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# Identification of microsatellite markers linked to Russian wheat aphid resistance genes *Dn4* and *Dn6*

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**Abstract** The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is a serious economic pest of wheat worldwide. Host plant resistance is the preferred method to control RWA infestations. The identification and mapping of RWA-resistant genes and the development of resistant wheat cultivars can be facilitated through the use of molecular markers. In the present study, microsatellite (SSR) markers linked to the RWA-resistant genes *Dn4* and *Dn6* were identified using several $F_2$ mapping populations derived from crosses of susceptible wheat cultivars and resistant sources. Two flanking microsatellite markers *Xgwm106* and *Xgwm337* are linked in coupling phase with *Dn4* on the short arm of wheat chromosome 1D at 7.4 cM and 12.9 cM, respectively. Two other microsatellite markers *Xgwm44* and *Xgwm111* are linked to *Dn6* in coupling phase near the centromere on the short arm of chromosome 7D at 14.6 cM and 3.0 cM, respectively. This is the first report on the chromosomal location of *Dn6*, which proved to be either allelic or tightly linked to *Dn1*, *Dn2* and *Dn5*. This result of *Dn6* location contradicts previous reports that *Dn6* was independent of *Dn1*, *Dn2* and *Dn5*. The linked markers can be conveniently used for marker-assisted selection in wheat breeding programs for the identification and/or pyramiding of *Dn4* and *Dn6* genes.

**Keywords** Wheat · Russian wheat aphid · Microsatellite markers · Plant 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 destructive pests of small grains in many areas of the world (Kovalev et al. 1991). It is a serious pest of wheat and barley in North America, South America, South Africa and Australia, causing major economic losses. Since its first detection in Texas in 1986, RWA has been identified as a major economic pest of wheat (*Triticum aestivum* L. AABBDD, 2n = 6*x* = 42) and barley (*Hordeum vulgare* L.) in the western United States (Legg and Amosson 1993). The use of resistant cultivars is an efficient, economical, and environmentally safe method to protect wheat from RWA while minimizing the use of insecticides (Smith 1999).

To-date, about 30,000 accessions of wheat and related Triticeae have been evaluated for RWA resistance since 1987 (Souza 1998; Smith 1999), and nine genes conferring wheat resistance to RWA have been described. The *D. noxia* (*Dn*) resistance genes *Dn1* and *Dn2* were identified in South Africa in the common wheat accessions PI 137739 and PI 262660, from Iran and Russia, respectively (Du Toit 1987, 1988, 1989). A recessive gene *dn3* is present in the *Aegilops tauschii* line SQ24 (Nkongolo et al. 1991a). The RWA resistance gene *Dn5* was identified in the Bulgarian wheat accession PI 294994 (Du Toit 1987; Marais and Du Toit 1993). *Dn7*, a gene derived from a rye accession, was transferred to the short arm of the 1RS·1BL translocation in wheat "Gamtoos" (Marais et al. 1994, 1998). *Dn8* and *Dn9* were identified in near-isogenic wheat lines derived from PI 294994, which is also the source of *Dn5* (Liu et al. 2001). Two other known dominant RWA resistance genes, *Dn4* and *Dn6*, originated from the Russian bread wheat accession PI 372129 and the Iranian wheat accession PI 243781, respectively (Nkongolo et al. 1991b; Saidi and Quick 1996).

Rapid breeding for, and deployment of, additional wheat cultivars resistant to RWA is urgently needed to reduce further losses from RWA outbreaks. However,

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 RWA resistant genes or genotypes.

