two resistance loci was r, then $r = 2\sqrt{n/N}$ where n and N are, respectively, the observed number of susceptible plants and the total number of plants in an F_2 population. For TC_1, $r = 2\times(n/N)$.

Table 1 Plant materials used for aphid screening, genetic mapping and crosses

Materials (supplier's original entry no.)	Source[a]	Notes and references
W7984 (TA4152L3)	B.S. Gill	Synthetic parent of ITMI population
Opata 85 (TA2931)	B.S. Gill	Parent of ITMI population
Aegilops tauschii (TA1651)	B.S. Gill	D-genome donor of W7984
Altar 84 (TA3369)	B.S. Gill	Durum wheat parent of W7984
Largo (CI 17895)	D.R. Porter	Synthetic line carrying *Gb3* (Joppa and Williams 1982)
TAM 107	The authors	Cultivar with *Gb2* (Porter et al. 1987)
Ae. tauschii (PI268210)	D.R. Porter	*Gb3* donor (Joppa and Williams 1982)
TAM 105	The authors	Cultivar susceptible to all greenbug biotypes
TAM 110	The authors	Cultivar with *Gb2* and *Gb3* (Lazar et al. 1997)
TAM 400	The authors	Cultivar susceptible to greenbug biotype E
Cutter	The authors	Cultivar susceptible to greenbug biotype E
Chinese Spring (CS)	G.H. Hart	Cultivar susceptible to greenbug biotype E
7DL deletion lines	B.S. Gill	Six deletion lines (7DL-1–7DL-6)
ITMI RILs	C.O. Qualset	113 RILs (lines no. 1–116, with the exception of 108, 109 and 115, which were not available)
(Largo × T107) F_7 RILs	The authors	130 RIL lines segregating at the *Gb3* locus

[a]The affiliations of D.R. Porter, B.S. Gill and C.O. Qualset are provided in the Acknowledgements. G.H. Hart is affiliated with the Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, USA

Greenbug resistance evaluation

Biotype E greenbug colonies were reared in the growth chamber of an isolated room at the Texas Agricultural Experiment Station, Bushland, Texas, USA with continuous evaluation of responses on known resistant and susceptible wheat lines. All RILs, F_2 and TC_1 plants and their parental lines were screened with biotype E greenbugs to determine the phenotypes of their responses to aphid infestation. TAM 105, TAM 107 and TAM 110 were included in all infestation experiments as susceptible or resistant controls. The reactions of Largo and W7984 to greenbug feeding were tested using five additional greenbug biotypes (B, C, G, I and K) and the South Carolina strain, all of which were kindly provided by Dr. David Porter (USDA-ARS, Stillwater, Oklahoma, USA).

The procedure we used for greenbug resistance evaluation followed that of Weng and Lazar (2002) except that infestation was performed on three-leaf-stage seedlings. In addition, each plant was scored qualitatively as either resistant or susceptible when the susceptible controls were almost dead. For each RIL, parental or control lines, 15–20 seedlings were evaluated in each experiment. χ^2-tests for goodness-of-fit were used to test for deviations of the observed data from the theoretically expected segregation.

Mapping strategy

A target mapping strategy was taken to identify microsatellite markers closely linked with *Gb3*. Since the allelism test showed that *Gbx* was linked to *Gb3*, which has been mapped to chromosome 7DL in an earlier study (Weng and Lazar 2002), a linkage analysis of *Gbx* with RFLP markers in 7DL was conducted using the ITMI mapping data (from Nelson et al. 1995; Li et al. 1999; Mingeot and Jacquemin 1999). On the resulting map, *Gbx* was linked with several RFLP markers physically located in the distal one quarter of 7DL. After an examination of the available 7DL genetic and deletion maps (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Guyomarc'h et al. 2002; Shi et al. 2003; Somers et al. 2004; Sourdille et al. 2004), we chose all of the microsatellite markers that are supposedly located in the distal one-quarter region of 7DL for carrying out a genetic analysis in the Largo × TAM 107 mapping population. First, a microsatellite map of *Gb3* was constructed with 54 F_7 RILs. Then, markers within 5 cM of the *Gb3* locus on this map were analyzed in more detail in a larger population consisting of 130 F_7 RILs. In addition, in order to validate the allelism test result for *Gbx* and *Gb3*, we carried out comparative mapping in the first 60 RILs of the ITMI population using all of the microsatellite markers linked to *Gb3*.

