

# Gene discovery in recombinant doubled haploid populations for breeding wheat resistance against aphids

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## ABSTRACT

Russian wheat aphid (RWA) (*Diuraphis noxia*) and greenbug (*Schizaphis graminum*) are two devastating pests of wheat worldwide. In Argentina another new aphid pest, *Sipha maydis*, infesting wheat and barley, appeared in 2002. These aphids cause severe damage to plant growth and production at the seedling and adult plant stages. RWA and greenbug have evolved several biotypes virulent to most of the resistance genes introduced into wheat cultivars. The fast deployment of resistance genes and the continuous appearance of new biotypes and pests requires the assessment of new sources of resistance for breeding plant-defence.

Resistance to aphids consists of three mechanisms: antixenosis, which prevents the insect selection of plant hosts; antibiosis, which imposes a reduced aphid longevity and fertility; tolerance, which allows the host to maintain a normal growth rate under infestation. Marker assisted genetic analysis and the use of recombinant doubled haploid (RDH) lines and RILs has allowed the mapping of novel resistance genes for Argentinean populations of aphids. Resistance QTLs against greenbug have been mapped onto several chromosomes. Antixenosis QTLs were located on chromosome 6A of a CS x Synthetic set of RDH, and on chromosome 7D of the ITMI RILs. QTLs for tolerance and antibiosis have been mapped onto chromosome 1B of the ITMI RIL population and 7D of the CS x Synthetic set of RDH. Resistance to RWA was accounted for by QTLs on different chromosomes. QTL for antixenosis to biotype 2 were located on chromosome 6A and tolerance and antibiosis on 1D and 7D of the CS x Synthetic set of RDH. Most of the QTLs for tolerance traits to *S. maydis* were mapped on the homoeologous group 1 and 2 chromosomes. These novel genes could be transferred into wheat cultivars by marker-assisted selection to enlarge the genetic base of defence against the aphid pests.

## INTRODUCTION

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko) and greenbug, *Schizaphis graminum* (Rond.), are the major insect pests of wheat worldwide, except for Australia (Burd et al. 2006). Both species inject toxins that breakdown chloroplasts (Voothuluru et al. 2006), and their attack leads to death of susceptible wheats if infestation is uncontrolled. Greenbug also damages systemically susceptible cultivars inhibiting the

phosphate influx and transport, and the differentiation of new roots and leaves (Castro et al. 1988; Giménez et al. 1997). RWA infested plants usually develop white, yellow, or purple longitudinal streaks on leaves and stems, exhibit rolled leaves, and often display a prostrate growth habit, with a reduced photosynthetic efficiency, a lower plant vigor, and in adult plants the emerging awns are trapped by the rolled flag leaf (Joyti et al. 2006). *S. maydis* is a new pest introduced into South America in 2002. This aphid infests the flag leaf and inhibits ear expansion. The young leaves of infested wheat and barley become chlorotic, which results in reduced plant growth and flag leaf expansion with a consequent decrease in yield (Corrales et al. 2006).

The most effective and environmentally sound strategy for controlling aphid damage is breeding for resistance. Plant resistance based on combinations of the different types of mechanisms of resistance is useful for controlling these pests and for preventing the occurrence of new aphid biotypes. Six genes for resistance have been introduced into wheat to control greenbug and another eleven to control RWA. No resistance gene against *S. maydis* has been reported yet. RWA and greenbug resistance genes are located on the 1A, 1D, 7D chromosomes and on the 1RS/1BL translocation (McIntosh et al. 2003). The chromosome location of aphid resistance genes in wheat is still very limited. Intervarietal single chromosome substitution lines have allowed the identification of wheat chromosomes carrying different types of aphid resistance (Castro et al., 1999, 2001). Mapping of recombinant doubled haploid (RDH) substitution lines and the ITMI RIL population, have permitted the identification and location of greenbug, RWA and *S. maydis* antixenosis, antibiosis and tolerance resistance genes.

## MATERIALS AND METHODS

**Aphids:** Greenbug, RWA and *S. maydis* isolates were collected on bread wheat growing in the fields of Buenos Aires and Córdoba provinces during the late spring between 2003 to 2007. The aphids were reared continuously on a susceptible wheat cultivar 'Buck Poncho' in a plant growth cabinet maintained at 20°C, 50% humidity, and 16:8 h day: night regime.