Molecular markers are powerful tools in marker-assisted selection (MAS) for breeding RWA-resistant cultivars. The development of molecular markers tightly linked to resistance genes not only facilitates the identification and mapping of plant insect-resistant genes, but also allows the MAS of resistant lines in breeding programs (Melchinger 1990). MAS greatly enhances the opportunity for gene pyramiding in desired germplasm and improved cultivars, and so expedites the process of breeding for multiple and durable resistance. The newly developed microsatellites [also called simple sequence repeats (SSRs), or simple/short tandem repeats (STRs)], which are reliable, highly polymorphic, chromosome group and arm specific, and easy to use, have been rapidly becoming a widely used DNA marker system (Weissenbach et al. 1992; Plaschke et al. 1995; Roder et al. 1998). Microsatellite maps of wheat have been constructed, with 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; Pestsova et al. 2000).

The RWA resistance genes *Dn1*, *Dn2*, *Dn5*, and an uncharacterized gene *Dnx*, are closely linked to *Xgwm111* on wheat chromosome 7DS (near the centromere). *Dn8* is linked to *Xgwm635* on the distal portion near the telomere of wheat chromosome 7DS, and *Dn9* is tightly linked to *Xgwm642* on chromosome 1DL (Liu et al. 2001). *Dn4* is loosely linked (11.6 cM) to the RFLP marker *ABC156* on wheat chromosome 1DS (Ma et al. 1998). *Dn6* was regarded as independent of *Dn1*, *Dn2* and *Dn5* (Saidi and Quick 1996), but was previously unmapped. The objectives of the present study were to identify microsatellite markers closely linked to *Dn4* and *Dn6*, to accurately map their location, and to facilitate their use in breeding for RWA resistance in wheat.

## Materials and methods

### Plant materials

Wheat seeds of resistant sources PI 372129 and PI 243781 were provided by the USDA/ARS National Small Grains Research Facility in Aberdeen, Idaho. Seeds of susceptible wheat cultivars were provided by the KSU Wheat Genetics Resource Center in Manhattan, Kan. Susceptible wheats were used as female parents to cross with resistant lines containing either *Dn4* or *Dn6*. The crosses were: Thunderbird × PI 372129 (*Dn4*), Wichita × PI 372129 (*Dn4*), Wichita × PI 243781 (*Dn6*), and AL359 × PI 243781 (*Dn6*). $F_1$ plants were self-pollinated to produce $F_2$ segregating populations. $F_2$ mapping populations, consisting of 140 to 200 individual plants developed from each cross, were evaluated for phenotypic reaction to RWA and genotyped using microsatellite markers that revealed putative linkage with *Dn4* and *Dn6* based on bulk segregant analysis (see below).

### DNA isolation

At the three-leaf stage of growth, the second leaf from each of the individual $F_2$ progeny plants or parent plants was cut into small segments. Genomic DNA was extracted using the modified CTAB procedure as described by Gill et al. (1991). DNA concentration was quantified spectrophotometrically.

### RWA resistance phenotyping

Wheat seedlings of parents, $F_1$ plants, and the segregating $F_2$ populations were evaluated for phenotypic reaction to RWA in the greenhouse according to the technique of Smith et al. (1991). Three days after leaf tissue was harvested for DNA isolation, each plant was artificially infested initially with five RWAs. Three weeks after RWA infestation, leaf rolling, leaf folding, and chlorosis/streaking in individual plants was scored and recorded using a 0–3 scale, when the plants of the control (susceptible parent) were dead or dying (rating of 3). Two major contrasting classes of plant reactions were distinguished for genetic and linkage analyses. Individual seedlings with no damage, or only a few chlorotic spots, or slight and loose rolling, were considered resistant (rating of 0 or 1), whereas those with heavy leaf streaking/bleaching, tight leaf rolling/folding, or dead plants, were considered susceptible (rating of 2 or 3). The chi-square test was used to test the goodness of fit to or deviation of the segregating $F_2$ populations from the theoretically expected Mendelian segregation ratios.

