RESEARCH ARTICLE

Chromosomal location of genomic SSR markers associated with yellow rust resistance in Turkish bread wheat (*Triticum aestivum* L.)

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Abstract

We have previously reported *Xgwm382* as a diagnostic marker for disease resistance against yellow rust in Izgi2001 × ES14 F_2 population. Among the same earlier tested 230 primers, one SSR marker (*Xgwm311*) also amplified a fragment which is present in the resistant parent and in the resistant bulks, but absent in the susceptible parent and in the susceptible bulks. To understand the chromosome group location of these diagnostic markers, *Xgwm382* and *Xgwm311*, in the same population, we selected 16 SSR markers mapped only in one genome of chromosome group 2 around 1–21 cM distance to these diagnostic markers based on the SSR consensus map of wheat. Out of 16 SSRs, *Xwmc658* identified resistant F_2 individuals as a diagnostic marker for yellow rust disease and provided the location of *Xgwm382* and *Xgwm311* on chromosome 2AL in our plant material.

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Introduction

Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating diseases of wheat throughout the world (Ma *et al.* 2001; Chen *et al.* 2002) and yellow rust epidemics have frequently been reported in Turkey and other countries (Kinaci and Kinaci 1991; Canihos *et al.* 1997; Chen 2005). Identification of yellow rust resistance genes and breeding of resistant varieties is an effective approach to minimizing wheat losses due to this disease. To date, more than 70 stripe rust resistance genes, officially or provisionally designated *Yr* for 'stripe rust', have been reported in wheat (McIntosh *et al.* 2003, 2007, 2009; Chen 2005; Cheng and Chen 2010). Most of these genes are race-specific and confer all-stage resistance, which can be detected at the seedling stage, but a few are expressed only at the adult plant stage. However, genes for resistance to yellow rust in many wheat cultivars are still unknown. Identification of resistance genes in wheat cultivars, even those overcome by new races of the yellow rust pathogen, is important for a better understanding of race changes and a better use of various resistance genes with various strategies (Chen 2005). Gene pyramiding, gene deployment and multiline cultivars were considered to be useful for prolonging race-specific resistance (McIntosh and Lagudah 2000). Only a few genes are effective in the seedling stage (Ma *et al.* 2001; Yang *et al.* 2003). Therefore, it is very important to identify new resistance genes for wheat breeding programmes.

The rapidly evolving technology of DNA markers helps to open a real possibility for developing functional markers as reliable genetic markers for use in plant breeding. Simple sequence repeats (SSRs) have become the preferred markers for the genetic analysis of cereals because

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they co-segregate with the trait and are therefore candidate markers for plant breeding (Hearnden *et al.* 2007) and they cover the whole genome of wheat, showing a much higher level of polymorphism and informativeness in hexaploid bread wheat (Chandna *et al.* 2010). The first microsatellite map in wheat possessed 279 microsatellites (Röder *et al.* 1998) and following this, 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 (Pestsova *et al.* 2000; Gupta *et al.* 2002). SSR markers have been reported for several yellow rust resistance genes, including *Yr5, Yr10, Yr15*, *Yr24* and *YrH52* (Peng *et al.* 2000; Sun *et al.* 2002; Wang *et al.* 2002; Zakari *et al.* 2003). Previously, we (Akfirat *et al.* 2010) identified *Xgwm382* as a diagnostic marker located on chromosome group 2 for disease resistance against yellow rust in Izgi2001 × ES14 F_2 population by Röder's *et al.* (1998) map. Several microsatellites have been identified which are linked to both insect and disease resistance genes in wheat (Chantret *et al.* 2000; Huang *et al.* 2000; Anderson *et al.* 2001; Liu X. M. *et al.* 2002).

In this study, we report a SSR marker, *Xgwm311*, linked to yellow rust resistance genes in the same F_2 population of Izgi2001 × ES14 using bulk segregant analysis (BSA) (Michelmore *et al.* 1991). In wheat, BSA was successfully applied mostly using RFLPs, RAPDs, SSRs and AFLPs to tag resistance genes for cereal cyst nematode (Eastwood *et al.* 1994), powdery mildew (Liu Z. *et al.* 2002; Mohler *et al.* 2005), leaf rust (William *et al.* 2003), *Septoria tritici* blotch (McCartney *et al.* 2003; Adhikari *et al.* 2004), or SSRs to map resistance genes for yellow rust (Börner *et al.* 2000; Ma *et al.* 2001), powdery mildew (Chantret *et al.* 2000; Huang *et al.* 2003) and to identify linked markers with yellow rust resistance (Ercan *et al.* 2010). The study has been undertaken to identify the chromosomal location of *Xgwm382* and *Xgwm311* markers in our plant material. Chromosomal localization will lead to the identification of genomic regions responsible for the expression of the trait of interest.

