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# Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat

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**Abstract** The Russian wheat aphid (RWA), *Diuraphis noxia* Mordvilko, is a serious economic pest of wheat and barley in North America, South America, and South Africa. Using aphid-resistant cultivars has proven to be a viable tactic for RWA management. Several dominant resistance genes have been identified in wheat, *Triticum aestivum*, including *Dn1* in PI 137739, *Dn2* in PI 262660, and at least three resistance genes (*Dn5*+) in PI 294994. The identification of RWA-resistant genes and the development of resistant cultivars may be accelerated through the use of molecular markers. DNA of wheat from near-isogenic lines and segregating $F_2$ populations was amplified with microsatellite primers via PCR. Results revealed that the locus for wheat microsatellite GWM111 (*Xgwm111*), located on wheat chromosome 7DS (short arm), is tightly linked to *Dn1*, *Dn2* and *Dn5*, as well as *Dnx* in PI 220127. Segregation data indicate RWA resistance in wheat PI 220127 is also conferred by a single dominant resistance gene (*Dnx*). These results confirm that *Dn1*, *Dn2* and *Dn5* are tightly linked to each other, and provide new information about their location, being 7DS, near the centromere, instead of as previously reported on 7DL. *Xgwm635* (near the distal end of 7DS) clearly marked the location of the previously suggested resistance gene in PI 294994, here designated as *Dn8*. *Xgwm642* (located on 1DL) marked and identified another new gene *Dn9*, which is located in a defense gene-rich region of wheat chromosome 1DL. The locations of markers and the linked genes were confirmed by di-telosomic and nulli-tetrasomic analyses. Genetic linkage maps of the above RWA resistance genes and markers have been constructed for wheat chromosomes 1D and 7D. These markers will be useful in marker-assisted breeding for RWA-resistant wheat.

**Keywords** Wheat · Russian wheat aphid · Microsatellite marker · Insect resistance · Gene mapping

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## Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko) (*Homoptera: Aphididae*), has been described as one of the most-damaging pests of small grains in the world (Kovalev et al. 1991). Since its detection in Texas in 1986, RWA has become a major economic pest of wheat (*Triticum aestivum* L., 2n = 6× = 42) and barley (*Hordeum vulgare* L.) in the western United States (Legg and Amosson 1993). The use of resistant cultivars is an effective, economical, and environmentally sound tactic to protect wheat from RWA while minimizing the use of insecticides.

Several thousand accessions of wheat and close wheat relatives have been evaluated for RWA resistance since 1987, and seven genes conferring wheat resistance to RWA have been identified. The *D. noxia* (*Dn*) resistance genes *Dn1* and *Dn2* were identified in South Africa in the common wheat accessions PI 137739 and PI 262660, and from Iran and Russia, respectively (du Toit 1987, 1988, 1989). The *Dn*1 gene was located on chromosome 7D using monosomic analysis (Schroeder-Teeter et al. 1994). The *Dn*2 gene was placed on chromosome 7DL (Ma et al. 1998). A recessive gene *dn3* is present in the *Aegilops tauschii* line SQ24 (Nkongolo et al. 1991b). The RWA resistance gene *Dn5*, identified in the Bulgarian wheat accession PI 294994, was placed on chromosome 7DL (long arm) using monosomic and ditelosomic analysis (du Toit 1987; du Toit et al. 1995; Marais and du Toit 1993). Two other dominant RWA resistance genes, *Dn4* (on chromosome 1DS) and *Dn6* (unlocated), origi-

nated from a Russian bread wheat accession PI 372129 and an Iranian wheat accession PI 243781, respectively (Nkongolo et al. 1989, 1991a; Saidi et al. 1996; Ma et al. 1998). *Dn7*, a gene derived from a rye accession, was transferred to 1RS of the 1RS•1BL translocation in wheat ''Gamtoos'' (Marais et al. 1994, 1998).