### Bulk segregant analysis (BSA)

Molecular markers putatively linked to *Dn* genes were identified by contrasting bulk segregant analysis (BSA) for a defined segregating $F_2$ population (Michelmore et al. 1991). For each population, two DNA bulks were assembled, using equal amounts of DNA from five resistant and five susceptible $F_2$ plants, respectively. DNA samples of resistant and susceptible parents, and resistant and susceptible bulks were amplified and screened for polymorphisms with microsatellite markers. Once a specific polymorphism between a resistant and susceptible bulk was identified by BSA screening, co-segregation analysis and mapping in the $F_2$ segregating population was carried out to confirm and determine the genetic linkage between a RWA resistance gene and a marker.

### Microsatellite analyses

Microsatellite primer sequences, chromosome locations, and PCR protocols were obtained from the GrainGenes Database at http://wheat.pw.usda.gov/, or as described by Roder et al. (1995, 1998), Plaschke et al. (1995) and Korzun et al. (1997), with modification of the location of *Xgwm111* (Liu et al. 2001). A total of eight microsatellite primer pairs specific for wheat chromosome 1D and 12 primer pairs specific for 7D were screened for linkage to *Dn4* and *Dn6* based on polymorphisms between contrasting parents, $F_2$ BSAs, and $F_2$ populations. Wheat microsatellite loci were designated as *Xgwm* [Gatersleben (Germany) wheat microsatellite], followed by a probe number, according to Roder et al. (1998), where "*X*" is the basic symbol for molecular marker locus with unknown function in wheat.

The microsatellite PCR was carried out as described by Roder et al. (1995) with minor modification (Liu et al. 2001). Each PCR reaction mixture contained 0.2 mM of each dNTP, 1.5–2.0 mM of $MgCl_2$, 1 × PCR buffer, 1 U of *Taq*-polymerase, 150 ng each of left and right flanking primers, and 60 ng of template DNA in a total volume of 25 µl. The PCR reaction was accomplished in an MJ Research thermocycler (Watertown, Mass., USA), at standard amplifications of 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, then with a final extension step at 72 °C for 10 min before cooling to 4 °C. PCR amplified

products (DNA fragments) were separated 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 (Digital Imagers).

### Linkage analysis and genetic mapping

Recombination frequencies (RF) or linkage relationships between microsatellite markers and *Dn* genes were calculated using maximum-likelihood equations with $F_2$ data for marker genotype and plant phenotype of the RWA reaction (Allard 1956). The linkage maps were constructed by converting RF to genetic map distance (cM) using the MapMaker computer program, version 3.0 (Lander et al. 1987) at LOD >3.0, and with the Kosambi mapping function (Kosambi 1944).

## Results and discussion

### Identification of polymorphic microsatellites

Of the 20 microsatellite primer pairs tested, chromosome-1D specific primers GWM106 and GWM337 amplified specific and polymorphic DNA fragments with the expected sizes related to the resistance of *Dn4*, and chromosome-7D primers GWM111 and GWM44 amplified specific DNA fragments associated with *Dn6*. To confirm the linkage and determine the linkage distances, microsatellite markers and the phenotypic reaction to RWA were scored for co-segregation analysis on each of the whole $F_2$ populations segregating for RWA resistance and susceptibility.

### Microsatellite markers linked to *Dn4*

Marker *Xgwm106*: the primer pair GWM106 amplified a 125-bp DNA fragment, which was present in PI 372129 (*Dn4*), but absent in the susceptible parent Thunderbird (Fig. 1). Linkage analysis on a population of 142 $F_2$ plants derived from a cross of Thunderbird × PI 372129 indicated that the GWM106 locus (*Xgwm106*) is a dominant marker linked to *Dn4* at a distance of 7.38 ± 2.26 cM on the short arm of wheat chromosome 1D.

Marker *Xgwm337*: the primer pair GWM337 amplified a polymorphic pattern displaying a 175-bp DNA fragment specific to the *Dn4* resistant parent PI 372129, and a 195-bp band amplified from the DNA of the susceptible parent Thunderbird (Fig. 2). Linkage analysis from the 142 $F_2$ plants derived from a cross of Thunderbird × PI 372129 revealed that *Xgwm337* is a co-dominant marker linked to *Dn4* at a distance of 12.93 ± 2.95 cM on the short arm of wheat chromosome 1D.