Microsatellite analysis

Twenty-six microsatellite markers were selected from the distal one-quarter region of 7DL, including ten BARCs (BARC48, 53, 76, 97, 105, 111, 121, 172, 235, 1046), ten WMCs (WMC 4, 94, 150, 157, 166, 273, 634, 671, 797, 824), CFD69, CFD175, CFA2040, GDM150, GWM037 and GWM428. Information on the primer sequences of WMC634, WMC671, WMC797 and WMC824 was kindly provided by Dr. Daryl Somers (Cereal Research Centre, Winnipeg, Canada). All other primer sequence information was downloaded from the GrainsGene database.

Total genomic DNA was extracted from young leaf tissue frozen in liquid nitrogen as previously described (Weng et al. 2000). Each PCR contained 50–100 ng of template DNA, 0.5 μM of each of two primers, 1 U *Taq* DNA polymerase, 2.5 µl 10× PCR buffer, 1.5 m M $MgCl_2$, 0.2 m M dNTPs in a total volume of 25 µl, and the analyses were performed in a PTC-200 thermocycler

(MJ Research, Waltham, Mass.). A one-fit-all, touch-down PCR program was designed for all primer sets and consisted of (in the sequence presented): a 3-min initial denaturation at 95°C; six cycles of 45 s at 94°C, 5 min at 68°C, 1 min at 72°C, with the annealing temperature being reduced by 2°C per cycle; eight cycles of 45 s at 94°C, 2 min at 58°C, 1 min at 72°C, with the annealing temperature reduced by 1°C per cycle; a final 25 cycles of 45 s at 94°C, 2 min at 50°C and 1 min at 72°C.

The polymorphic bands between Largo and TAM 107 amplified from 12 of the 26 microsatellite primer sets could be resolved in a 2.5% agarose gel in a 1× TBE buffer. For the remaining 14 markers, PCR products were size-fractioned in denaturing polyacrylamide gels and the banding patterns visualized by silver staining. Polyacrylamide gel electrophoresis (PAGE) and silver staining procedures followed Weng and Lazar (2002) except that the running time per PAGE was shorter in the present study (1.5 h).

Deletion mapping

We investigated the banding patterns of three microsatellite markers flanking the *Gb3* locus in group-7 aneuploid stocks and 7DL deletion lines. Microsatellite loci were assigned to deletion bins based on the presence or absence of bands in specific deletion lines, as according to Weng et al. (2000).

Data analysis

The images of the microsatellite band patterns in ethidium bromide (EB)-stained agarose gels were recorded by instant films. Microsatellite profiles in silver-stained PAGE gels were reproduced electronically with a scanner or printed on an APC film (Promega, Madison, Wis.). Band patterns were scored manually. Linkage analysis of the resistance loci *Gb3* or *Gbx* with molecular markers was performed with MAPMAKER 3.0 (Lander et al. 1987) using a minimum LOD threshold of 3.0 and the Kosambi function (Kosambi 1944).

Results

Inheritance of greenbug resistance in W7984

On the basis of our germplasm screening tests for aphid resistance, W7984 was highly resistant to biotype E greenbug feeding. To identify the origin of this aphid resistance, we examined all of the parental lines involved in the development of the ITMI population with respect to their reactions to biotype E greenbug infestation and found that Opata 85 (another parent of the ITMI population) and Altar 84 (durum wheat parent of W7984) were both susceptible but that *Ae. tauschii* accession TA1651, the D-genome donor of W7984, was resistant. We therefore concluded that TA1651 was the original source of resistance to biotype E greenbugs in W7984. The responses of W7984 and Largo to an infestation of five additional greenbug biotypes (B, C, G, I and K) and the South Carolina strain were also compared, but no difference was found between the two lines.

We then looked at the inheritance mode of greenbug resistance in W7984 by examining the phenotypes of the responses of 112 ITMI F_8 RILs to biotype E greenbug feeding. Of the 112 ITMI F_8 RILs, 62 were homozygous-resistant, 49 were homozygous-susceptible and one was heterozygous-resistant, which suggested that greenbug resistance in W7984 is controlled by a single, dominant gene (test against the 1:1 expected ratio, $\chi^2 = 1.52$, $P = 0.22$).