Previous results have shown complicated relationships among some of the known *Dn* genes. Du Toit (1989) reported that *Dn1* and *Dn2* were independently inherited. However, the results of Saidi and Quick (1996) indicated that *Dn1* and *Dn2* were probably allelic at the same locus. There are also conflicting results about the number and type of resistance genes in PI 294994. Marais and Du Toit (1993) assigned the single dominant gene symbol *Dn5* to PI294994, and proposed that *Dn5* and *Dn1* are linked on chromosome 7DL. Elsidaig and Zwer (1993) indicated that resistance in PI 294994 was controlled by one dominant and one recessive gene. However, allelism tests by Saidi and Quick (1996) showed that as many as three genes confer resistance in PI 294994; two of them were dominant, and at least one was allelic with *Dn1* and *Dn2*. A third RWA resistance gene in PI 294994 is likely on chromosome 1D because PI 294994 and PI 372129 (*Dn4*) share a linkage of RWA resistance genes (Saidi and Quick 1996) and *Dn4* was located on chromosome 1DS (Ma et al. 1998). Zhang et al. (1998) inferred the presence of three resistance genes in PI 294994, two on 7DL and one on 1DS.

PI 220127, a winter wheat accession from Afghanistan, was identified as a new RWA resistance source by Harvey and Martin (1990). An improved wheat germplasm derived from PI 220127 was developed in the U.S. by Martin and Harvey (1997), but the inheritance of this resistance is not known.

''Halt'', the first RWA-resistant U.S. wheat cultivar expresses *Dn4* resistance that is effective in controlling RWA (Quick et al. 1996). Other U.S. cultivars containing *Dn1* or *Dn2* are also being bred in Idaho (Souza et al. 1997a, b). Rapid breeding for, and deployment of, additional wheat cultivars resistant to RWA is urgently needed to reduce further losses from RWA outbreaks. Conventional plant phenotype selection is cumbersome, time-consuming, and sometimes inconclusive, suggesting a critical need to develop new, more efficient and accurate techniques to identify the RWA resistance genes.

Molecular marker-assisted selection (MAS) for host resistance (Melchinger 1990) is useful in identifying and mapping new genes for RWA resistance in wheat. Loci of the RFLP markers *XksuA1* and *ABC 156* are linked to the *Dn*2 gene on wheat chromosome 7DL, and to the *Dn*4 gene on 1DS, at a distance of 9.8 and 11.6 cM, respectively (Ma et al. 1998). However, these two markers will not be useful for detecting or pyramiding *Dn2* and *Dn4*, as the linkage is not tight enough (Ma et al. 1998). Myburg et al. (1998) also identified four RAPD markers linked to the *Dn2* resistance gene ranging from 3.3 cM to 4.4 cM, but gave no information about *Dn2* location.

Newly developed microsatellite markers are rapidly becoming a widely used DNA marker system (Plaschke et al. 1995; Roder et al. 1998). Several microsatellite maps of wheat have been constructed, with the microsatellite loci evenly distributed along the chromosome lengths to provide excellent coverage of the wheat genome (Korzun et al. 1997; Peil et al. 1998; Roder et al. 1998). The objectives of the present study were to identify wheat microsatellite markers closely linked to the *Dn1, Dn2* and *Dn5* genes, and the unknown genes in PI 294994, as well as the unknown gene *Dnx* in PI 220127, and to map their chromosome locations.

## Materials and methods

### Plant materials and DNA analysis

The identification of molecular markers linked to resistance genes was accomplished by contrasting near-isogenic lines (NILs) for the gene of interest (Martin et al. 1991), or by bulk segregant analysis (BSA) for a defined $F_2$ segregating population from a single cross (Michelmore et al. 1991).

### Near-isogenic lines (NILs)

Wheat NILs used in this study were developed and kindly supplied by the Small Grain Institute, Bethlehem, South Africa (du Toit 1989). The susceptible recurrent parents, Betta, Karee and Tugela, were crossed respectively to each of the resistant donor parents, PI 137739 (*Dn1*), PI 262660 (*Dn2*) and PI 294994 (*Dn5*). The resulting populations were backcrossed five times with the corresponding susceptible parents using single-seed (plant) selection in each generation. The final backcross lines were selfed twice to produce defined homozygous-resistant lines. Once the putative markers were identified as related to a resistant NIL, the corresponding $F_2$ segregating population was evaluated to confirm the linkage using co-segregation analysis, and to determine the genetic distance between a marker and a *Dn* resistance gene.