Although the linkage for either *Xgwm106* or *Xgwm337* to *Dn4* (7.38 cM and 12.93 cM respectively) is not close enough for marker-assisted selection (MAS) of *Dn4*, these two markers do flank *Dn4* on both sides (see Fig. 5), making them more useful and accurate if they are combined to tag *Dn4* for MAS. The recombination frequency (RF) between *Xgwm106* and *Dn4* is 6.86% with a Kosambi map distance of 7.38 cM. The RF between *Xgwm337* and *Dn4* is 11.39% with a map distance of 12.93 cM. If the selection accuracy in MAS using either *Xgwm106* or *Xgwm337* to tag *Dn4* is 93.14% (=1–6.86%) or 88.61% (=1–11.39%), respectively, then the selection accuracy using both markers together to tag *Dn4* will increase to 99.22%. (Based on the product rule of probability, the frequency of double-crossover between these two markers is 6.86% × 11.39% = 0.78%; thus the combined selection accuracy will be 1–0.78% = 99.22%.) If a single marker is used for MAS, such a high accuracy of selection could only be achieved if the marker is tightly linked to the gene within 0.78 cM.

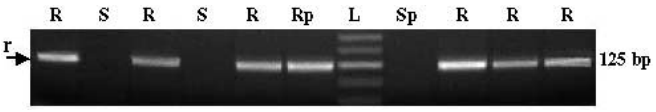


**Fig. 1** DNA bands amplified from $F_2$ progeny of Thunderbird (susceptible parent, Sp) × PI 372129 (resistant parent containing *Dn4*, Rp), using microsatellite primer pair GWM106 and electrophoresed in a 2% agarose gel; 142 $F_2$ plants were evaluated. *R* = RWA-resistant $F_2$ plants, *S* = RWA-susceptible $F_2$ plants, *L* = 25–bp DNA ladder, *r* [UNICODE 279E] = 125 bp resistance-related band

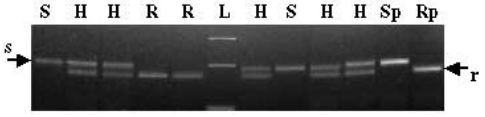


**Fig. 2** DNA fragments amplified from $F_2$ progeny of Thunderbird (Sp) × PI 372129 (Rp, *Dn4*) using primer pair GWM337 and electrophoresed in a 2% agarose gel. *r* [UNICODE 279E] = resistant band (175 bp), *s* [UNICODE 279E] = susceptible band (195 bp), *R* = homozygous resistant, *S* = susceptible, *H* = heterozygous $F_2$ plants. *L* = 100-bp DNA ladder; 142 $F_2$ plants were evaluated

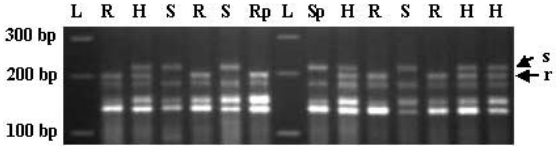


**Fig. 3** DNA bands amplified from $F_2$ progeny of Wichita (Sp) × PI243781 (Rp, *Dn6*) using primer pair GWM111 and electrophoresed in a 2% agarose gel. *r* [UNICODE 279E] = resistant band (200 bp), *s* [UNICODE 279E] = susceptible band (220 bp), *L* = 25-bp ladder. *R* = homozygous resistant, *S* = susceptible, *H* = heterozygous $F_2$ plants; 137 $F_2$ plants were evaluated

### Microsatellite markers linked to *Dn6*

Marker *Xgwm111*: the primer pair GWM111 amplified a 200-bp DNA band specific to PI 243781(*Dn6*), and a 220-bp band from the DNA of the susceptible parent Wichita (Fig. 3). Linkage analysis of 137 $F_2$ progeny derived from the cross of Wichita (S) × PI 243781 (*Dn6*), indicated that the GWM111 locus (*Xgwm111*) is a co-