Allelic relationships of greenbug resistance loci in W7984 and Largo

To clarify the allelism of the two greenbug resistance loci in W7984 (*Gbx*) and Largo (*Gb3*), we developed F_2 and TC_1 populations derived from the two lines and subsequently investigated the segregation of their phenotypic responses to greenbug feeding. The results are presented in Table 2. Segregation data were tested against the expected ratios of 15 resistant (R) to one susceptible (S) in the F_2 population and 3R:1S in TC_1 plants, assuming independent segregation of greenbug resistance in W7984 and Largo. Chi square tests indicated that greenbug resistance in the two lines is conditioned by different but linked loci. The estimated genetic distance between the two loci was 8.75 cM in the F_2 population and varied from 10.3 cM to 20.0 cM in the TC_1 population, depending on the female parent involved.

Genetic mapping of greenbug resistance locus *Gbx* in W7984 with molecular markers

Since we were able to determine that *Gbx* and *Gb3* are linked loci and given the fact that *Gb3* resides on 7DL (Weng and Lazar 2002), we carried out a linkage analysis of *Gbx* in the ITMI population using 19 RFLP markers that had previously been mapped to this arm and found that *Gbx* was indeed located on 7DL and linked with eight RFLP markers (map not shown). The nearest marker was *Xwg420* and was 2.1 cM proximal to *Gbx* (Fig. 1a). Physically, *Xwg420* is located in the distal one-quarter region of 7DL (Erayman et al. 2004).

Microsatellite map for greenbug resistance gene *Gb3* in Largo

Based on the map information of *Gbx* in 7DL and the allelic relations between *Gb3* and *Gbx*, we selected

Table 2 Segregation of responses to biotype E greenbug infestation in F_2 and testcross (TC_1) plants derived from W7984 and Largo (*R* resistant to biotype E greenbug feeding, *S* susceptible)

Materials	R:S ratio	Ratio tested	χ^2	*P*	Genetic distance (cM)
F_2 plants					
(W7984 × Largo) F_2	523R:1S	15:1	60.3	< 0.001	8.75
TC_1 plants					
CS × (W7984 × Largo) F_1	173R:15S	3:1	47.7	< 0.001	15.96
TAM 111 × (W7984 × Largo) F_1	55R:3S	3:1	12.16	< 0.001	10.34
TAM 400 × (W7984 × Largo) F_1	36R:4S	3:1	4.8	0.03	20.00
Cutter × (W7984 × Largo) F_1	125R:11S	3:1	14.1	< 0.001	16.18
TC_1 Sum	389R:33S	3:1	66.41	< 0.001	15.64