### DNA isolation and bulk segregant analyses (BSA)

The uncharacterized RWA-resistant source, PI 220127 wheat, was crossed to the susceptible Sando's selection 4040 (Martin and Harvey 1997). From each of 105 individual $F_2$ plants at the four-leaf stage, two 10-cm leaf segments were harvested from the primary leaves for genomic DNA isolation. Genomic DNA was isolated from wheat-seedling leaves in these and all other experiments using the modified CTAB / phenol-extraction and ethanol-precipitation method described by Gill et al. (1991). DNA concentration was quantified spectrophotometrically. Sufficient $F_3$ seeds were harvested from each of 66 $F_2$ plants, planted and evaluated for RWA phenotypic response. Eight homozygous-resistant $F_{2:3}$ families, eight susceptible $F_{2:3}$ families, and eight heterozygous $F_{2:3}$ families were selected based on phenotype test results. The resistant, susceptible, and heterogeneous $F_2$ plant DNA was bulked respectively by combining an equal amount of $F_2$ DNA from the corresponding selected progenies in each category. Once a specific polymorphism between resistant and susceptible bulks was identified by BSA screening, individual co-segregation analysis, based on the associations between marker genotype and RWA reaction phenotype, was carried out on the total $F_2$ segregating population to determine the genetic linkage between a RWA resistance gene and a marker.

### Aneuploid analysis

Genomic DNA from normal euploid ''Chinese Spring'' (CS) wheat, the nulli-tetrasomic (NT) stocks N1D-T1 A and N7D-T7 A of CS, and ditelosomic (Dt) stocks Dt1DL, Dt7AS, Dt7BS and Dt7DS of

**Table 1** Phenotypic segregation ratios and chi-square analyses of $F_2$ wheat plant reaction to Russian wheat aphid infestation

| Parents | | $F_2$ plants (#) | |
|---|---|---|---|
| Female × Male | R : S[a] | $\chi^2$ (3:1)[b] | *P* |
| S4040 × PI220127 | 48R : 18S | 0.18 | 0.69 > 0.05 |
| Karee × Karee-*Dn1* | 68R : 33S | 3.17 | 0.08 > 0.05 |
| Karee × Karee-*Dn2* | 67R : 28S | 1.11 | 0.31 > 0.05 |
| Karee × Karee-*Dn8* | 74R : 34S | 3.50 | 0.07 > 0.05 |

[a] R = resistant, S = susceptible
[b] significance limit of $\chi^2$ ($P$ = 0.05, $df$ =1) =3.84

CS, as well as Dt7DS and Dt7DL of Thatcher (TC), were amplified by PCR using the specific microsatellite primers GWM635, GWM642 and GWM111. The presence or absence of the corresponding characteristic PCR band was compared among euploid CS, Dt CS or TC, and NT CS stocks, to determine the specific locus of the primer or marker. All euploid CS, aneuploid CS and TC lines are maintained and kindly provided by the Wheat Genetics Resource Center, Kansas State University.

### Phenotypic RWA assessment

Wheat seedlings of parents and segregating $F_2$ populations and/or $F_3$ families derived from selfing individual $F_2$ plants were artificially infested in the greenhouse with RWA and rated according to the technique of Smith et al. (1991). For segregation analysis, approximately 100 plants from each of the four $F_2$ populations: Karee × Karee-*Dn1*, Karee × Karee-*Dn2*, Karee × Karee-*Dn8* and PI 220127 × Sando's 4040, were scored. Three days after leaf tissue was harvested for DNA isolation, plants were infested with RWA and evaluated for phenotypic reaction. In addition, 66 $F_{2:3}$ families from the cross PI 220127 × Sando's 4040 were screened for reactions to RWA. In these tests, RWA damage to individual plants was rated using a 0–3 scale based on leaf folding, leaf rolling and chlorosis /streaking, when the plants of the susceptible control were dead or dying (rating of 3). Individual seedlings with no damage or only a few chlorotic spots were considered resistant (rating of 0 or 1), while those with leaf streaking, leaf rolling, leaf folding or dead plants, were considered susceptible (rating of 2 or 3).