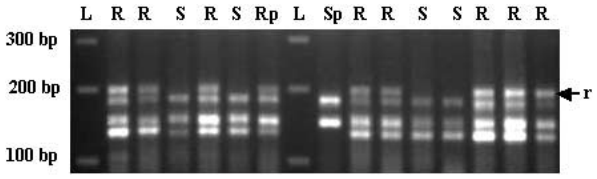


**Fig. 4** DNA bands amplified from $F_2$ progeny of AL 359 (Sp) × PI 243781 (Rp, *Dn6*) with primer pair GWM111 and electrophoresed in a 2% agarose gel. *r* [UNICODE 279E] = resistance-related band (200 bp), *L* = 100-bp ladder. *R* = resistant, *S* = susceptible $F_2$ plants; 189 $F_2$ plants were evaluated

dominant marker tightly linked to *Dn6* at a distance of 3.35 ± 1.57 cM on wheat chromosome 7D. In addition, GWM111 only produced null and no specific bands from another susceptible parent (AL359) (Fig. 4). Linkage analysis of the 189 $F_2$ plants derived from the cross AL953 (S) × PI 243781 (*Dn6*), showed *Xgwm111* to be a dominant marker tightly linked to *Dn6* at a distance of 2.82 ± 1.10 cM. A balanced average linkage distance of 3.04 ± 1.30 cM was calculated based on the results of the two $F_2$ populations as above. We conclude that *Xgwm111* is tightly linked to *Dn6* at 3.04 ± 1.30 cM on the short arm of wheat chromosome 7D.

Marker *Xgwm44*: the primer pair GWM44 amplified a 180-bp band in PI 243781(*Dn6*), and a 190-bp band in the susceptible Wichita (data not shown). Linkage analysis on 137 $F_2$ plants derived from the cross of Wichita (S) × PI 243781 indicated that the GWM44 locus (*Xgwm44*) is linked as a co-dominant marker to *Dn6* at a distance of 14.63 ± 3.18 cM on the short arm of wheat chromosome 7D.

## Segregation and inheritance of RWA resistance genes

Resistance in both PI 372129 (*Dn4*) and PI 243781 (*Dn6*) has been documented as being controlled by a single dominant gene (Nkongolo et al. 1991a; Saidi and Quick 1996). Among the 189 $F_2$ plants of a mapping population from the cross AL359(S) × PI 243781 (*Dn6*) (Table 1), both the ratio of resistance to susceptibility, and the ratio of presence to absence of the linked dominant marker *Xgwm111* segregated in a ratio consistent with 3:1 ($P$ = 0.718, and 0.602, respectively). These data and other $F_2$ segregation data (Table 1) are basically consistent with the previous inheritance model of one dominant gene.

However, the *Dn4* gene showed a distorted segregation ratio in the 142 $F_2$ progeny derived from the RWA-infested $F_1$ plants of the cross Thunderbird (S) × PI 372129 (Table 1). Segregation for RWA resistance in this $F_2$ population deviated significantly from the expected Mendelian segregation ratio of 3R:1S ($\chi^2$ = 4.141, $P$ = 0.044). The resistance-linked dominant marker *Xgwm106* also showed a highly significant deviation from the expected 3:1 ratio ($\chi^2$ = 12.855, $P < 0.001$). A similar trend of distorted segregation also occurred in the $F_2$ populations that derived from RWA-infested $F_1$ plants of the crosses Wichita (S) × PI 372129 (*Dn4*), and Wichita (S) × PI 243781 (*Dn6*), respectively, although the deviation was not significant at the $P < 0.05$ statistical level (Table 1). These data showed a deviation trend toward a deficient susceptible portion and an excessive resistant portion that deviated from the expected 3R:1S segregation ratio in $F_2$ populations derived from RWA-infested $F_1$ plants, contrasting to the normal segregation in the corresponding $F_2$ populations derived from un-infested $F_1$ plants. It is likely that the RWA-resistant gene

