An EST-SSR Marker, *bu099658*, and its Potential Use in Breeding for Yellow Rust Resistance in Wheat

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Abstract

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EST-SSR markers, derived from the A and B genomes of wheat were used to identify molecular markers associated with yellow rust resistance. For this purpose, bulk segregant analysis was performed using 114 EST-SSR primer pairs. They were screened on the parent genotypes and resistant/susceptible DNA pools from the cross between Izgi2001 (resistant male parent) × ES14 (susceptible female parent) at the seedling and adult plant stage. An EST-SSR marker, *bu099658*, generated the 206 bp DNA fragment that was present in the resistant parent and resistant bulk, but it was not present in the susceptible parent and the susceptible bulk. To investigate its association with *Yr* genes, 20 individuals of NILs were also amplified with BU099658 and the 206 bp marker fragment was obtained only in *Yr1/6* × Avocet S. Additionally, *bu099658* was screened on 65 genotypes which possessed different *Yr* genes/gene combination(s) and *Yr1*. The results indicate a close linkage of *bu099658* with the *Yr1* gene.

Keywords: Bulk Segregant Analysis (BSA); Marker-Assisted Selection (MAS); *Puccinia striiformis* f.sp. *tritici*; *Triticum aestivum* L.; *Yr1*

Wheat (*Triticum aestivum* L.) is the most important and strategic cereal crop for the majority of the world countries because of its basic nutrition supply. The largest proportion of yield losses in wheat production worldwide each year is due to rust diseases. Yellow rust (stripe rust), caused by *Puccinia striiformis* f.sp. *tritici*, is one of the major devastating factors worldwide in common wheat (*Triticum aestivum* L.). Turkey is the 10th largest wheat-producing country in the world with an average of 20 million tonnes per year and the economic damage caused by yellow rust as yield losses is quite serious. Growing resistant cultivars is considered the most effective, low-cost, and environmentally safe approach to controlling yellow rust (Line & Chen 1995). Several rust resistance genes have been identified and used in breeding for resistance but new variants of the pathogen overcome the resistance over a period of time. Molecular markers are becoming available for many genes and their use in marker-assisted selection will certainly have a considerable impact on practical breeding (Priyamvada & Tiwari 2011).

Molecular markers tightly linked to yellow rust resistance (*Yr)* genes are very useful for the introduction of resistance genes into wheat breeding programmes by Marker-Assisted Selection (MAS) (Todorovska *et al.* 2009). Moreover, they are very valuable tools to select the suitable genotypes for gene pyramiding to improve the new resistant genotypes containing two or more *Yr* genes (Chen 2007). This being the purpose, many molecular markers, such as Restriction Fragment Length Polymorphisms (RFLPs) (Helentjaris *et al.* 1986), Random Amplified Polymorphic DNAs (RAPDs) (Williams *et al.* 1990), Amplified Fragment Length Polymorphisms (AFLPs) (Vos *et al.* 1995), Resistance Gene Analogs (RGAs) (Kanazin *et al.* 1996) and Simple Sequence Repeats (SSRs) (Akkaya *et al.* 1992) have been widely used in plants. The successful utility of DNA markers has been shown in different wheat breeding programmes, namely SCAR marker (SC-Y15) for *Yr17* (Sharp *et al.* 2001), SSR markers (*Xgwm413* and *Xgwm273*) for *YrH52* (Peng *et al*. 2000) and SSR marker (*Xgwm498*) for *Yr26* (Yildirim *et al.* 2004*)*, for developing resistant wheat cultivars. Worldwide, yellow rust resistance genes *Yr1–Yr48* and many provisionally designated genes have been identified in wheat and its relatives (Huang *et al.* 2011). However, more markers are needed for identifying and mapping the genes.

Express Sequence Tags derived from SSRs (EST-SSRs), as genetic markers, have been evaluated in several studies and these tend to be considerably less polymorphic than those from genomic DNA for wheat (Eujayl *et al*. 2001). However, EST-SSRs have received a lot of attention because they are derived from the transcribed region of genes responsible for the traits of interest. This feature can provide opportunities for gene discovery and enhance the role of markers by assaying variation in the transcribed and known gene function (Andersen & Lubberstedt 2003). Recently, an increasing number of ESTs being deposited in databases for wheat (1.361.764; http://www.ncbi.nlm.nih.gov/dbEST) and EST-SSRs can be rapidly developed from the *in silico* analysis of these databases at low cost. EST-SSRs are already available for wheat (www.wheat.pw.usda.gov) and are transferred to adapted varieties. To date, few EST-SSR markers linked to yellow rust resistance have been reported (Ercan *et al.* 2010; Jia *et al.* 2011).

