# A Microsatellite Marker for Yellow Rust Resistance in Wheat

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Bulk segregant analysis (BSA) was used to identify molecular markers associated with yellow rust disease resistance in wheat (*Triticum aestivum* L.). DNAs isolated from the selected yellow rust tolerant and susceptible $F_2$ individuals derived from a cross between yellow rust resistant and susceptible wheat genotypes were used to established a "tolerant" and a "susceptible" DNA pool. The BSA was then performed on these DNA pools using 230 markers that were previously mapped onto the individual wheat chromosomes. One of the SSR markers *(Xgwm382)* located on chromosome group 2 (A, B, D genomes) was present in the resistant parent and the resistant bulk but not in the susceptible parent and the susceptible bulk, suggesting that this marker is linked to a yellow rust resistance gene. The presence of *Xgwm382* was also tested in 108 additional wheat genotypes differing in yellow rust resistance. This analysis showed that 81% of the wheat genotypes known to be yellow rust resistant had the *Xgwm382* marker, further suggesting that the presence of this marker correlates with yellow rust resistance in diverse wheat germplasm. Therefore, *Xgwm382* could be useful for marker assisted selection of yellow rust resistances genotypes in wheat breeding programs.

**Keywords:** marker assisted selection, disease resistance, SSR marker, wheat

## Introduction

Wheat yellow rust caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* is an important disease of wheat (*Triticum aestivum* L.) in many parts of the world (Wang et al.

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2008). Growing resistant wheat cultivars is the most economical and environmentally safe approach to eliminate the use of fungicides and to reduce crop losses due to yellow rust.

Seedling resistances to yellow rust are mostly race-specific and are conditioned by the interaction of resistance alleles in the host and avirulence alleles in the pathogen (Torabi and Nazari 1998). Adult plant resistance which has been reported to be more durable than seedling resistance (Dyck and Kerber 1985) can also be race-specific (McIntosh et al. 1995). Genes for adult plant resistance which are effective against a wide spectrum of rust pathotypes can provide horizontal resistance (Robinson 1976).

With the use of microsatellite markers and a genetic linkage map, various wheat genes that control yellow rust resistance have been successfully tagged (Prasad et al. 2003). In this study, we have used the bulk segregant analysis (BSA) technique (Michelmore et al. 1991) in combination with microsatellites to identify potential molecular marker(s) associated with yellow rust resistance in wheat. These markers shall be used for selecting yellow rust resistance in segregating populations. A number of genes affect yellow rust resistance in wheat. So far, 40 resistance genes at 37 loci *(Yr1-Yr37)* and 23 temporarily designated genes have been identified (McIntosh et al. 2005). Molecular markers have been linked to many yellow rust resistance genes in wheat, such as *Yr5, Yr10, Yr15, Yr17, Yr24, Yr26, Yr29, Yr32, Yr34* and *YrH52* (Chague et al. 1999; Robert et al. 2000; Peng et al. 2000; Sun et al. 2002; Wang et al. 2002; Zakari et al. 2003; William et al. 2003; Eriksen et al. 2004; Bariana et al. 2006; Wang et al. 2008). These markers provide an important tool to plant breeders for marker-aided wheat breeding and also for pyramiding resistance genes in the absence of distinguishable rust virulences (Kaur et al. 2008).

A microsatellite map of wheat containing 279 loci amplified by 239 primer sets (Röder et al. 1998) is available as a valuable resource for tagging and mapping genes that control a number of important traits (Schmidt et al. 2004; Khlestkina et al. 2007; Niu et al. 2008). In this study, we used these primer sets to identify markers linked to yellow rust resistance genes in wheat by bulk segregant analysis. Here, we report the identification of *Xgwm382* marker that is associated with yellow rust resistance and can potentially be used to select yellow rust resistant wheat germplasm.