**Table 1** Segregation analyses for the *Dn4* and *Dn6* genes and linked microsatellite markers in $F_2$ populations from crosses between susceptible (female) and resistant (male) wheats

| Cross female(S) × male(R) | Gene or marker | # of $F_2$ plants | # of Observed[c] | | | Observed ratio | Expected ratio | $\chi^2$ | $P$[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | $X_1X_1$ | $X_1X_2$ | $X_2X_2$ | | | | |
| Thundb × PI372129[a] | *Dn4* | 142 | 117 | | 25 | 4.7:1 | 3:1 | 4.141 | 0.044 |
| | *Xgwm106* | 142 | 125 | | 17 | 7.3:1 | 3:1 | 12.855 | <0.001 |
| | *Xgwm337* | 142 | 44 | 67 | 31 | 1:1.5:0.7 | 1:2:1 | 2.830 | 0.245 |
| Wichita × PI372129 [a] | *Dn4* | 150 | 119 | | 31 | 3.8:1 | 3:1 | 1.502 | 0.230 |
| Wichita × PI372129 [b] | *Dn4* | 145 | 111 | | 34 | 3.3:1 | 3:1 | 0.186 | 0.689 |
| AL359 × PI24378 [a] | *Dn6* | 189 | 144 | | 45 | 3.2:1 | 3:1 | 0.143 | 0.718 |
| | *Xgwm111* | 189 | 145 | | 44 | 3.3:1 | 3:1 | 0.298 | 0.602 |
| Wichita × PI243781 [a] | *Dn6* | 137 | 110 | | 27 | 4.1:1 | 3:1 | 2.046 | 0.172 |
| | *Xgwm111* | 137 | 40 | 72 | 25 | 1:1.8:0.6 | 1:2:1 | 3.642 | 0.179 |
| | *Xgwm44* | 137 | 43 | 66 | 28 | 1:1.5:0.7 | 1:2:1 | 3.467 | 0.193 |
| Wichita × PI243781 [a] | *Dn6* | 194 | 155 | | 39 | 4.0:1 | 3:1 | 2.481 | 0.124 |
| Wichita × PI243781 [b] | *Dn6* | 197 | 153 | | 44 | 3.5:1 | 3:1 | 0.746 | 0.415 |

[a] $F_2$ progeny derived from the $F_1$ wheat plants that were artificially infested with RWA for 20 days (all showed resistant) at the seedling stage

[b] $F_2$ progeny derived from un-infested $F_1$ wheat plants

[c] Phenotype or genotype: $X_1X_1$ = homozygous resistant, $X_2X_2$ = homozygous susceptible, $X_1X_2$ = heterozygous (if not detectable, then grouped together with $X_1X_1$)

[d] $P < 0.05$ = significant deviation from the expected segregation ratio, $P > 0.05$ = fit to the expected segregation ratio of the $F_2$ population

promotes gamete competition under the selection pressure of RWA infestation.

Significant deviations from expected Mendelian segregation ratios of molecular markers or physical loci have been reported on chromosomes 1D, 3D, 4D, 5D and 7D (near the centromere) in *Aegilops tauschii*, the diploid D-genome progenitor of bread wheat (Faris et al. 1998; Boyko et al. 1999). Deviation phenomena and distortion loci have also been reported in other species, and the loci were regarded as segregation distorters, distortion factors, distortion genetic elements, and genetic selection factors (Lyttle 1991; Faris et al. 1998), but they have not been identified as associated with genes of specific function. Our present results provide some evidence of the distorted segregation of RWA resistance genes in RWA-infested or the induced wheat population. Further investigation will be necessary to demonstrate the mechanism.