**Plant material:** Four sets of recombinant doubled haploid (RDH) lines involving variation for a single chromosome were derived from the F<sub>1</sub> of "Chinese Spring" (CS) and "Chinese Spring (Synthetic)" substitution lines and used as mapping populations. These precise genetic stocks of 80, 120, 85 and 110

RDH lines were for chromosomes 1B, 7D, 6A and 1D and were developed using the maize cross technique, at the John Innes Centre, Norwich, UK, and at the IPK, Gatersleben, Germany. The four sets of RDH lines and the ITMI population of 117 RILs were screened for antixenosis, antibiosis and tolerance types of resistance against greenbug and RWA.

Another set of 110 RDH lines, developed at the John Innes Centre, UK, was tested for resistance against *S. maydis*.

#### Procedures

**Antixenosis:** The experiments were performed by allowing aphids a free choice among plants at a similar growth stage (second fully expanded leaf). The trials consisted of 10 replicates for each genotype in every population. The number of adult aphids on every plant was recorded 24 h after infestation (Castro et al. 2001, 2005).

**Antibiosis:** Two first instar nymphs were placed on each genotype and allowed to develop into adults. Nymphs were observed every day, and the time from birth to adulthood (d), and the daily reproduction were recorded until adults ceased reproducing. The total fecundity (TF), the longevity (L) and d were used as measures of the antibiotic type of resistance (Castro et al., 2004).

**Aphid Tolerance:** Germinating seeds of each RDH line or RIL and the parental lines were sown in plastic trays and maintained under natural conditions of light and temperature in a glasshouse, in La Plata, Argentina (34° 55' SL, 57° 57' WL). At the 2nd fully expanded leaf stage, every plant in half of the trays was infested with 10 adult aphids. The rest of the trays were kept uninfested as controls. The Foliar Area (FA), the Aerial Fresh Weight (AFW), the Aerial Dry Weight (ADW), the Root Fresh Weight (RFW), the Root Dry Weight (RDW), the Chlorophyll content (CI), the Total Number of Leaves (TNL) and the Expanded Leaves (EL) were evaluated weekly and the changes for plant growth traits were calculated. The relative growth was determined as the difference between the mean value of the infested plants and that registered on the controls for every plant trait.

All experiments were conducted as randomized complete blocks. The data were analyzed by ANOVA using PROC GLM (SAS 1998), and the Tukey Multiple range Test was used to test the differences between means.

**Mapping analysis:** For mapping, wheat microsatellites located on the different chromosomes were chosen using published data. Primer sequences, fragment sizes and the chromosome arm locations of microsatellite markers were published by Röeder et al. (1998). The programs MAPMAKER V 3.0 (Lander et al. 1987), and QTL Café (<http://www.biosciences.bham.ac.uk/labs/kearsey>) (Seaton et al. 2002) were used for QTL discovery and location, using single marker ANOVA, marker

regression and interval mapping approaches on the means of the traits from ANOVA analyses.

## RESULTS AND DISCUSSION

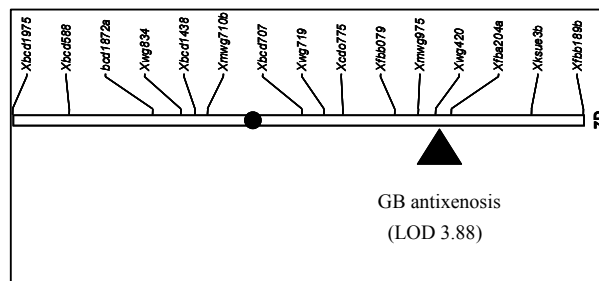
Initial analysis with molecular markers showed that antixenosis, antibiosis and tolerance to greenbug and RWA were not based on single major genes. Sixteen QTLs conferring antixenosis, antibiosis and tolerance to greenbug, RWA and *S. maydis* were located on the RDH and RIL sets (Table 1).