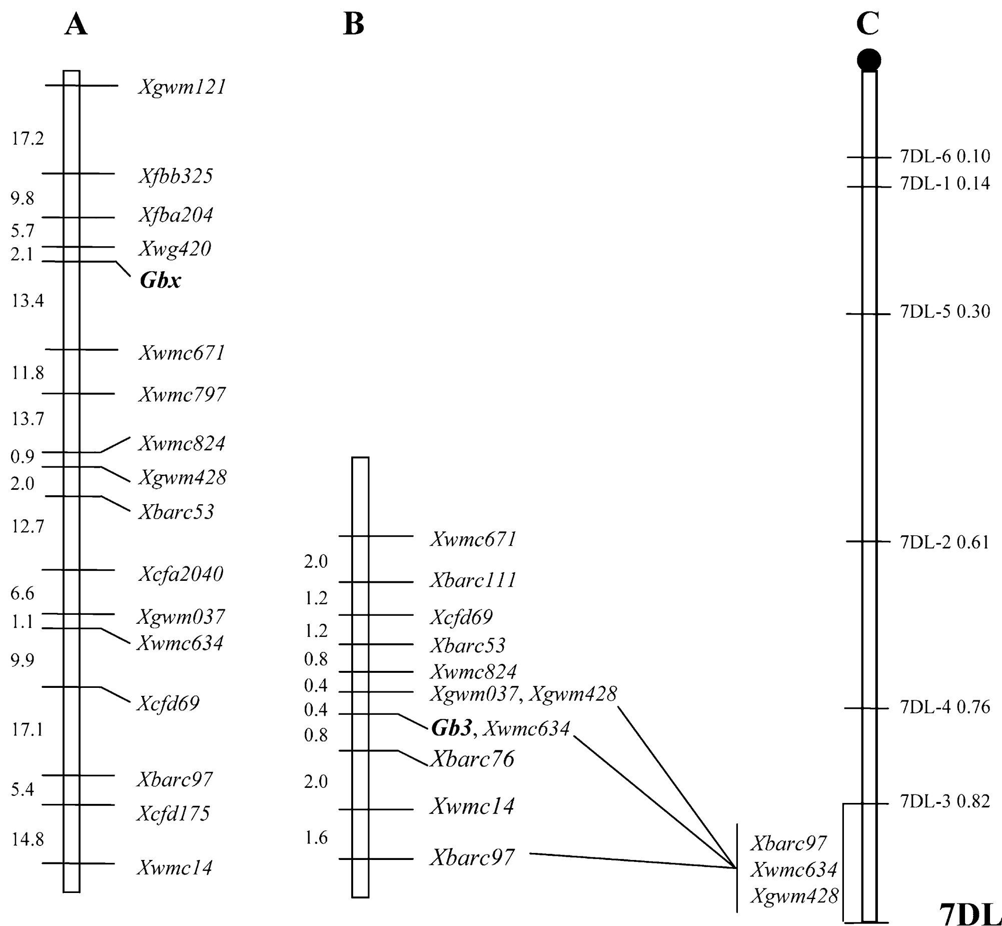

Fig. 1 Microsatellite linkage maps of chromosome 7DL for greenbug resistance genes *Gbx* in W7984 (**a**) and *Gb3* in Largo (**b**) and the deletion map of 7DL for selected microsatellite markers (**c**). The *bottom* of each linkage map represents the distal end of 7DL. *Vertical bars* designate chromosome arm 7DL and the *numbers to the left* of the chromosome arm are the genetic distances between adjacent markers in centiMorgans. On **c**, the *black circle* indicates the location of the centromere. The deletion-line (*DL*) breakpoint and the fraction length are indicated, respectively, by the *horizontal lines to the left* and the *numbers to the right* of each deletion-line symbol. The breakpoint positions are drawn appropriately to scale. *Solid lines* between **b** and **c** connect collinear loci

available microsatellite markers that mapped distal to *Xwg420* in 7DL for linkage analysis with *Gb3*. The mapping population consisted of 130 F_7 RILs derived from Largo × TAM 107. Among the 130 RILs, 72 were homozygous-resistant, 56 were homozygous-susceptible and two were heterozygous to biotype E greenbug feeding, indicating a normal segregation ratio at the *Gb3* locus (test against a 1:1 expected ratio, $\chi^2 = 2.0$, $P = 0.16$).

All but three (GDM150, BARC1046, WMC157) of the 26 microsatellite markers were polymorphic between Largo and TAM 107. WMC273 and CFA2040 detected two loci each. Genetic mapping of *Gb3* was conducted in two phases. First, all polymorphic markers were applied to a mapping population of 54 RILs. Of the 25 loci detected by 23 polymorphic microsatellites, 20 showed linkage with *Gb3* in this population (loci detected by BARC235, WMC166, WMC273 and one of the two loci detected by FBA2040 were not linked; map not shown), of which 11 were within 5 cM of *Gb3*. These 11 markers were analyzed in an additional 76 RILs, and the resulting genetic map is shown in Fig. 1b. It emerged that microsatellite marker *Xwmc634* was co-segregating with *Gb3* and that four flanking markers (*Xbarc76*, *Xgwm037*, *Xgwm428* and *Xwmc824*) were within 1 cM of *Gb3*.