The objective of the present study was to identify associated EST-SSR markers for *Yr* genes that can be used for MAS in wheat breeding programmes. Here we report on the identification of *bu099658* marker and its close association with *Yr1*gene.

MATERIAL AND METHODS

Plant material. A cross between the yellow rust resistant and susceptible Turkish bread wheat cultivar, Izgi2001 and ES14, respectively, was made in the wheat breeding programme of the Anatolian Agricultural Research Institute (AARI). Izgi2001 has *Yr1* resistance gene according to the result of the gene postulation study conducted by Colin Wellings and Zafer Mert at the Plant Breeding Institute, Sydney University (Personal communication). The F_2 individuals derived from Izgi2001 × ES14 were evaluated for yellow rust resistance at the seedling stage in the greenhouse and adult stage in the field. Randomly selected 100 F_2 individuals were used for an inheritance study of the marker locus. Additionally, 20 near isogenic lines (NIL) (Table 1) and a total of 65 wheat genotypes carrying *Yr1* or different *Yr* gene/gene combination(s) (Table 2) that were supplied from Australia, Syria and Denmark were used for the identification of linkage between *bu099658* marker locus and *Yr1* gene.

Inoculation and disease assessment. Five hundred F_2 individuals derived from the cross were evaluated for yellow rust resistance both at the seedling stage in the greenhouse and at the adult stage in the field. For the inoculations, urediospores of a *Pst* isolate

Table 1. NIL 06 sets used to study the association of *bu099658* with *Yr1* gene

No.	Pedigree	Gene
1	*Yr1/6* × Avocet S	*Yr1*
2	*Yr5/6* × Avocet S	*Yr5*
3	*Yr6/6* × Avocet S	*Yr6*
4	*Yr7/6* × Avocet S	*Yr7*
5	*Yr8/6* × Avocet S	*Yr8*
6	*Yr9/6* × Avocet S	*Yr9*
7	*Yr10/6* × Avocet S	*Yr10*
8	*Yr11/3* × Avocet S	*Yr11*
9	*Yr12/3* × Avocet S	*Yr12*
10	*Yr15/6* × Avocet S	*Yr15*
11	*Yr17/6* × Avocet S	*Yr17*
12	*Yr18/3* × Avocet S	*Yr18*
13	*Yr24/3* × Avocet S	*Yr24*
14	*Yr26/3* × Avocet S	*Yr26*
15	*YrSP/6* × Avocet S	*YrSP*
16	*YrSK/3* × Avocet S	*Yr27*
17	Jupateco R (S)	*Yr18+*
18	Jupateco S	
19	Avocet R	*YrA*
20	Avocet S	

Table 2. Wheat genotypes screened for validation of the association between *bu099658* and the *Yr1* gene