### Microsatellite primer screening and identification

DNA from the three sets of NILs and one set of $F_2$ bulk segregants was screened with microsatellite primers for polymorphisms related to RWA resistance. Putative markers and linked genes were confirmed and mapped using segregation analysis of the respective $F_2$ populations segregating for resistance genes. For microsatellite PCR, the chromosome-specific primers flanking the SSR blocks were used. Primer sequences, chromosome locations, and PCR protocols were obtained from the GrainGenes Database at <http://wheat.pw.usda.gov/>, and from Roder et al. (1995, 1998), Plaschke et al. (1995), and Korzun et al. (1997). A total of 40 pairs of microsatellite primers on wheat chromosomes 7 A, 7B, 7D o 1D, were tested to detect polymorphisms between contrasting parents, NILs, or $F_2$ progenies. Wheat microsatellite loci were designated as *Xgwm* (Gatersleben wheat microsatellite) according to Roder et al. (1998).

PCR amplifications are conducted as described in Roder et al. (1995) with modification. The PCR reaction mixture contained 0.2 mM of each DNTP, 1.8–2.0 mM of MgCl, 1 × PCR buffer, 1 U of Taq-polymerase, 150 ng each of left and right flanking primer, and 60 ng of template DNA in a total volume of 25 µl. PCR was performed in an MJ Research thermocycler, at 94°C for 3 min, followed by 44 cycles of 94°C for 1 min, 50, 55, or 60°C (based on primer annealing temperature) for 1 min, and 72°C for 2 min, with final incubation at 72°C for 10 min before cooling to 4°C.

Amplification products (DNA fragments) were resolved by electrophoresis in 2% agarose gels (Fisher Biotech) at 4 V/cm in 0.5 × TBE buffer. Gels were stained with ethidium bromide (0.5 µg/ml). DNA banding patterns were visualized with UV light and recorded by an AMBIS Radioanalytic Imaging System (Digita Imagers). Approximate fragment sizes were estimated by comparison with internal size standards (25-bp or 100-bp DNA ladders).

### Linkage analysis and genetic mapping

Recombination frequencies (RF) or linkage relationships between microsatellite markers and RWA resistance genes were calculated using maximum-likelihood equations with $F_2$ data for marker genotype and plant phenotype for RWA reaction (Allard 1956) using MapMaker (Lander et al. 1987). RF was also estimated using the probability formula for NILs, where the proportion of genomic DNA from the recurrent parent after *n* backcrosses is estimated as: $P = 1–1/2^n$, and the recombination frequency (RF probability) between the target gene and the linked marker must be: $RF < 1/2^n$. RF was transformed to genetic linkage distance (cM) using the Kosambi mapping function (Kosambi 1944).

## Results and discussion

### Inheritance of RWA resistance

Among the 66 $F_{2:3}$ families from the cross Sando's 4040(S) × PI220127(R), 14 families were homozygous resistant, 34 segregated for resistance, and 18 were susceptible. Results of chi-square analysis demonstrated that the original $F_2$ progenies of these families genotypically segregated in a ratio of 1RR : 2Rr : 1rr ($\chi^2$ = 0.55, $P$ = 0.76), and phenotypically segregated in a ratio of 3R:1 S ($\chi^2$ = 0.18, $P$ = 0.69). Both ratios fit the inheritance model of a single dominant gene. These results indicated that the RWA resistance in PI220127 is controlled by a single dominant gene, which is temporarily designated as *Dnx*. Resistance in Karee-*Dn1*, Karee-*Dn2* and Karee-*Dn8* was confirmed as being controlled by single dominant genes, because each $F_2$ population derived from the Karee-*Dn1*, Karee-*Dn2* and Karee-*Dn8* NILs segregated for a RWA reaction consistent with a single dominant gene inheritance model in a ratio of 3R:1 S (Table 1).

### Microsatellite markers linked to RWA resistance genes

Out of 40 primer pairs tested in this study, three amplified specific DNA fragments related to RWA resistance, based on polymorphism between the DNA of resistant and susceptible plants (Table 2).

The primer pair GWM111 produced four specific PCR amplification bands respectively, related to each of four different resistance genes (Table 2). A 210-bp band was present in the DNA of PI 137739 (*Dn1*) and the DNA of all three related resistant NILs (Betta-*Dn1*, Karee-*Dn1* and Tugela-*Dn1*), but was absent in the DNA