Materials and methods

Plant materials and disease scoring

A cross between the yellow rust resistant Izgi2001 and susceptible ES14 Turkish wheat (*Triticum aestivum* L.) cultivars was made in the wheat breeding programme of the Anatolian Agricultural Research Institute (AARI, Eskisehir, Turkey). The parental cultivars and F_2 generations were evaluated for yellow rust resistance at both seedling stage in the greenhouse and adult stage in the field by applying uredospores collected from the experimental research sites of the Central Research Institute for Field Crops (CRIFC, Ankara, Turkey). Disease scoring was conducted as Akfirat *et al.* (2010).

Microsatellite screening in combination with BSA

Leaf tissue samples were collected, frozen in liquid nitrogen, and ground to a powder using the Retsch MM301 system (Haan, Germany). Genomic DNA was exracted as described by Weining and Langridge (1991). The microsatellite analysis with 230 SSR primer pairs (Röder *et al.* 1998) to screen F_2 population of Izgi2001 × ES14 were performed by using BSA. BSA analysis was performed by mixing equal amounts of DNA from 30 resistant plants and 30 susceptible plants representing the resistant and susceptible bulks, respectively. PCR amplifications were performed in reaction mixture volumes of 25 μL, each containing 100 ng of genomic DNA, 1× PCR buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.25 μM of each primer, and 0.5 U *Taq* polymerase (MBI Fermentas, St Leon-Rot, Germany). Reactions were performed in Biorad Mycycler thermocycler (CA, USA) as follows; 3 min at 94°C, 1 min at 94°C, 1 min at 50, 55, 60°C (depending on the annealing temperature), 1 min at 72°C for 40 cycles with 10 min final extension at 72°C before cooling to 4°C. The products were separated on 2% agarose gels in a 0.5% TBE buffer. Putative polymorphisms among bulks and parents were checked by repeated amplifications and all the individuals contributing to the respective pools were tested separately.

Chromosomal location of Xgwm382 and Xgwm311

In a second study 17 microsatellite markers (table 1) mapped only in one genome of chromosome group 2 (A, B, D) around 1–21 cM from *Xgwm382* and *Xgwm311* were used in microsatellite analysis. PCR reactions were performed same as BSA.

Sequencing and fragment analysis

Sequence analysis of the fragment was performed on the isolated plasmid with M13-47 sequencing primer using GeXP GenomeLab Genetic Analysis System (Beckman Coulter, IN, USA) according to the manufacturer's instructions. Fluorescent-labelled forward primers were synthesized according to manufacturer's instructions for fragment analysis. PCR mixture was prepared as described previously except fluorescent-labelled forward primer was included instead of the unlabelled one. The fluorescently-labelled PCR products were mixed with size standard-400 and the volume was completed to 30 mm^3 with sample loading solution. The electrophoretic separation was performed by using GeXP GenomeLab Genetic Analysis System and the data was analysed by fragment analysis module of the system.

Statistical analysis

The numbers of plants of the two phenotypic categories (resistant and susceptible) found in the 94 F_2 individuals of Izgi2001 × ES14 were compared with theoretical Mendelian segregation ratios by a chi-square test, using the data for the

Table 1. SSR markers mapped only in one genome of chromosome group 2 (A, B, D) around 1–21 cM distance to *Xgwm382* and *Xgwm311*.

Marker	Chromosome group 2	Distance to *Xgwm382**	Markers	Chromosome group 2	Distance to *Xgwm382*
Xbarc76	A	9	*Xgwm539*	D	9
Xwmc658	A	0	*Xgwm349*	D	7
Xwmc332	B	21	*Xcfd239*	D	6
Xwmc627	B	20	*Xcfd161*	D	5
Xwmc361	B	13	*Xwmc167*	D	3
Xwmc317	B	8	*Xgwm320*	D	1
Xwmc356	B	3	*Xbarc59*	D	1
Xgwm526	B	6	*Xgwm301*	D	7

*Distances (cM) are based on consensus map of wheat according to the Somers *et al.* (2004).

observed values and the expected values, and the number of resistance genes was estimated.