No.	Genotype	*Yr* gene(s)	*bu099658* fragment (206 bp)	Seed source
1	Buster-1	*Yr1*	+	
2	Buster-2	*Yr1*	+	
3	Galahad	*Yr1*	+	
4	Hobbit	*Yr1*	–	
5	Ritmo	*Yr1*	+	
6	Chinese 166	*Yr1*	+	Sydney University, Australia
7	Forno	*Yr1*	+	
8	ISR678.1	*Yr1*	+	
9	ISR678.39	*Yr1*	+	
10	ISR 678.40	*Yr1*	+	
11	ISR.679.19	*Yr1*	+	
12	ISR 679.20	*Yr1*	+	
13	Chinese 166	*Yr1*	+	
14	Suwon 92 × Omar	*Yr1*	–	
15	AVS/ 6 × *Yr 1*	*Yr1*	+	
16	Chinese 166	*Yr1*	+	
17	Avocet S	*Yr1*	+	
18	IBIS	*Yr1*, *Yr2*	+	
19	Tadorna	*Yr1*, *Yr2*	–	
20	Fenman	*Yr1*, *Yr2*	–	ICARDA, Syria
21	Stetson	*Yr1*, *Yr9*	–	
22	Bounty	*Yr1*, *Yr13*	+	
23	Galahad	*Yr1*, *Yr2*, *Yr14*	+	
24	Maris Ranger	*Yr1*, *Yr3a*, *Yr4a*, *Yr6*	–	
25	Virtue	*Yr1*, *Yr3a*, *Yr4a*, *Yr13*	+	
26	Mardler	*Yr1*, *Yr2*, *Yr3a*, *Yr4a*, *Yr13*	–	
27	Hustler	*Yr1*, *Yr2*, *Yr3a*, *Yr4a*, *Yr13*	–	
28	Avocet	*Yr32*	–	
29	Carstens V	*Yr32, Yr25* +	–	
30	VPM 1	*Yr17,* +	–	
31	Avocet	*Yr10*	–	
32	Moro	*Yr10*	–	
33	Avocet	*Yr9*	–	
34	Sleipner	*Yr9*, +	–	
35	Heines Kolben	*Yr6*, *Yr 2*	–	
36	Suwon 92 /Omar	*So/Yr4*	–	Aarhus University, Denmark
37	Hybrid 46	*Yr4,* +	–	
38	Vilmorin23	*Yr3,* +	–	
39	Heines VII	*Yr2, Yr25*+	–	
40	Kalyansona	*Yr2,* +	–	
41	Spaldings Prolific	*Sp*, *Yr25*	–	
42	Strubes Dickkopf	*Sd*, *Yr25*	–	
43	Avocet *Yr8*	*Yr8*	–	
44	Chinese 166	*Yr1*	+	
45	Compair	*Yr8,* +	–	

Table 2 to be continued

No.	Genotype	*Yr* gene(s)	*bu099658* fragment (206 bp)	Seed source
46	Avocet *Yr7*	*Yr7*	–	
47	Lee	*Yr7, +*	–	
48	Avocet *Yr6*	*Yr6*	–	
49	Heines Peko	*Yr6, Yr2, Yr25+*	–	
50	TP 981	*Yr25?*	–	
51	Ambition	several	–	
52	Oakley	several	–	
53	Avocet S	*U2*	–	
54	Avocet *Yr5*	*Yr5*	–	
55	Avocet *Yr24*	*Yr24*	–	Aarhus University, Denmark
56	Brigadier	*Yr9, Yr17+*	–	
57	Anja	*U1*	–	
58	Opata	*Yr27, Yr18*	–	
59	Cortez	*Yr15*	–	
60	Cartago	none	–	
61	Chinese 166 (winter type)	*Yr1*	+	
62	Moro (winter type)	*Yr10*	–	
63	*Yr1/6* × Avocet S	*Yr1*	+	
64	*Yr10/6* × Avocet S	*Yr10*	+	
65	Chinese 166 (winter type)	*Yr1*	+	

which was virulent for *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr11*, *Yr12*, *Yr17*, *Yr18*, *Yr27*, *YrA+* and avirulent for *Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *YrSP*, *YrCV* genes were used. The most resistant and susceptible F_2 individuals at the seedling and adult stage were selected for Bulk Segregant Analysis (BSA) (Michelmore *et al.* 1991) after 15–20 days following the inoculation. The infection type was recorded, using the 0–9 scale of McNeal *et al.* (1971) at the seedling stage and the 0-100 scale of the modified Cobb scale (CI) at the adult stage (Roelfs *et al.* 1992).

Based on the global virulence mapper (http://wheatrust.org/international-services/yellow-rust/global-virulence-mapper/), since 2010 yellow rust isolates have been avirulent for *Yr1* in the Middle East including Turkey, Azerbaijan and Syria whereas the effectiveness of *Yr1* gene has been decreasing in Northern Europe (Figure 1).

DNA isolation and EST-SSR screening. Genomic DNA of leaves was extracted as described by Weining and Langridge (1991). Aliquots of DNA from 28 resistant and 28 susceptible plants from F_2 segregating population were mixed, respectively, to produce resistant and susceptible bulks of both growth stages to be used for BSA. 114 EST-SSR markers derived from A and B genomes of wheat (Gadaleta *et al.* 2009) were employed to determine the markers associated with yellow rust resistance.