## Materials and Methods

*Evaluation of Yellow Rust Resistance:* A cross between the yellow rust resistant and susceptible Turkish wheat cultivars, Izgi2001 and ES14, respectively, was made in the wheat breeding program of the Anatolian Agricultural Research Institute (AARI). The $F_2$ individuals derived from the cross were evaluated for yellow rust resistance at both seedling stage in the greenhouse and adult stage in the field. For the inoculations, uredospores were collected from the experimental research sites of the Central Research Institute of Field Crops (CRIFC). Inoculum is virulent for *Yr2, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr17, Yr18, Yr27, YrA+* and avirulent for *Yr1, Yr5, Yr10, Yr15, Yr24, YrSP, YrCV* genes. The most resistant and susceptible $F_2$ progenies at the seedling and adult stage were selected after 15–20 days following inoculations. The infection type was recorded using the 0–9 scale of

McNeal et al. (1971) at seedling stage and 0–100 scale of Cobb at adult stage (Peterson et al. 1948).

*Bulked segregant analysis (BSA):* Leaf tissues were ground using the Retsch MM301 and total genomic DNA was extracted as described by Weining and Langridge (1991). Aliquots of DNA from 27 resistant and 27 susceptible plants from $F_2$ segregating population were mixed, respectively, to produce resistant and susceptible bulks of both growing stages for BSA. A 230 microsatellite markers distributed onto the A, B and D genomes (Röder et al. 1995, 1998) were initially screened in the parents. For polymorphic markers, the bulks were also taken into analysis.

PCR for microsatellite analysis, contained 100 ng of genomic DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP (MBI Fermentas, Germany), 0.25 μM of each primer, and 0.5U Taq polymerase (MBI Fermentas, Germany). Amplification was performed in Applied Biosystems Gene Amp PCR System 9700 thermocycler as follows; 3 min at 94 °C; 40 cycles with 1 min at 94 °C, 1 min at 50, 55, 60 °C (depending on the suggested annealing temperature), 1 min at 72°C, followed by 10 min final extension at 72 °C. The PCR products were separated on 2% agarose gel (SeaKem) and visualized under UV light after ethidium bromide staining. Band sizes were determined by comparison with a 50 bp DNA ladder (MBI Fermentas). Putative polymorphisms among bulks were checked by repeated amplifications, and all individuals contributing to the resistant and susceptible bulks were tested separately.

*Fragment analysis by using fluorescence-based capillary electrophoresis in resistant and susceptible genotypes:* Fluorescently labelled forward primer was synthesized according to GenomeLab GeXP System manufacturer's (Beckman Coulter) instructions. PCR mixtures were prepared as described previously, except fluorescently labelled forward primer was included instead of the unlabeled one. The electrophoretic separation was carried out on GenomeLab GeXP System and the data were analysed by the "GeXP Software for Fragment Analysis Module" Version 10.

## Results

*Selection of $F_2$ individuals based on their response to yellow rust:* To establish resistant and susceptible bulks that can be used in BSA to tag yellow rust resistance in wheat; we first undertook disease inoculation assays on the parental genotypes. In the greenhouse assays, the disease score of Izgi2001 (resistant parent) was "0" while that of ES14 (susceptible parent) was "8". In the field assays, CI (co-efficient of infection) value of Izgi2001 was "0" while that of ES14 was "80". These assays confirm that the parental lines greatly differ in their resistance to yellow rust and the rust resistance phenotype of Izgi2001 was detectable in both seedling and the adult plant stages. Infection type and CI values of selected resistant (R) $F_2$ individuals were between "0" and "1" at seedling and score at adult plant stage, while susceptible (S) $F_2$ individuals were "8–9" at seedling stage and "60–90" at adult plant stage, respectively.

*Screening of bulks with wheat microsatellite primers*: Two hundred and thirthy microsatellite primer pairs were initially tested to see whether they reveal polymorphic bands between the resistant (Izgi2001) and susceptible (ES14) parents. One hundred and thirteen primer pairs (49.1%) amplified only monomorphic fragments in Izgi2001 and ES14. Most of the remaining primer pairs (112; 48.7%) produced polymorphic amplification products between two parents. These polymorphic primers were also screened against resistant and susceptible bulks of both growing stages. Except for *Xgwm382,* other primer pairs did not produce such polymorphic band profiles. *Xgwm382,* assigned to chromosome group 2 [F: 5' -GTC AGA TAA CGC CGT CCA AT –3'; R: 5'- CTA CGT GCA CCA CCA TTT TG –3' with $T_m$: 60 ºC] amplified a DNA fragment of 118 bp that was present in the resistant parent and the resistant bulks but not in the susceptible ones, which implied the association between the *Xgwm382* microsatelite locus and yellow rust resistance. The 118 bp fragment, indicated by an arrow, was present in 25 out of 27 individuals in the resistant bulks of both stages (Fig. 1A, B). This fragment was absent in 24 $F_2$ individuals of seedling stage and 25 of adult stage out of 27 individuals (Fig. 2A, B). New generation fluorescence-based capillary electrophoresis system was also used for the verification of the exact sizes of fragments generated by the *Xgwm382*. Figure 1A shows the fragment profile of the Izgi2001 with two peaks labelled as 86.99 bp and 118.52 bp while Fig. 2B indicates that of the ES14 with two peaks 87.05 bp and 90.93 bp.