## Microsatellite polymorphism and marker inheritance

The majority of documented microsatellite markers have been shown to exhibit a co-dominant inheritance (Weissenbach et al. 1992; Rafalski and Tingey 1993; Roder et al. 1998). However, our previous results (Liu et al. 2001) and the results of the present study indicate that microsatellite polymorphism among $F_2$ plants and marker inheritance were basically determined by the parents used for a cross as well as the primer pair of the marker.

Microsatellite primer pair GWM111 amplified RWA resistance-related fragments in the resistance sources of *Dn1*, *Dn2*, *Dn5* and *Dnx*, and the respective resistant $F_2$ progeny, but no corresponding fragments were detected in the susceptible parents and susceptible progeny. In this case, microsatellite marker *Xgwm111* was inherited in a dominant manner as evidenced by only resistance related bands (Liu et al. 2001). Although *Xgwm44* and *Xgwm111* are close neighbors on chromosome 7DS, GWM44 detected no polymorphism between the resistant parents of *Dn1*, *2*, *5* and *Dnx*, and the respective susceptible parents we used (data not shown).

In the present study, GWM111 amplified a dominant resistance-related fragment in the $F_2$ population derived from AL359 (S) × PI 243781 (*Dn6*), and both GWM44 and GWM111 detected co-dominant bands in the $F_2$ population derived from Wichita (S) × PI 243781 (*Dn6*) (Figs. 3, 4, 5). In the $F_2$ population derived from Thunderbird (S) × PI 372129 (*Dn4*), GWM106 amplified a dominant R-related fragment, while GWM337 detected co-dominant bands (Figs. 1, 2).

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 another, but with no apparent alteration in any other fragments. Missing amplification products also occurred in 22 out of 40 closely related wheat cultivars and lines amplified with primer pair GWM106 (Plaschke et al. 1995). A possible explanation for the dominance of microsatellite markers with null-alleles is the nucleotide-sequence alterations within the priming recognition sites of susceptible plants. These alterations are likely to be due to point mutations, insertions, deletions or inversions, which lead to primer mismatches, and the absence of the corresponding PCR band. The co-dominance of microsatellite markers may be due to alterations in fragment length between the two priming recognition sites, such as the deletion or insertion of repetitive DNA sequences, which can lead to different numbers of repeats within the microsatellite region (Bowcock et al. 1994).

## Linkage or allelic relationships among *Dn1*, *2*, *5*, *6* and *Dnx*

In the present study, we report the first mapping of *Dn6* in PI 243781 to a specific wheat chromosome locus. The microsatellite marker *Xgwm111* is tightly linked to *Dn6* at a distance of 3.0 ± 1.3 cM, and mapped *Dn6* to the short arm of wheat chromosome 7D near the centromere. *Xgwm111* is also tightly linked to *Dn1* in PI 137739, *Dn2* in PI 262660, *Dn5* in PI 294994, and *Dnx* in PI 220127 at distances of 3.8 ± 2.0, 3.1 ± 1.8, 3.2 ± 1.6, and 1.5 ± 1.5 cM, respectively (Liu et al. 2001). The linkage distance and standard errors may vary in different $F_2$ mapping populations. Thus *Dn6* is either tightly linked, or allelic, to *Dn1*, *2*, *5* and *Dnx*. Further allelism tests are in progress to determine the exact relationship. This result conflicts with the finding of Saidi and Quick (1996) who reported that *Dn6* was independent of *Dn1* and *Dn2*, as $F_2$ plants from crosses of PI 243781 with PI 137739 (*Dn1*), PI 262660 (*Dn2*), and PI 372129 (*Dn4*) segregated in 15R:1S ratios. As a result, the RWA resistance gene in PI 243781 was designated as a new gene, *Dn6* (Saidi and Quick 1996).

