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Full Length Research Paper

Yr10 gene polymorphism in bread wheat varieties

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Yellow rust resistance locus *Yr10* located on chromosome 1B in Moro and originated from the Turkish line PI178383 was investigated in terms of polymorphism in seven winter type bread wheat cvs. (*Triticum aestivum* ssp. *Aestivum*) Altay2000, İzgi2001, Sönmez2001 (yellow rust resistant), Aytın98, ES14, Harmankaya99 (yellow rust susceptible) and PI178383 as control. Exon 1 (1 - 833 bp) and Exon 2 (1989 - 3630 bp) parts of *Yr10* were amplified with three primers. Amplification was not observed with E2A primers in Harmankaya99, İzgi2001 and Sönmez2001 cvs, while amplification products were observable at all tested varieties with the other primers. PCR results showed that E2A reverse primer is not able to anneal to the three varieties mentioned above. Sequence analysis and bioinformatics analysis proved that there has been single nucleotide changes especially in the second exon. The most similar sequences to the first exon of Harmankaya99, İzgi01 and Sönmez2001 are AF509535 (*Aegilops tauschii* NBS-LRR-like gene), AF509534 (*A. tauschii* NBS-LRR-like gene sequence) and AF509534, respectively. These results could be helpful in revealing divergence between resistant and susceptible varieties.

Key words: *Triticum aestivum* L., yellow rust, resistance gene, PCR, sequence analysis.

INTRODUCTION

Wheat (*Triticum aestivum* ssp. *aestivum)* is one of the most important cereal crops in the world for both human food and animal feed. Characterization of disease resistance genes has great importance for the transfer of agronomically important genes to commercial varieties. Gene based molecular markers (Peng et al., 2000; Wang et al., 2002; Chen et al., 2003) when using PCR based gene specific primers, present an opportunity not only for selection of desired varieties but also provide the information about sequence polymorphisms in genes. However direct sequence information gives more valuable data for understanding of resistance mechanisms. Yellow rust or stripe rust caused by the fungus *Puccinia striiformis* f.sp. *tritici*, is one of the most damaging diseases affecting bread wheat in temperate regions (Mallard et al., 2005). Pathogen utilizes water and nutrients of the host and reduces leaf area and yield. Growing resistant varieties is the most effective and economically method of disease control (Röbbelen and Sharp, 1978; Line and Chen, 1995). In this research, we investigated sequence variations using bioinformatics tools in *Yr10* locus which is one of the main resistance genes to yellow rust (stripe rust) caused by *P. striiformis* from seven winter type bread wheat cvs.

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Table 1. Primers used in the study.

Primer name	Forward (5' 3')	Reverse (5' 3')	Product (bp)
E1	CTTGCTGGCGACCTGCTTA	TGTTTCGCTCCACGCTGACT	754
E2 Upstream	TGGTAGTAGAGTAATCGCAACA	TCTTCAGATTTGGAGGTAGG	377
E2A Downstream	TGGAAATGGATAGGCGAAGG	AAATCAATGAAGCCGCAACC	872

MATERIALS AND METHODS

Plant material

Altay2000, İzgi2001, Sönmez2001 (Yellow rust resistant), Aytın98, ES14, Harmankaya99 (Yellow rust sensitive) and PI178383 (Control) were screened for *Yr10* gene polymorphisms. Seeds were obtained from Anatolian Agricultural Research Institute.

Structure of *Yr10*

Yr10 is found in Turkish line PI178383 and is also the first yellow rust resistance gene, which is sequenced (GenBank No: AF149112) (Authors; Laroche A, Frick, M.M., Huel, R., Nykiforuk, C., Conner, B., Kuzyk, A.). *Yr10* is a dominant gene which confers race specific resistance to yellow rust. It is 3630 bp long and consists of two exons interrupted by an intron. Its mRNA transcript is 2475 bps long (GenBank No: AF149114).

Primer design

PCR primers were designed to amplify both exon 1 and exon 2 using the program Primer Premier Version 5.00. One pair (E1) was designed for the first exon, two pairs (E2, E2A) were designed for the second exon (Table 1).