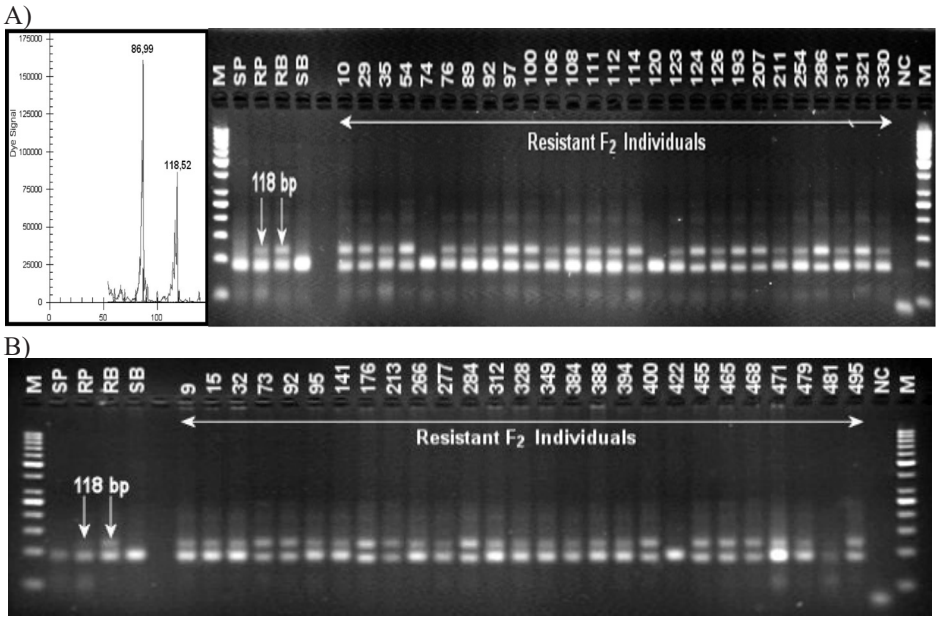


*Figure 1.* The microsatellite reaction amplified by *Xgwm382* primer pair produced 118 bp fragment indicating the yellow rust resistant phenotype at seedling (A) and adult (B) stages. The corresponding fragment was shown by capillary (left) and agarose gel (right) electrophoresis. M: 50 bp DNA Ladder, SP: Susceptible parent (ES14), RP: Resistant parent (Izgi2001), RB: Resistant bulk, SB: Susceptible bulk, NC: Negative control (free of DNA)