Results

Selection of F_2 individuals based on their response to yellow rust

We first undertook disease inoculation assays on the parental genotypes to establish resistant and susceptible bulks that can be used in BSA to tag yellow rust resistance in wheat. The disease scores were performed as in Akfirat *et al.* (2010).

Screening of bulks with microsatellites

Two hundred and thirty microsatellite primer pairs released by Röder *et al.* (1998) were initially tested to see whether they reveal polymorphic bands between the resistant Izgi2001 and susceptible ES14 parents. One hundred and seventy three primer pairs (75.2%) amplified monomorphic fragments in Izgi2001 and ES14. The remaining primer pairs (51; 22.2%) produced polymorphic amplification products between two parents. These polymorphic primers were also screened against resistant and susceptible bulks. *Xgwm311* (F: 5′-TCA CGT GGA AGA CGC TCC-3′; R: 5′-CTA CGT GCA CCA CCA TTT TG-3′ with T_m: 60°C) amplified a DNA fragment of 142.31 bp that was present in the resistant parent and the resistant bulks but not in the susceptible ones, showing the association between the *Xgwm311* microsatellite locus and yellow rust resistance. While the 143 bp fragment was present in 28 out of 30 individuals in the resistant bulk (figure 1a), it was absent in 23 out of 30 F_2 susceptible individuals in the susceptible bulk (figure 1b).

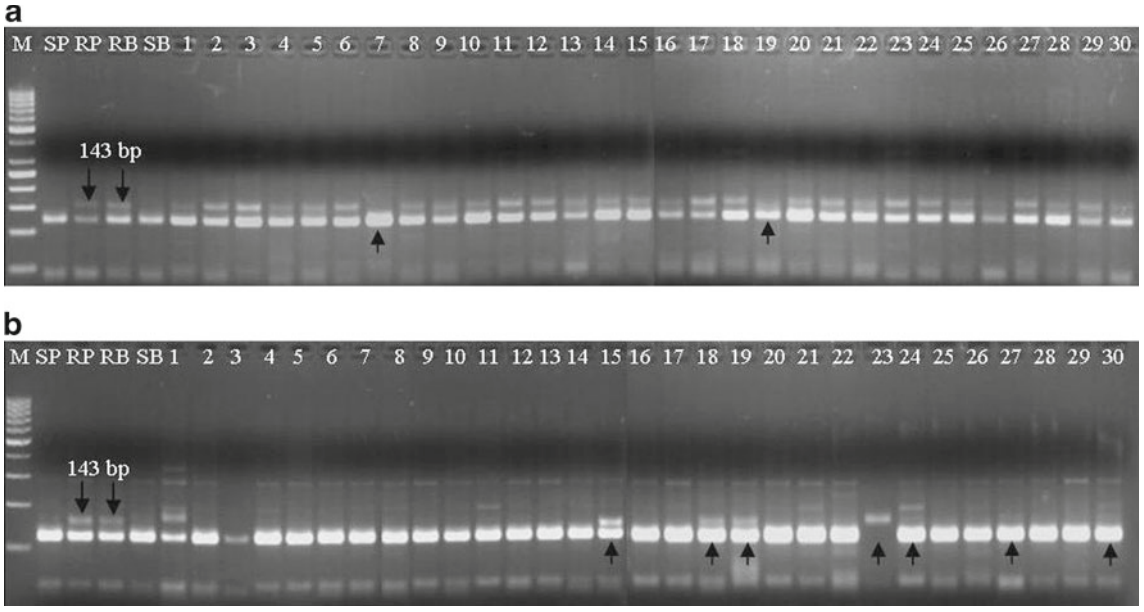

Figure 1. Amplification products of microsatellite PCR obtained by *XGWM311* primer pair in genomic DNA of the parents and (a) resistant and (b) susceptible F_2 hybrids (1 to 30). M, 50-bp DNA ladder; SP, susceptible parent (ES14); RP, resistant parent (Izgi2001); RB, resistant F_2 bulk; SB, susceptible F_2 bulk. Arrows show different samples in the F_2 bulks.