**Table 2** Chromosome locations of wheat microsatellite markers linked to resistance genes in four sources of Russian wheat aphid resistance

| Gene | Source | Marker | PCR band (bp) | Location | Linkage distance (cM) |
|---|---|---|---|---|---|
| *Dn1* | PI 137739 | *Xgwm111* | 210 | 7DS | 3.82 ± 0.20 |
| *Dn2* | PI 262660 | *Xgwm111* | 200 | 7DS | 3.05 ± 0.18 |
| *Dn5* | PI 294994 | *Xgwm111* | 220 | 7DS | <3.20 |
| *Dnx* | PI 220127 | *Xgwm111* | 225 | 7DS | 1.52 ± 0.15 |
| *Dn8* | PI 294994 | *Xgwm635* | 100 | 7DS | <3.20 |
| *Dn9* | PI 294994 | *Xgwm642* | 180 | 1DL | <3.20 |

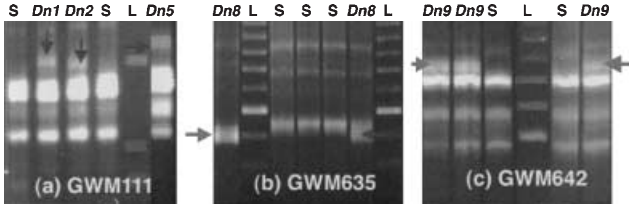


**Fig. 1** Polymorphic DNA bands in RWA-resistant near-isogenic lines (NILs), amplified with primer pairs (**a**) *GWM 111*, (**b**) *GWM 635*, and (**c**) *GWM 642*, and electrophoresed in 2% agarose gels. *Dn1, 2, 5, 8, 9 = resistance genes* in NILs, *S* = susceptible parent, *L* = 100-bp DNA ladder (**a**) or 25-bp DNA ladder (b and c), → = specific resistance-related bands described in the text and Table 2

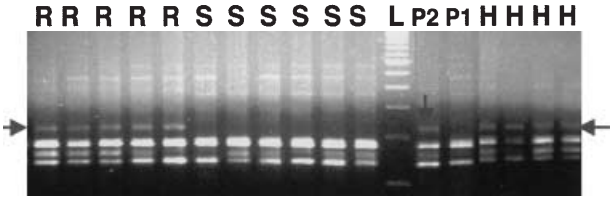


**Fig. 2** PCR bands amplified from the DNA of selected $F_2$ progenies from the cross Sando's 4040 (P1, susceptible) × PI 220127 (P2, resistant) using primer pair *GWM111*, and electrophoresed in a 2% agarose gel. *R* = homozygous RWA resistant, *H* = heterozygous and segregating in $F_{2:3}$ families, *S* = RWA susceptible, *L* = 100-bp DNA ladder, → = 225-bp resistance band

of the susceptible recurrent parents (Fig. 1a). Similarly, a 200-bp band was present in the DNA of PI 262660 (*Dn2*) and the DNAs of all three resistant NILs of *Dn2*, but absent in the DNA of the susceptible recurrent parents (Figs. 1a and 3). A 220-bp band was present in the DNA of PI 294994 (*Dn5*) and the resistant NIL Betta-*Dn5*, but was absent in the susceptible recurrent parent Betta (Fig. 1a). A 225-bp band was present in the DNA of PI 220127 (*Dnx*), the resistant $F_2$ DNA bulk, the DNA of all 49 resistant $F_2$ plants (both homogeneous and heterogeneous), and the DNA of 1 of 17 susceptible F2 plants, but was absent in the DNA of the susceptible parent Sando's 4040, as well as 16 of 17 susceptible $F_2$ plants (Fig. 2).

The alterations in fragment length related to *Dn1*, *Dn2*, *Dn5* and *Dnx* detected by GWM111 could be a result of either deletion or insertion of repetitive DNA sequences leading to different numbers of repeats within the microsatellite region. Concurrently, Jing et al. (2000) found that a drought tolerance gene in an ancient Chinese wheat landrace ''Pingyao Bai Mai'' and its derived varieties was putatively linked to the locus *Xgwm 111* on chromosome 7D. In greenhouse tests, we noticed that drought stress significantly accelerates wheat damage from plant sap sucking / feeding by RWA. In addition, the RWA occurs most seriously in the arid and semi-arid cereal-producing regions of the world. The relationship between drought tolerance and RWA resistance in wheat warrants further investigations of the genetic and physiological mechanisms involved.