PCR analysis

Genomic DNA was extracted from leaves of 23 day-old seedlings according to Song and Henry (1995). Genomic DNA was quantified spectrophotometrically. PCR was performed at 25 µl final volume containing 1X enzyme buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 2 µM forward and 2 µM reverse primer, 100 ng template DNA and 1 U Taq polymerase (M186A, Promega). Amplifications were performed with an initial denaturation at 94°C for 2 min, 30 cycles of 94°C for 30 s, 55°C for 30 s (E1, E2A primers) or 50°C for 30 s (E2 primers) or 60°C (E2 forward and E2A reverse primers), 72°C for 1 min and completed after a final elongation at 72°C for 7 min. PCR products were run on 1% nusieve agarose gels and stained with 0.5 µg/ml ethidium bromide.

Sequencing

PCR products were excised from agarose gel and recovered using a purification kit (Wizard SV Gel and PCR Clean-Up System A9281, Promega). After the checking of purified bands on agarose gels, 754 bp products from 7 varieties and 1311 bp product from 4 varieties were sent to sequencing (IONTEK). Multiple sequence alignments were calculated using CLUSTALW (Thompson et al., 1994) (http://www.ebi.ac.uk/clustalw/). First and second exon sequences were compared in the nucleotide collection database (nr/nt) using BLASTN (discontiguous megablast) (Altschul et al., 1990) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS AND DISCUSSION

PCR amplification

E1 and E2 primer pairs gave the expected product size in 7 varieties (Figure 1a, b). But E2A primer did not amplify any product in 3 varieties (Harmankaya99, İzgi2001, Sönmez2001) (Figure 1 c). In order to find out which primer could not bind to template, E2 forward primer (upstream region of the second exon) and E2A reverse primer (downstream region of the second exon) were used together. After gradient PCR, a band of expected size (1300 bp) was amplified at 60°C annealing temperature in 4 varieties (PI178383, Altay2000, Aytın98, ES14) (Figure 1d). We hypothesized E2A reverse primer cannot bind to genomic DNA in 3 varieties mentioned above.

Multiple alignment

The most similar varieties to the first exon of *Yr10* are PI178383 (98%) and Altay2000 (99%) (Table 2). Harmankaya99, İzgi01 and Sönmez2001 are the least similar varieties to the first exon of *Yr10* (Table 2). The most similar variety to the second exon of *Yr10* is PI178383 (97%) (Table 3). Multiple alignment results are also presented as phylogram trees (Figures 2 and 3). The best BLASTN matches for exon 1 sequence of Harmankaya99, İzgi01 and Sönmez2001 are AF509535 (*Aegilops tauschii* NBS-LRR-like gene) (91%), AF509534 (*Aegilops tauschii* NBS-LRR-like gene sequence) (95%) and AF509534 (98%) respectively. Spielmeyer and Lagudah (2003) hybridized probe *RgaYr10* to genomic DNA of *A. tauschii* line AUS18911. They isolated four clones representing at least four different *RgaYr10* gene family members. These clones were sequenced and submitted to GenBank as AF509533, AF509534 and AF509535.

The results obtained from this work indicate that (1) *Yr10* gene sequence is present in all of these varieties, (2) the divergence between the varieties is raised from the variations in the second exon and (3) the first exon is

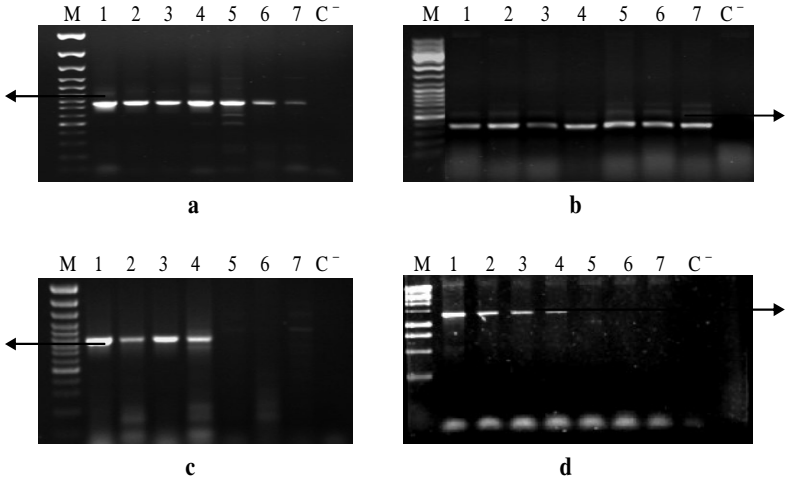

Figure 1. Amplification products of E1 (a), E2 (b), E2A (c) and E2 forward and E2A reverse (d) primers. Product length 754 bp, 377 bp, 872 bp and 1311 bp respectively. M. Marker, 1. PI178383, 2. Altay2000, 3. Aytın98, 4. ES14, 5. Harmankaya99, 6. İzgi2001, 7. Sönmez2001, C^- Negative control.