Identification of chromosomal location for Xgwm382 and Xgwm311

We screened the F_2 population of Izgi2001 × ES14 by 16 microsatellite markers (table 1) which were mapped only in one genome of chromosome group 2 (A, B, D) around 0–21 cM distance to *Xgwm382* and *Xgwm311* for BSA. Polymorphism was tested among resistant and susceptible bulked F_2 individuals together with their parental lines. *Xwmc658*, assigned to chromosome group 2A (F: 5′-CTC ATC GTC CTC CTC CAC TTT G-3′; R: 5′-GCC ATC CGT TGA CTT GAG GTT A-3′ with T_m: 60°C) amplified a DNA fragment of 181.99 bp that was present in the resistant parent and the resistant bulks but not in the susceptible ones, which shows the association between the *Xwmc658* microsatellite locus and yellow rust resistance. While the 181.99 bp fragment was present in 28 out of 30 individuals in the resistant bulks (figure 2a). This fragment was absent in the 30 selected susceptible plants (figure 2b). As a result of BSA, the two markers on chromosome 2A (*Xgwm311* and *Xwmc658*) produced polymorphic bands between the parents and F_2 populations.

Segregation of the Xgwm311 and Xwmc658 loci

To determine the inheritance of the *Xgwm311* and *Xwmc658* loci, PCR amplification was performed in 94 F_2 individuals of Izgi2001 × ES14. In this analysis, 71 resistant plants produced 142.31 bp fragment while 23 plants did not, which fits a 3:1 ratio (χ^2 test: 3.68, $P = 0.25$–0.50). This result demonstrates that the resistance in Izgi2001 revealed by *Xgwm311* is most likely controlled either by a dominant gene and/or the *Xgwm311* marker may link a major resistance gene for yellow rust. For *Xwmc658*, 83 resistant plants produced the 181.99 bp fragment while 17 plants did not, which also fits the ratio of 3:1, ($\chi^2 = 3.64$, $P = 0.05$).

Fragment analysis data

New generation florescence-based capillary electrophoresis system was used for the verification of the exact sizes of fragments generated by *XGWM311* and *XWMC658*. The fragment profile of the Izgi2001 with two peaks were labelled as 142.31 bp and 116.51 bp (figure 3a) while ES14 had a peak only 116.48 bp (figure 3b) amplified by *XGWM311*. DNA sequencing data for Izgi2001 amplified by *XGWM311* showed $(AG)_{26}$ bases. Fragment analysis of *Xwmc658* by florescence-based capillary electrophoresis in Izgi2001 was labelled as 181.99 bp (figure 4a) while ES14 had one peak, 205.29 bp (figure 4b). In Izgi2001, DNA sequencing amplified by *XWMC658* resulted in $(AG)_{22}$ bases.