Table 1: QTLs identified for antixenosis antibiosis and tolerance to greenbug, RWA and *S. maydis* in the ITMI RILs and in five populations of RDH

Trait	Aphid	Chromosome/ Stock
Antixenosis	Greenbug	7D/ ITMI 6A / CS(Syn6A)
	RWA	
	Biotype 1	7D/ CS(Syn7D)
	Biotype 2	6A/ CS(Syn6A)
Antibiosis	Greenbug	7D/ CS(Syn7D)
	RWA	7D/ CS(Syn7D)
TF Longevity	RWA	7D/ CS(Syn7D)
	Greenbug	1B/ ITMI 7D/ CS(Syn7D)
	RWA	7D/ CS(Syn7D)
Tolerance	Greenbug	7D/ ITMI
	RWA	1D/ CS(Syn1D) 7D/ CS(Syn7D)
	<i>S. maydis</i>	1A/ S x R 1B/ S x R 2A/ S x R 2D/ S x R

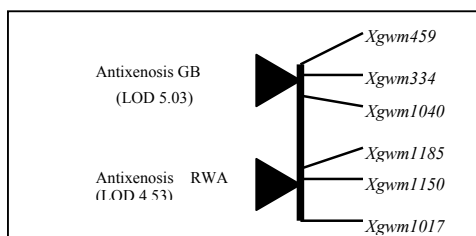
Variation for antixenosis to greenbug was significantly associated with 7D marker loci in the ITMI RIL population (Fig 1) and to 6A markers in the CS(Syn6A) set (Fig 2). The increasing alleles for antixenotic resistance in both mapping populations were provided by the ‘Synthetic’ parent.

Figure 1: Linkage map of chromosome 7D (ITMI set) showing the location of quantitative trait loci (QTLs) determining antixenosis to greenbug



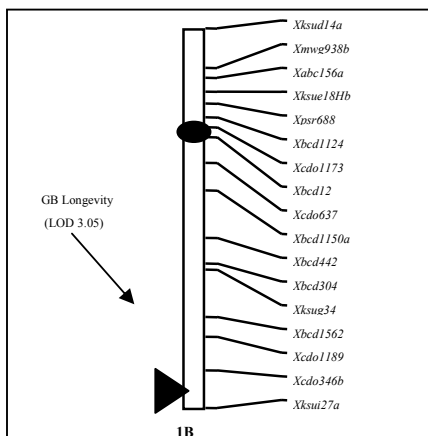
Antixenosis against RWA biotype 1 was significantly associated with 7D [CS(Syn7D)] marker loci, and antixenosis against RWA biotype 2 was linked to 6A markers (Fig 2). Greenbug longevity was significantly reduced in those aphids hosted by genes on chromosome 1B of the ITMI population and genes on chromosome 7D [CS(Syn7D)]. The L was significantly associated with loci on 1B (Fig 3) and 7D. The variation for greenbug d was explained by the same QTL located on

**Figure 2:** Linkage maps of chromosome 6A showing the location of quantitative trait loci (QTLs) determining antixenosis to greenbug and Russian wheat aphid.



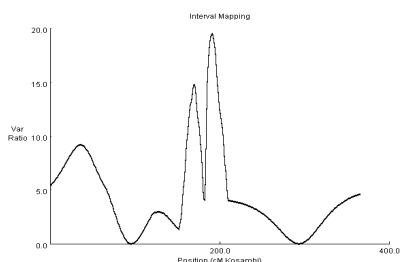
CS(Syn7D). The increasing alleles for antibiotic resistance were provided by ‘Synthetic’ parent in both sets.

**Figure 3:** Linkage map of chromosome 1B of the ITMI population of RILs showing the location of quantitative trait loci (QTLs) determining antixenosis against greenbug by means of aphid longevity.



Antibiosis to RWA was significantly associated with 7D [CS(Syn7D)] molecular markers (Fig. 4) with the increasing alleles provided by ‘Synthetic’.

**Figure 4:** QTL interval mapping profile for antibiosis against RWA by means of the total fertility (total number of nymphs born from each aphid) on chromosome 7D [CS(Syn7D)].



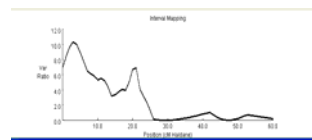
Most of the tolerance traits against greenbug and RWA biotype 1 were associated with 7D (ITMI) and with CS(Syn1D) and CS(Syn7D) molecular markers, respectively (Table 1).

The tolerance traits to *S. maydis* showed significant associations with molecular markers on chromosomes 1A, 1B, 2A and 2D of the Spark x Rialto set of RDH lines (Fig. 5).

The sixteen genes found, providing different types of resistance against aphids are novel and not allelic to other aphid resistance genes. Their use can enable the creation of new lines, resistant to aphids. Their

introduction into other genotypes already carrying other greenbug and/or RWA resistance genes will result in gene pyramiding.

**Figure 5:** QTL interval mapping profile for tolerance to *S. maydis* by means of the Foliar Area on chromosome 1B of the Spark x Rialto set of RDH lines



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