Fragment analysis by capillary electrophoresis. A fluorescently labelled BU099658 forward primer was used for the determination of the polymorphic DNA fragment. PCR mixtures were prepared according to the GenomeLab GeXP System manufacturer's (Beckman Coulter, Brea, USA) instructions. The electrophoretic separation was performed using the GenomeLab GeXP Genetic Analysis System and the data was analysed by a fragment analysis module of the system. Each experiment was replicated at least three times to verify the reproducibility of the marker analysis.

RESULTS AND DISCUSSION

Infection type and CI values of selected resistant F_2 individuals were between 0 and 1 at the seedling stage and about 20 at the adult plant stage, while susceptible F_2 individuals were 8–9 at the seedling stage and 60–90 at the adult plant stage. The isolates used in this study are avirulent for *Yr1* based on the gene postulation study (supplementary info). Based on disease scoring data, the 28 most resistant and 28 most susceptible F_2 seedlings were taken into consideration for BSA.

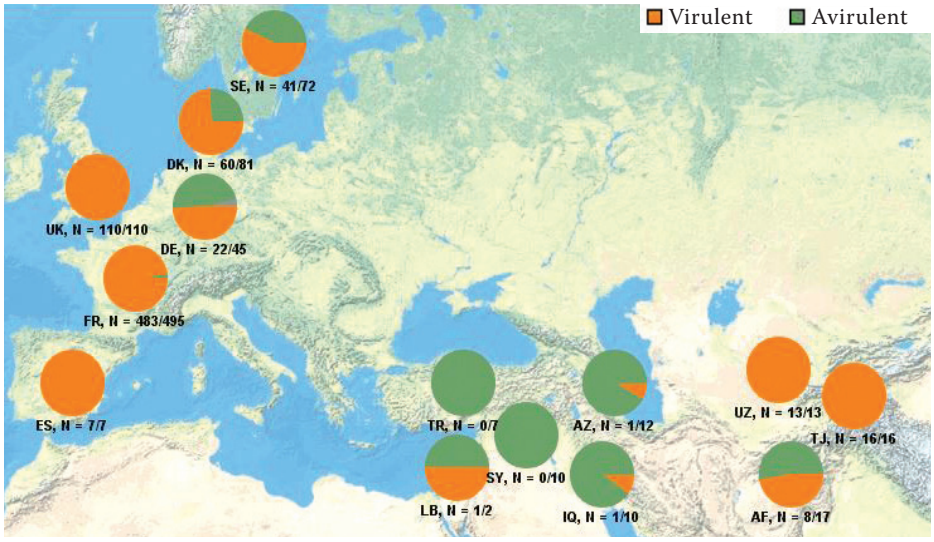

Figure 1. Map of *Yr1* virulence in Northern Europe and Middle East during 2010–2012; data provided by: Institut National de la Recherche Agronomique (France), Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (Germany and Austria), National Institute of Agricultural Botany (United Kingdom) and Aarhus University (Denmark and Sweden); N – No. of virulent *Pst* isolates/No. of analysed *Pst* isolates in a country

Among the 114 EST-SSR markers (Table 3) employed in this study, 99 of them (87%) revealed a monomorphic band profile between the two parents. The remaining 15 markers (13%) amplified polymorphic DNA fragments except for *bu099658*, while others did not produce such polymorphic band profiles between bulks. BU099658 amplified a 206 bp DNA fragment that was present in the resistant parent and in the resistant bulks, but not in the susceptible ones both for seedling and adult plant stages (Figure 2). A linkage between the *bu099658* locus mapped on chromosome group 2A and 2B and yellow rust resistance was confirmed in 28 resistant F_2 individuals at both growth stages. The 206 bp fragment, indicated by an arrow, was present in all 28 individuals in the resistant bulks, but not in the susceptible ones at both stages (Figure 2).

A new generation fluorescence-based capillary electrophoresis system was also used for the verification of the sizes of fragments generated by the BU099658. Figure 3 shows the fragment profile of the Izgi2001 with six peaks labelled as 168.20; 206.20; 222.43; 225.61; 227.70 bp and 230.75 bp, and also shows the fragment profile of the ES14 with five peaks 168.80; 222.54; 225.58; 227.57 bp and 230.84 bp. The 206 bp fragment was amplified in the resistant parent and resistant bulks at both growth stages but not in the susceptible ones.