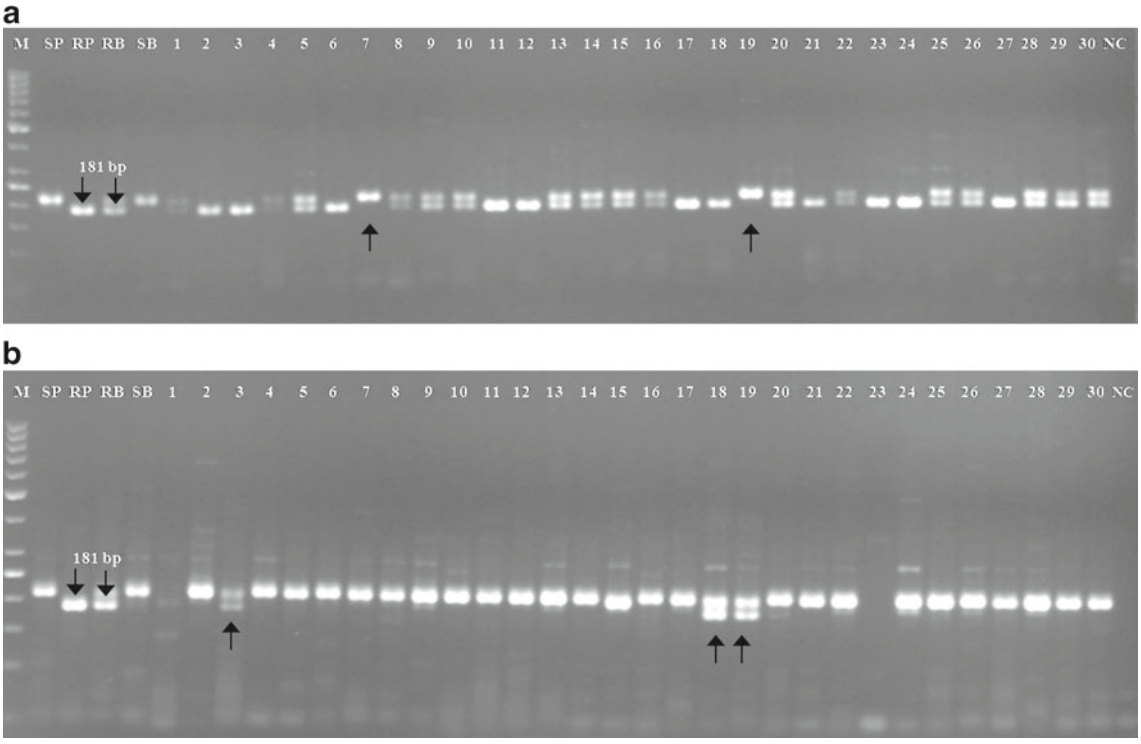

Figure 2. Amplification products of microsatellite PCR obtained by *XWMC658* primer pair in genomic DNA of the parents and (a) resistant and (b) susceptible F_2 hybrids (1 to 30). M, 50-bp DNA ladder; SP, susceptible parent (ES14); RP, resistant parent (Izgi2001); RB, resistant F_2 bulk; SB, susceptible F_2 bulk. Arrows show different samples in the F_2 bulks.