Comparative mapping of *Gbx* in W7984

The allelism test had indicated that *Gbx* and *Gb3* are two different but linked loci. A comparative genetic mapping analysis of *Gbx* with microsatellite markers linked to *Gb3* validated this result. Because the ITMI mapping data for most of the *Gb3*-linked microsatellite markers (Fig. 1b) were not publicly available, we carried out an independent linkage analysis of these markers with *Gbx* using the first 60 RILs of the ITMI population. The linkage map of *Gbx* is shown in Fig. 1a. Three RFLP markers, *Xwg420*, *Xfba204* and *Xfbb325*, were also integrated into this map using the ITMI map data from the GrainGene database. Other RFLP markers were also linked to *Gbx* but were much too far away from *Gbx* in genetic distance and are therefore not included in Fig. 1a. By comparing Fig. 1a with Fig. 1b, it is obvious that *Gbx* and *Gb3* are different loci and that *Gbx* seems to be more proximal than *Gb3* in 7DL.

Physical location of *Gb3* in 7DL

DNAs from Chinese Spring, six group-7 nulli-tetrasomic line, ditelosomic 7DS and six 7DL deletion lines (7DL-1–6) were amplified with microsatellites BARC97, GWM428 and WMC634, which detected loci co-segregating with or flanking the *Gb3* locus (Fig. 1b). All three microsatellite markers were located in the distal bin delimited by deletion point 7DL-3 and the telomere (Fig. 1c). The deletion mapping profile for WMC634 is shown in Fig. 2.

Discussion

We have identified a new *Ae. tauschii*-derived greenbug resistance locus, *Gbx*, in the synthetic hexaploid wheat germplasm line W7984. Taking advantage of the availability of the ITMI genetic maps, we mapped *Gbx* to chromosome arm 7DL of wheat (Fig. 1a). Classical segregation analysis indicated that *Gb3* in Largo and

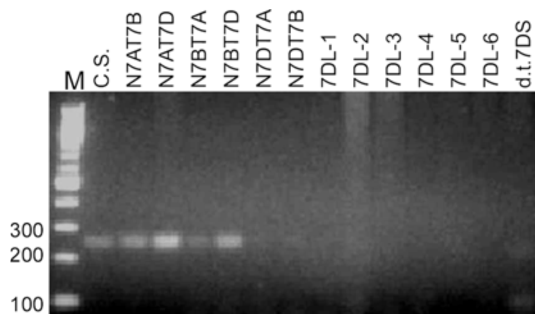

Fig. 2 Agarose gel profile of PCR products amplified by microsatellite marker WMC634 from Chinese Spring and its aneuploid and deletion stocks. *M* = Size marker, *CS* = Chinese Spring, *N* = nullisomic, *T* = tetrasomic, *d.t.* = ditelosomic. *Numbers to the left* of the marker lane are fragment sizes in basepairs

Gbx in W7984 are two linked loci (Table 2). Again using the ITMI linkage map as a reference, we chose microsatellite markers as a means to map *Gb3* genetically in Largo. Most (20/23) of the microsatellite markers selected were associated with *Gb3*, and five markers either co-segregated with *Gb3* or were within 1 cM of it (Fig. 1b), suggesting that the target mapping strategy we adopted in this study was very efficient.

Weng and Lazar (2002), using 90 F_2 plants from a Largo × TAM 107 population, mapped *Gb3* in chromosome 7DL at a distance of 33.1 cM from microsatellite marker *Xgwm428*. In the present study, *Xgwm428* and *Gb3* were found to be 0.4 cM apart (Fig. 1c). This discrepancy in genetic distance between the two loci in the two experiments may have been caused by the different populations analyzed (90 F_2 vs. 130 F_7 RILs) or by phenotyping errors in the responses of the F_2 plants to greenbug feeding. In the earlier study of Weng and Lazar (2002), F_2 plants were rescued following greenbug infestation for advancing to the next generation. Consequently, symptoms of greenbug feeding might not have been fully developed before pesticide application. Some of the F_7 RILs used in the present study were developed from the F_2 plants used in their 2002 experiments. Our initial screening test on these F_7 RILs indicated that several of the F_2 plants used by Weng and Lazar (2002) were incorrectly phenotyped with respect to their greenbug feeding responses. In the present study, the scoring of all plants subjected to greenbug feeding was taken when all of the susceptible controls (TAM 105 and TAM 107) were almost dead, which helped to reduce the chances of incorrectly phenotyping the F_2 and TC_1 plants in the allelism test.