Table 3. Screened EST-SSR markers (polymorphic markers used in this study are shown in bold); primer sequences are reported on the website http://wheat.pw.usda.gov

TC91851	TC74823	TC65966	**TC88833**	CA681959	BJ262177	BQ170801
TC69046	TC91851	TC84481	TC70788a	CA716967	BJ239878	BQ805704
TC87195a	TC71236	TC85303	TC70788b	CA594434	BJ306922	BQ246417
TC87195b	TC77302	TC84464	TC67645	CA668788	**BJ213673**	**BE419757**
TC85294	**TC88560**	TC85125a	TC77994	CA724675	BJ267382	BE427655
TC95235	TC80528	TC85125b	TC77993	CA594434	BJ318987	BF483631
TC84551	TC89014	TC92445	TC101037	CA681959	BJ227727	**BU099658**
TC91645	TC87011	**TC86533**	TC70722	CA662535	BJ253815	NP234852a
TC69046	TC85050	TC69177	CA741577	CA677684	BJ237020a	NP234852b
TC85294	**TC77481**	TC95791	CA703897	CA623872	**BJ237020b**	AL825137ab
TC81688a	TC80528	TC84481a	CA594434	CA499601	BJ262177	AL825137b
TC81688b	**TC80528**	TC84481b	CA597228	CA499463a	BJ261821	
TC88378	TC69937	TC85035a	CA679329	**CA499463b**	BJ236800a	
TC90641	TC72953	TC85035b	CA651264	CA694714	BJ236800b	
TC90640	TC86610	TC85037a	**CA677684**	CA663888	BJ213673	
TC82001	TC89014	TC85037b	CA658758	CA707573	BQ607256	
TC81096	TC82742	TC67416	CA695634	CA668775	BQ838884	

NILs were screened by the BU099658 primer pair to validate associations between the marker locus and *Yr* gene. As expected, while only *Yr1* including *Yr1/6* × Avocet S produced a 206 bp marker fragment, others did not. In addition, 21 genotypes carrying *Yr1*, 10 genotypes carrying *Yr1* in combination with other *Yr* gene/genes and 34 genotypes lacking *Yr1* (Table 2) were used for validation. Genotypes carrying the *Yr1* gene including Chinese 166, as the reference sources for this gene, were used by Bansal *et al*. (2009) for validation of the linkage between *Yr1* and *stm673acag*. The marker fragment (206 bp) belonging to the *bu099658* locus was amplified in all of the 19 genotypes carrying *Yr1* and another 4 individuals carrying *Yr1* gene in combination with other *Yr* gene/genes, which demonstrated the same pattern with the resistant parent, Izgi2001. The remaining genotypes gave exactly the same amplification profile with the susceptible parent, ES14, and did not amplify the marker fragment (Table 2).

In order to determine the inheritance of the *bu099658* locus, PCR amplification was performed in 100 F_2 individuals of Izgi2001 × ES14. In this analysis, 71 plants produced the polymorphic 206 bp marker fragment, while it was not produced by 29 plants, which fits a 3:1 ratio (χ^2 test, P = 0.25–0.50). This chi-squared analysis supported monogenic inheritance of Izgi2001 resistance to yellow rust.