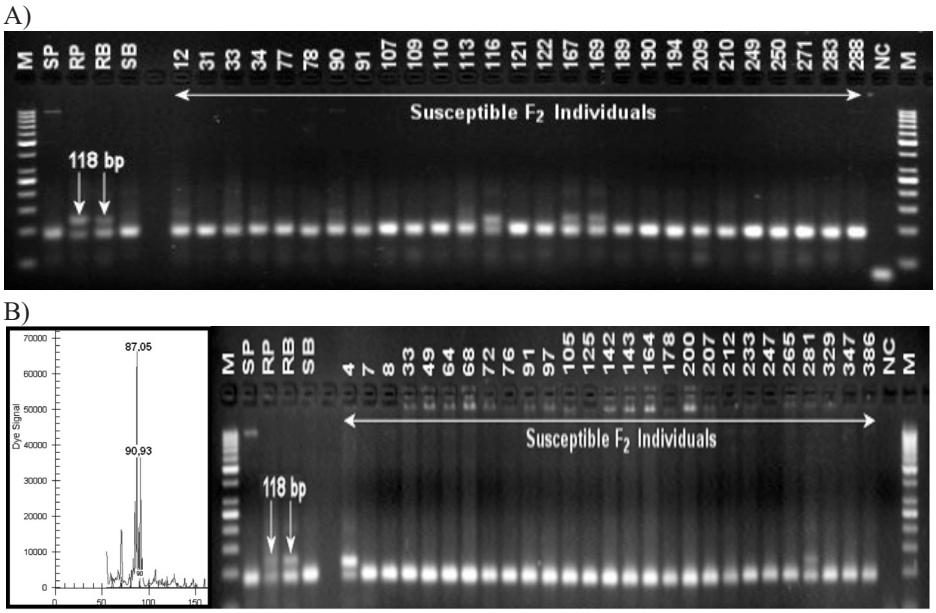


*Figure 2*. The absence of 118 bp fragment indicating the yellow rust susceptible phenotype at seedling (A) and adult (B) stages was shown by capillary (left) and agarose gel (right) electrophoresis. M: 50 bp DNA ladder, SP: Susceptible parent (ES14), RP: Resistant parent (Izgi2001), RB: Resistant bulk, SB: Susceptible bulk, NC: Negative control (free of DNA)

*Segregation of the Xgwm382 locus:* In order to determine the inheritance of the *Xgwm382* locus, PCR amplification was performed additionally in 98 $F_2$ individuals of Izgi2001 × ES14. In this analysis, 78 resistant plants produced the 118 bp fragment *(Xgwm382)* while 20 plants did not, which fits a 3:1 ratio ($X^2$ test, $P = 0.25–0.50$). This result indicates that the tolerance in Izgi2001 revealed by *Xgwm382* is most likely controlled either by a dominant gene or the *Xgwm382* marker linked a major resistance gene for yellow rust.

*Screening of wheat genotypes using Xgwm382 marker:* A total of 108 wheat genotypes with known yellow rust resistance (38 resistant and 70 susceptible) provided by AARI and CRIFC were screened by using *Xgwm382* primer pair and a 118 bp fragment was observed in 81% of resistant genotypes while all the susceptible genotypes did not produce a fragment.

## Discussion

Tolerance to yellow rust is one of the most important objectives of wheat breeding programs in all wheat growing regions of the world. Resistance to rust diseases may comprise genes effective at both the seedling and adult growth stages, genes either effective at the post-seedling or adult stages only, or combinations of both types (Zwer and Qualset

1994). Genes effective at the seedling stage tend to contribute greater effects on the rust phenotype (Eriksen et al. 2004). Selection for increased resistance could be greatly accelerated if yellow rust disease resistance can be fixed in an early segregating generation through selection of resistant and susceptible genotypes. To ensure optimal cost-effectiveness, molecular markers used for marker-assisted selection should permit efficient screening of large populations (Huang and Röder 2004). Genetic associations of many microsatellite markers with stripe rust resistance genes have been reported (Bariana et al. 2002). In the present study, 230 microsatellite primer pairs reported by Röder et al. (1998) were used in BSA and one microsatellite marker, *Xgwm382,* was found closely linked to yellow rust disease resistance.

The *Xgwm382* amplified identical PCR fragment with the size of 118 bp in resistant parent and resistant $F_2$ individuals both for seedling and adult plant stage for yellow rust disease. Similar to our work, Khlestkina et al. (2007) identified *Xgwm533* as a diagnostic marker for non-specific adult plant disease resistance against stripe rust in spring wheat (a cross between 'Lgst. 79-74'-resistant and 'Winzi'-susceptible). The 117 bp allele of *Xgwm533* was found in about 35% of the cultivars analysed, however, none of them possessed the expected disease resistance. Thus, the utilization of *Xgwm533* as a diagnostic marker seems to be restricted to certain gene pools. In contrast to this, 118 bp allele of *Xgwm382* marker was found in approximately 81% of resistant Turkish wheat genotypes in our work and it appears that it linked to yellow rust resistance in diverse wheat germplasm.

*Xgwm382* also displays linkage to the powdery mildew resistance gene, *Pm4b,* PmPS5B (Zhu et al. 2005; Yi et al. 2008) and the leaf rust resistance gene, *Lr50* (Brown-Guedira et al. 2003). Development of markers for different resistance genes could be very important for pyramiding different resistance loci into one variety in wheat breeding. These results indicate that *Xgwm382* has a potential use for large scale screening of segregating populations for different fungal diseases.

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