Based on the segregation data of the responses to greenbug feeding in the F_2 and TC_1 populations, we estimated the genetic distance between *Gbx* and *Gb3* to be 8.75 cM from the F_2 data and, on average, 15.6 cM from the TC_1 data (Table 2). While the three-way cross in TC_1 might have increased the recombination between the resistance loci, the higher proportion of susceptible plants in the TC_1 progenies may also be due to the suppression of greenbug resistance in certain genetic backgrounds (for example, TAM 400 in Table 2). The latter has been observed in a number of synthetic hexaploid wheat lines (Lage et al. 2003).

The allelic relationship of *Gbx* and *Gb3* was further validated by comparative mapping using the same set of microsatellite markers in similar populations (60 ITMI F_8 RILs for *Gbx* vs. 130 F_7 RILs of Largo × TAM 107 for *Gb3*). All common microsatellite markers were mapped in the same chromosome region (Fig. 1a,b). The order of these markers was largely consistent on the two maps (Fig. 1a, b) and on the consensus wheat microsatellite map of Somers et al. (2004), and the minor discrepancies in marker order that do occur between the two maps may be due to different population sizes and genetic backgrounds (60 ITMI F_8 RILs vs. 130 F_7 RILs of Largo × TAM 107). It was also postulated that the RILs of the ITMI population might have been mixed or

incorrectly numbered during seed increase or distribution. To check for this possibility, we compared the genotypes of the first 60 RILs for GWM037 and GWM428 from this study with those of Röder et al. (1998) (from GrainsGene database). We found that three and four RILs showed different genotypes at the *Xgwm428* and *Xgwm037* loci, respectively, but that only one RIL (Line 35) exhibited the difference at both loci. The reason for this discrepancy is not known, but it seems that the authenticity of the 60 ITMI RILs was largely conserved. More direct evidence supporting our results that *Gb3* and *Gbx* are two different loci came from marker WMC634. *Xwmc634* co-segregated with *Gb3* (Fig. 1b), but it was located more than 50 cM from *Gbx* (Fig. 1a).

Since all of the evidence we collected supports that conclusion that *Gb3* and *Gbx* are two different loci, we suggest that this new greenbug resistance locus in W7984 be designated *Gb7* according to the name convention of greenbug resistance genes (Tyler et al. 1987; Porter et al. 1997). Although W7984 and Largo showed similar responses to infestation by greenbugs of biotypes B, C, E, G, I, K and the South Carolina strain, biotype E greenbug population dynamics on the two lines were quite different (Weng et al. 2004). It is not known if W7984 and Largo have different responses to other greenbug biotypes or if there are different mechanisms of resistance between the two lines.

In this study, three microsatellite markers flanking the *Gb3* gene, *Xbarc97*, *Xgwm428* and *Xwmc634*, were physically mapped in the bin delimited by deletion breakpoint 7DL-3 and the telemetric end, which is 18% of the physical length of 7DL (Fig. 1c). Several other microsatellite markers closely linked with *Gb3* (Fig. 1b), such as *Xbarc53*, *Xbarc76*, *Xbarc111*, *Xcfd69* and *Xgwm037*, were also deletion-mapped into the same bin (Shi et al. 2003; Sourdille et al. 2004). This information is important for the following reasons. First, the distal bin of 7DL where *Gb3* resides is a gene- and recombination-rich region (Hohmann et al. 1994; Erayman et al. 2004), suggesting that it might be possible to clone *Gb3* using the map-based cloning strategy. Second, for saturation mapping of the *Gb3* region with additional markers, only those physically located in this bin would be necessary. On the other hand, it is not known if *Gb7* is also located in the same bin as *Gb3*. Although we do know it should be in the distal one-quarter region of 7DL (distal to deletion breakpoint 7DL-4 in Fig. 1c) because the three RFLP markers located proximal to *Gb7*, *Xwg420*, *Xfba204* and *Xfbb325*, were found in this region (Erayman et al. 2004). In practice, the microsatellite map of *Gb3* constructed in this study will provide a valuable tool in marker-assisted selection for *Gb3*-conferred greenbug resistance in marker-aided wheat breeding programs.

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