In this study, we reported on the detection of the *bu099658* EST-SSR marker, linked to the seedling and adult plant resistance to yellow rust. An F_2 population from a cross between Izgi2001 and ES14 was visually assessed for seedling infection type in the greenhouse and adult plant infection in the field. In repeated amplifications, the presence of the 206 bp EST-SSR marker may significantly enhance the selection of wheat genotypes for yellow rust resistance. Screening of NILs and 65 wheat genotypes which have *Yr1* or other *Yr* genes by *bu099658* showed that only *Yr1/6* × Avocet S from NILs and all of the 19 genotypes carrying *Yr1* from validation sets amplified the 206 bp EST-SSR marker fragment. These results supported our suggestion that this marker fragment is closely linked to the *Yr1* gene. Bariana and McIntosh (1993) predicted a distal location of *Yr1* on chromosome 2AL based on recombination studies of rust resistance loci. Bansal *et al.* (2009) reported the genetic relationship between the genes *Yr1* and *Sr48* on chromosome 2AL. The close linkage was identified between *Yr1* and the PCR-based molecular marker *stm673acag*. Genotyping with *stm673acag* amplified a 120-bp fragment in 8 of 9 wheat genotypes carrying *Yr1,* also used in this study for validation. However, the line ISR679.20 amplified a 124-bp allele present in Australian cultivars lacking *Yr1*. This marker failed to differentiate Avocet S × 6/Chinese 166 (*Yr1*) and Avocet S by amplifying a 120-bp product in both genotypes. Thus, the line ISR679.20 and Avocet S were genotyped as false negative and false positive by the author. In contrast, BU099658 did not produce any false positive or false negative results for *Yr1* in our work. Therefore, we estimated that this marker could be useful for

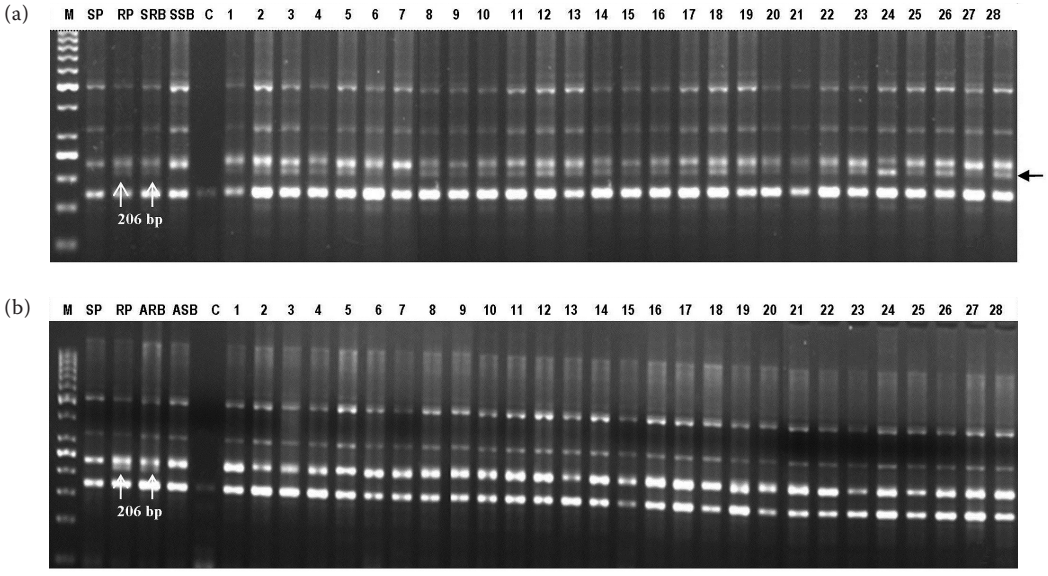

Figure 2. Bulk segregant analysis of the *bu099658* marker in resistant and susceptible F_2 individuals at both stages: resistant F_2 individuals at the seedling stage (a), susceptible F_2 individuals at the adult stage (b); SRB – seedling resistant bulk, SSB – seedling susceptible bulk, ARB – adult resistant bulk, ASB – adult susceptible bulk, C – negative control, 1–28: F_2 individuals in the resistant/susceptible bulk; black and white arrows show the 206 bp *bu099658* marker fragment