GWM635 amplified a specific 100-bp band in DNA in both the resistant parent PI 294994 (*Dn8* donor) and the resistant NIL Karee-*Dn8*, but was absent in the DNA of the recurrent susceptible parent Karee (Fig. 1b). GWM 642 specifically amplified a 180-bp band related to the RWA resistance in PI 294994 (also a *Dn9* donor) and the resistant NIL Betta-*Dn9*, but was absent in the recurrent susceptible parent Betta (Fig. 1c).

## Inheritance of microsatellite markers

The majority of documented microsatellite markers have been shown to be inherited in a co-dominant manner (Rafalski and Tingey 1993; Roder et al. 1998). However, the microsatellite markers *Xgwm111*, *Xgwm635* and *Xgwm 642*, linked to six RWA resistance genes in this study, were inherited in a dominant manner, because they detected only resistance related bands, and $F_2$ populations segregated in a 3:1 ratio (R:S) for the presence or absence of the resistant band (data not shown). Gill et al. (1991) demonstrated that the D genome of wheat possesses a high percentage of null alleles characterized by the absence of RFLP fragments in one parent that are present in the other, but with no apparent alteration in any other fragments. A possible explanation for the dominance of these microsatellite markers with null alleles is most-likely due to nucleotide-sequence alterations within the priming recognition sites of susceptible plants. These alterations are due to point mutations, insertions, deletions or inversions, which lead to no primer matching, and the absence of the corresponding PCR band.

## Aneuploid analysis

The chromosome location of the identified markers was verified by matching the polymorphic DNA fragment

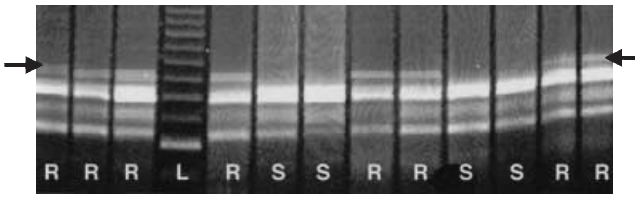


**Fig. 3** PCR bands amplified from the DNA of selected $F_2$ progenies from the cross Karee × Karee-*Dn2* using primer pair *GWM111*, and electrophoresed in 2% agarose gel. *R* = RWA-resistant, *S* = susceptible. *L* = 25-bp DNA ladder. → = 200-bp resistance-related band

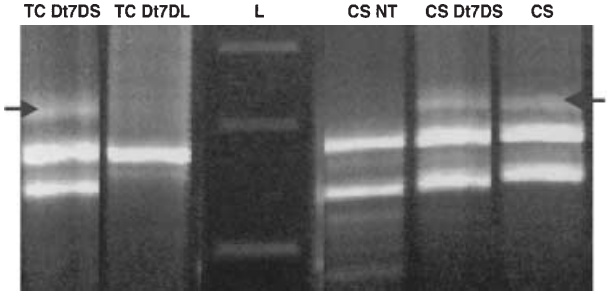


**Fig. 4** Comparison of PCR bands amplified using *GWM111*, electrophoresed in 2% agarose gel. Arrows indicate the specific 205-bp band present in "Chinese Spring" (*CS*) wheat, CS and "Thatcher" (*TC*) ditelosomic (*Dt*) 7DS wheat, but absent in CS Dt 7DL, CS nulli-tetrasomic (NT) N7DT7 A, and TC Dt7DL wheat, indicating that *Xgwm111* is located on chromosome arm 7DS. *L* = 100-bp DNA ladder

bands linked to the RWA resistant genes with the corresponding bands present or absent in euploids and aneuploids. Missing specific bands in a nullitetrasomic (NT) line indicates that a given marker is located on that corresponding chromosome. The presence of a band in a ditelosomic (DT) line for a respective chromosome arm is subsequently indicative of the marker being mapped to the corresponding chromosome arm. As was expected, *Xgwm635* was located on chromosome 7DS, and *Xgwm642* was located on 1DL (data not shown), which is consistent with the locations of these markers on the microsatellite map of wheat (Roder et al. 1998). However, a characteristic 205-bp PCR band (Fig. 4) amplified by GWM 111 in Chinese Spring (CS), corresponding to polymorphic bands related to RWA resistance, was present in normal CS, as well as in both the CS Dt7DS and Thatcher (TC) Dt7Ds lines (lacking the long arm of chromosome 7D), but was absent in the TC Dt7DL line (lacking the short arm of 7D) and the CS N7DT7 A line (lacking 7D, but possessing an additional pair of 7 A chromosomes). These results clearly indicate that *Xgwm111* is located on chromosome 7DS, instead of on 7DL as previously reported by Roder et al. (1998). Our results are in accordance with the molecular and cytogenetic data of Werner et al. (1992) and Friebe et al. (1996), who verified that all previously available CS ditelosomic 7D stocks (designated as either CS Dt7DS or CS Dt7DL) are in fact Dt7DS.