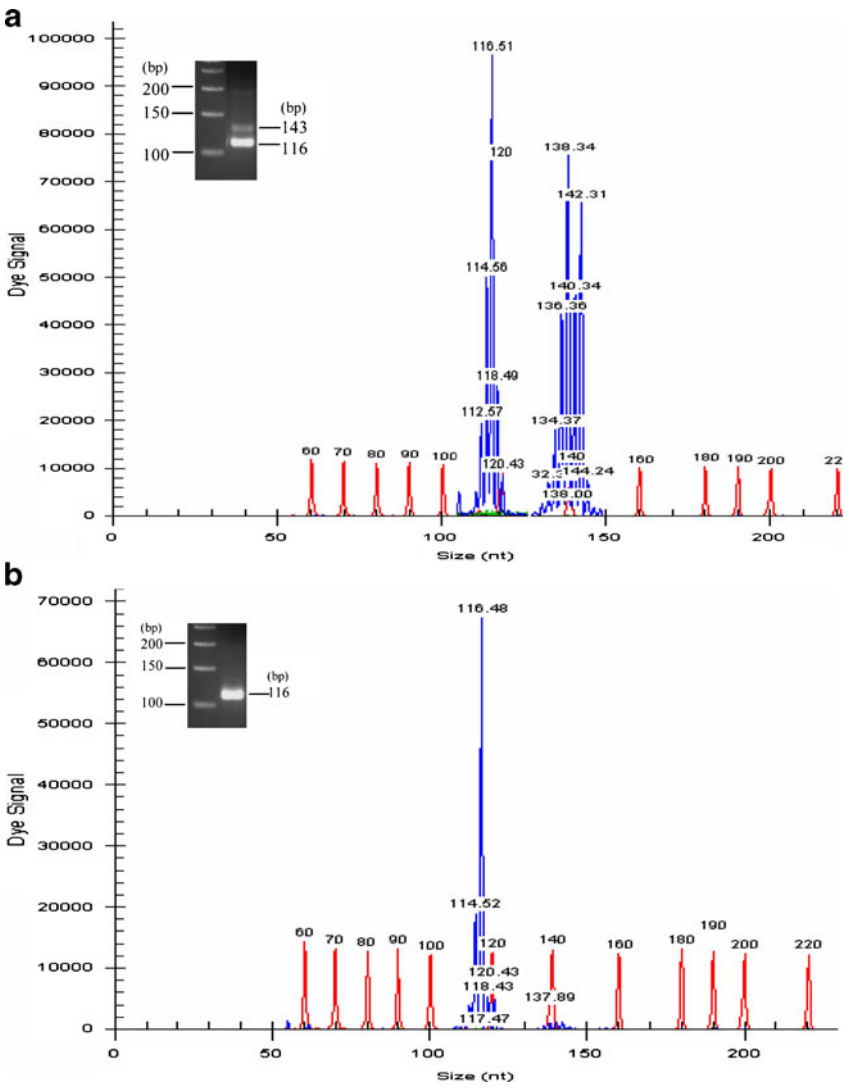

Figure 3. Fragment analysis of *Xgwm311* by fluorescence-based capillary electrophoresis in (a) resistant Izgi2001 and (b) susceptible ES14 parents.

Discussion

The breeding of resistant varieties is the key measure to control yellow rust disease, but the conventional breeding method is of low efficiency. MAS can significantly improve the breeding efficiency (Yu *et al.* 2004). A fundamental prerequisite for MAS application in conventional breeding is the availability of tightly linked DNA markers. This can dramatically increase the speed at which resistant varieties are developed and it can thus be an effective tool for plant breeding (Koebner and Summers 2003). Markers can be used to better characterize parental material, thereby improving the efficiency and effectiveness of parental selection for crossing and to track genes in segregating progenies through the selection process (William *et al.* 2007). A number of useful marker-trait associations have been reported for wheat, namely powdery mildew resistance (Chantret *et al.* 2000; Zhu *et al.* 2006; Liu *et al.* 2008), karnal bunt resistance (Kumar *et al.* 2007), leaf rust resistance (Gupta *et al.* 2006), septoria resistance (Adhikari *et al.* 2004), mycosphaerella resistance (Adhikari *et al.* 2003) and water stress tolerance (Altinkut and Gozukirmizi 2001; Altinkut *et al.* 2003).

SSR loci are associated with significant levels of sequence polymorphism and rust resistance genes and a growing number of SSR loci have been incorporated into the wheat genetic map (Röder *et al.* 1998; Somers *et al.* 2004). The majority of documented microsatellite markers are inherited in a codominant manner (Röder *et al.* 1998). However, the microsatellite markers *Xgwm311* and *Xgwm382*, linked to the powdery mildew resistance gene in Zhu *et al.* (2004) and Russian wheat aphid resistance in Liu *et al.* (2001), were inherited in a dominant manner, because they detected only resistance-related bands and segregation of the presence or absence of resistance band in tested segregating population fitted a 3:1 ratio. So, markers *Xgwm311* and *Xgwm382* are 'resistance-dominant' markers. Similar to this, *Xgwm311, Xgwm382* and