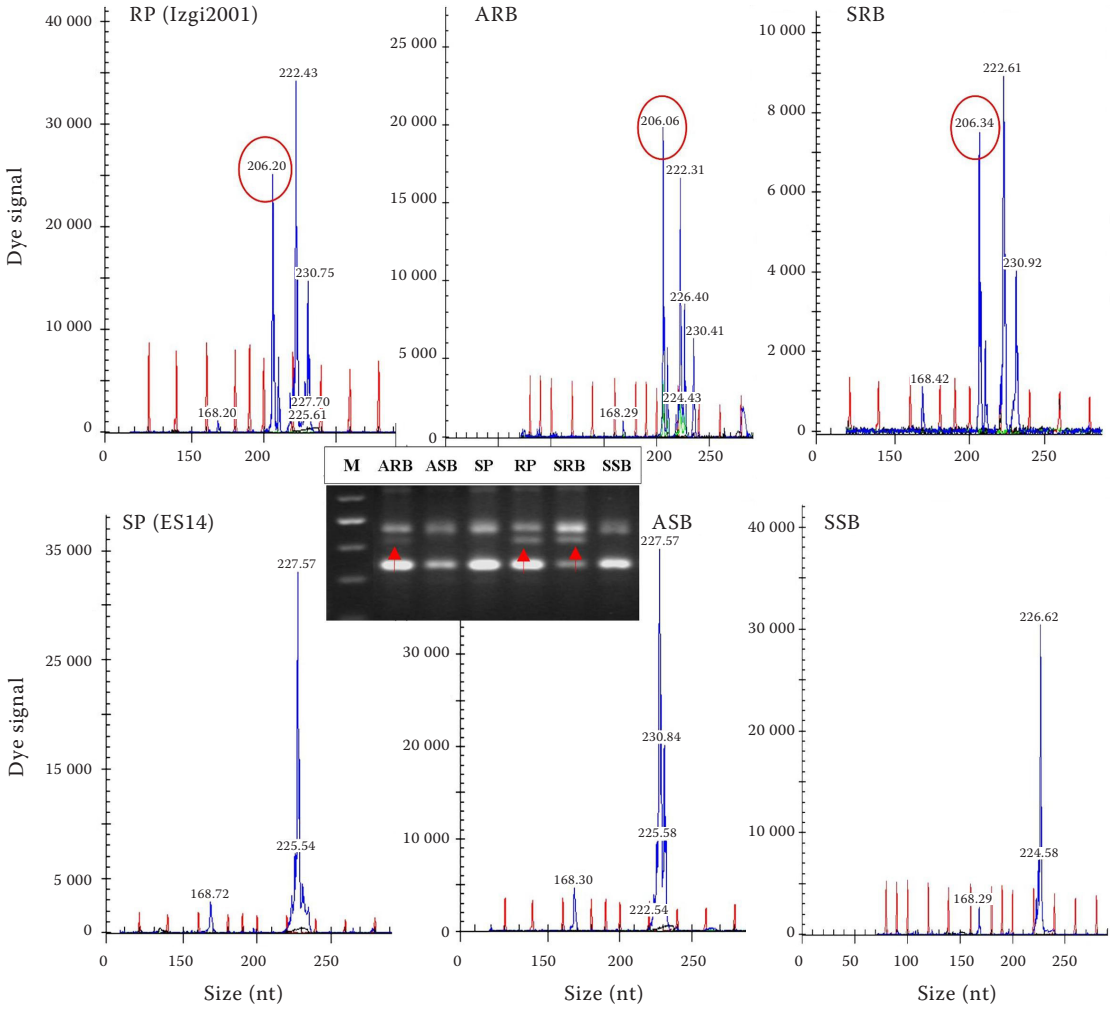

Figure 3. Fluorescence-based capillary electrophoresis; the *bu099658* marker in parents and F_2 resistant and susceptible bulks at both stages; red arrows and circles show the 206 bp marker fragment; M – DNA size marker (50 bp), ARB – resistance bulk at the adult stage, ASB – susceptible bulk at the adult stage, SP – susceptible parent (ES14), RP – resistance parent (Izgi01), SRB – resistance bulk at the seedling stage, SSB – susceptible bulk at the seedling stage

MAS in breeding programmes aimed at the large scale for the screening of segregating populations for *Yr1*. Bansal *et al.* (2009) mapped markers *Xgwm311* and *Xgwm382* with a 5 and 5.6 cM proximal distance to *Yr1* in an Arina/Forno RIL population. Previously, we also detected the presence of *Xgwm382* (Akfirat-Senturk *et al*. 2010) and *Xgwm311* (Akfirat-Senturk *et al.* 2013) markers in resistant germplasm of the Izgi2001 × ES14 cross. According to the genetic map presented in Somers *et al.* (2004), the marker *Xgwm311* was the most distal marker on chromosome 2AL, followed by the marker *Xgwm382*. Identification of a close or loose genetic association between *Yr1* and *bu099658* will be confirmed by linkage mapping in the Izgi2001 × ES14 population in our forthcoming studies.

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