It is critical that the parents used to test for allelic relationships must be absolutely pure and homozygous. Otherwise, conclusions about allelism may be incorrect. The *Dn1* and *Dn2* genes were proposed as separate genes for PI 137739 and PI 262660, respectively, as Du Toit (1989) reported an $F_2$ segregation ratio of 266R:20S (15:1) from the cross PI 137739 × PI 262660. However, Du Toit (1989) found two susceptible plants out of 20 plants of PI 262660, and showed that the $F_1$ plants derived from the cross of PI 262660 × Tugela(S) segregated in a ratio of 93R:8S, indicating that the PI 262660 seed source was either heterozygous or heterogeneous (mixed). The seed homozygosity or purity of PI 243781(*Dn6*) that we used in the present study is reliable (USDA/ARS National Small Grains Research Facility). All the tested 52 $F_1$ plants derived from the cross of PI 243781 (male) × Wichita or AL359 (female) were resistant to RWA, which indicated that the resistant source in this study is pure and homozygous.

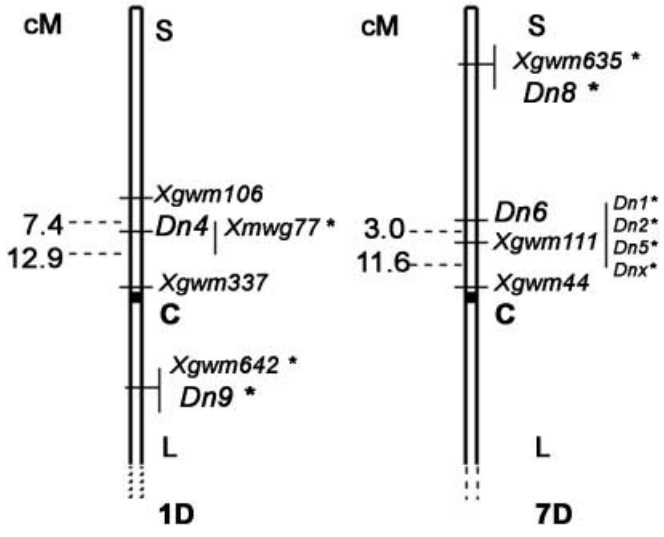


**Fig. 5** Genetic linkage maps of RWA resistance genes (*Dn*) and the linked molecular markers on wheat chromosomes 1D and 7D. *S*, *L* = short or long chromosome arm, *C* = centromere position. *The orientation between markers and genes is unresolved

## Genetic mapping of *Dn4* and *Dn6*

The identified chromosome locus-specific microsatellite markers linked to *Dn4*, *Dn6*, and other *Dn* genes, provide a powerful tool to accurately map RWA resistance genes to specific chromosome loci. The genetic linkage maps of *Dn* genes on chromosomes 1D and 7D, including *Dn4* and *Dn6* from the present study, previously tagged *Dn* genes (Liu et al. 2001), and the linked markers are shown in Fig. 5. The maps were integrated into the previously published microsatellite framework map (Roder et al. 1998) by adjusting *Xgwm111* from 7DL to 7DS (Liu et al. 2001). A RFLP marker *Xmwg77* is inferred as being tightly linked to *Dn4* by comparing the relative genetic map position of *Xmwg77*, which is between *Xgwm106* and *Xgwm337* (Roder et al. 1998).

## Concluding remarks

Microsatellite marker *Xgwm111* is closely linked to *Dn6* at 3.0 cM on wheat chromosome 7DS in the same area as *Dn1*, *Dn2*, *Dn5* and *Dnx*. *Xgwm44* is distantly linked to *Dn6* at 14.6 cM on 7DS. Linkage to *Dn4* was obtained with *Xgwm106* and *Xgwm337* at 7.4 cM and 12.9 cM, respectively, on chromosome 1DS. The closely linked marker *Xgwm111* can be used in marker-assisted selection (MAS) of *Dn6* for RWA-resistant lines in wheat breeding programs. Although the linkage for either *Xgwm106* or *Xgwm337* to *Dn4* is not close enough for MAS, these two markers are very useful if they are combined to tag *Dn4* for MAS. Work is currently in progress to pyramid *Dn4* and *Dn6* into a single wheat genotype using the linked markers.

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