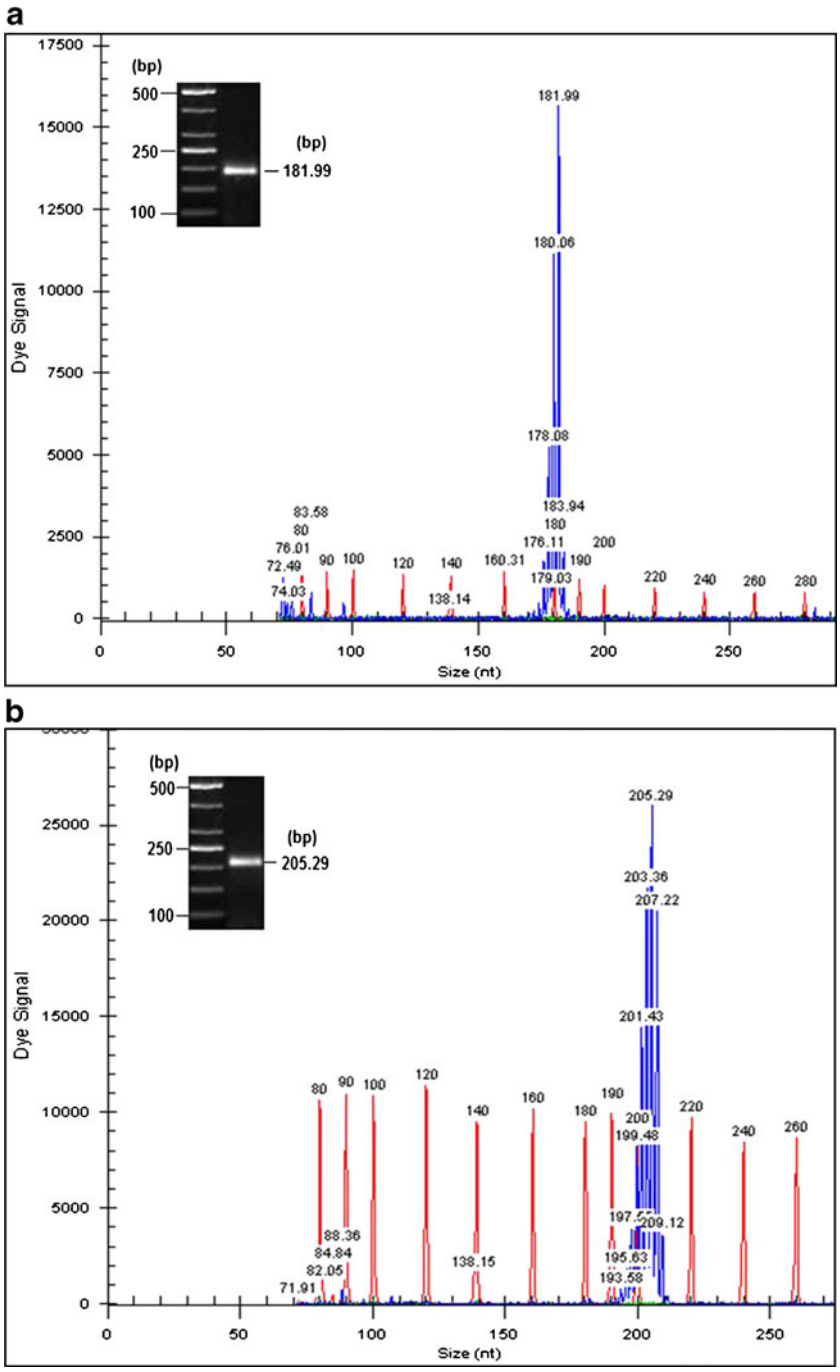

Figure 4. Fragment analysis of *Xwmc658* by florescence-based capillary electrophoresis in (a) resistant Izgi2001 and (b) susceptible ES14 parents.

Xwmc658 linked to yellow rust resistance in our germplasm were inherited in a dominant manner. The possible explanation for the dominance of these microsatellite markers with null alleles is most likely due to nucleotide-sequence alterations within the binding site for a DNA primer and results due to a primer site close to the microsatellite (Gupta and Varshney 2000; Liu *et al.* 2001).

As shown in previous studies, *Xgwm382* and *Xgwm311* loci are related to different wheat fungal diseases (Gilani *et al.* 2006; Kuraparthy *et al.* 2007; Runli *et al.* 2008; Buerstmayr *et al.* 2009). This study demonstrated that *Xgwm311, Xgwm382* and *Xwmc658* loci on chromosome 2AL were related to yellow rust resistance in Turkish germplasm. Previously, markers *Xgwm311* and *Xgwm382* mapped 5.0 and 5.6 cM proximal to *Yr1* based on the genetic linkage map presented by Bansal *et al.* (2009). In other study, *Xwmc658* and *Xgwm356* located on 2AL from 219 SSR primer combinations were found linked to *YrHV*

(temporarily designated). *YrHV* was 8.5 cM from *Xgwm356* and 5.6 cM from *Xwmc658*, respectively, the two sites linked to *YrHV* were validated by a portion of BC_1F_1 individuals and F_3 lines (Lu *et al.* 2009). In another study, marker *Xgwm311* was the most distal marker in chromosome 2AL, followed by marker *Xgwm382* (Somers *et al.* 2004).

Our results can be speculated that this region could have resistant genes for yellow rust based on our screening data derived from Izgi × ES14 F_2 populations with *Xgwm311, Xgwm382* and *Xwmc658* and may provide an insight into the genetic control of yellow rust resistance in wheat cross between highly resistant and highly susceptible wheat genotypes. Marker enrichment for this region would assist in resolving the map locations and distances for our future linkage mapping studies, thus improve the possibilities for marker-assisted selection.

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