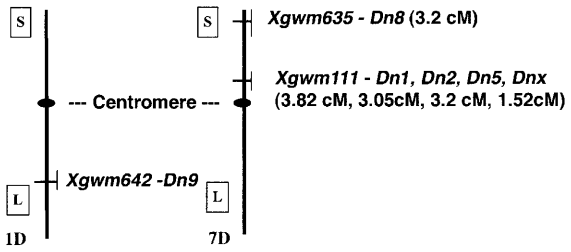


**Fig. 5** Genetic linkage maps of RWA resistance genes and the linked microsatellite markers on wheat chromosomes 1D and 7D. *S*, *L* = short or long arm. The orientation between markers and genes on the maps is unresolved

## Linkage analysis and genetic mapping

Specific DNA allelic bands amplified by locus-specific GWM primers, co-segregated with their respective linked resistant gene(s), suggesting that the location of the resistant gene and the locus of the primer are closely linked. The locus of the corresponding primer and the resulting specific PCR band were both used as markers to identify each linked gene and to specifically map the gene to a location near the primer locus.

The genotypes of markers and the phenotypes of RWA reactions were scored in the $F_2$ populations and/or NIL lines. Different sizes of specific PCR bands (210, 200 and 220 bp) related to *Dn1*, *Dn2* and *Dn5* respectively, were amplified by primer GWM111 (Figs. 1 and 3, and Tables 1 and 2). These three genes are tightly linked to *Xgwm111* at distances of 3.82, 3.05 and 3.20 cM, respectively. So *Dn1*, *Dn2* and *Dn5* are either allelic at the same locus, or different but tightly linked to each other, proximally on the short arm of chromosome 7D, near the centromere (Fig. 5), instead of on 7DL as previously reported. A second resistance gene in PI 294994, here designated as *Dn8*, was located <3.2 cM from *Xgwm 635* near the distal end of chromosome 7DS. *Dn8* is most likely the same gene that Zhang et al (1998) suggested to be loosely linked to *Dn5*. A third resistance gene in PI 294994, which we designate as *Dn9*, was located <3.2 cM from *Xgwm642* in the middle of chromosome 1DL, within a defense gene-rich region (Boyko et al. 1999; Boyko, unpublished). These results clearly indicate that there are at least three RWA resistance genes in wheat PI 294994, and clarify the previous confusion about the complicated relationships among *Dn1*, *Dn2* and *Dn5*, and fix their correct chromosome-arm locations.

The single dominant resistance gene *Dnx* in PI 220127 wheat is most likely a new RWA resistance gene. A specific 225-bp PCR band, amplified by GWM111, co-segregated with *Dnx* (Fig. 2), which was located 1.52 cM away from *Xgwm111* near the centromere of 7DS. Results indicated that *Dnx* is linked, but different from *Dn1*, *Dn2* and *Dn5* (Table 2, Fig. 5). Whether *Dnx* is allelic to *Dn6* (unmapped) or a new gene, remains to be determined by additional marker and/or allelic analyses.

The identified microsatellite markers linked to RWA resistance genes, the estimated sizes of PCR fragments

related to resistance genes, their chromosome locations and genetic distances, are summarized in Table 2. The linkage maps, shown in Fig. 5, were constructed using the linkage data from this study, and were integrated with the previously published microsatellite framework map (Roder et al. 1998) with the adjustment of *Xgwm111* from 7DL to 7DS.

## Concluding remarks

The identified microsatellite markers closely linked to the RWA resistance genes described in this study offer rapid and accurate determination of the chromosome location of these genes, and should enable practical marker-assisted selection of RWA resistance genes in wheat improvement. It is now also possible to pyramid several RWA resistance genes into a single improved wheat genotype for durable RWA resistance. Work is currently in progress for the RWA resistance genes *Dn4*, *Dn6*, and unknown RWA resistance genes, using molecular markers.

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