Table 2. Comparison of the first exon sequences with ClustalW.

Sequence 1	Product size (bp)	Sequence 2	Product size (bp)	Score
AF149112	754	PI178383	755	98
AF149112	754	Altay2000	757	99
AF149112	754	Aytın98	710	88
AF149112	754	ES14	747	95
AF149112	754	Harmankaya99	741	77
AF149112	754	İzgi01	729	82
AF149112	754	Sönmez2001	743	79
PI178383	755	Altay2000	757	98
PI178383	755	Aytın98	710	88
PI178383	755	ES14	747	95
PI178383	755	Harmankaya99	741	78
PI178383	755	İzgi01	729	84
PI178383	755	Sönmez2001	743	79
Altay2000	757	Aytın98	710	89
Altay2000	757	ES14	747	95
Altay2000	757	Harmankaya99	741	77
Altay2000	757	İzgi01	729	82
Altay2000	757	Sönmez2001	743	80
Aytın98	710	ES14	747	92
Aytın98	710	Harmankaya99	741	78
Aytın98	710	İzgi01	729	79
Aytın98	710	Sönmez2001	743	80
ES14	747	Harmankaya99	741	77
ES14	747	İzgi01	729	82
ES14	747	Sönmez2001	743	78
Harmankaya99	741	İzgi01	729	76
Harmankaya99	741	Sönmez2001	743	79
İzgi01	729	Sönmez2001	743	90

Table 3. Comparison of the second exon sequences with ClustalW.

Sequence 1	Product size (bp)	Sequence 2	Product size (bp)	Score
AF149112	1311	PI178383	1242	97
AF149112	1311	Altay2000	815	71
AF149112	1311	Aytın98	952	45
AF149112	1311	ES14	877	71
PI178383	1242	Altay2000	815	70
PI178383	1242	Aytın98	952	46
PI178383	1242	ES14	877	72
Altay2000	815	Aytın98	952	53
Altay2000	815	ES14	877	92
Aytın98	952	ES14	877	49

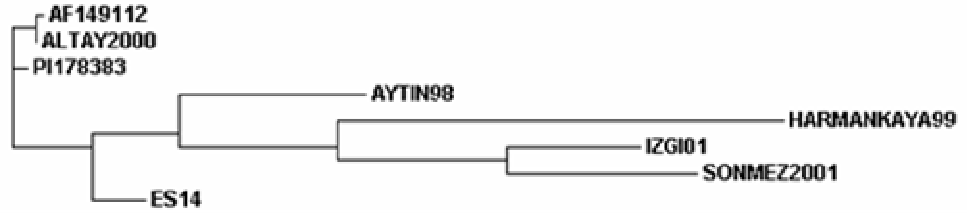

Figure 2. Phylogram tree of the first exon sequences.

Figure 3. Phylogram tree of the second exon sequences.

more conserved than the second one. Sequence variations could effect gene expression and could weaken yellow rust resistance. Research on yellow rust resistance genes performed by other authors (Sun et al., 2002; Chen et al., 2003; Yan et al., 2003; Yildirim et al., 2004; Li et al., 2006) generally depend on marker development. Wang et al. (2002) found out microsatellite markers such as Xpsp3000 linked to *Yr10*. These authors crossed PI178383 with Yumai 18, a susceptible com-mon wheat variety from China and investigated inheritance of the *Yr10* gene. They showed that the resistance to strain CYR31 was determined by a single dominant gene. Bozkurt et al. (2007) isolated RGAs using homology based PCR to target conserved regions (NBS) from bread wheat varieties. They found one RGA similar to *Yr10* of wheat. A *Yr10*-like protein (*RGAYr10*) gene (GenBank No: EU428764) was identified in *Dasypyrum breviaristatum* recently (Tang and Yang, unpublished, NCBI). However, there is no report on *Yr10* sequence polymorphisms in different wheat varieties. Although the PCR and sequencing results are not directly related to phenotypic data and cannot discern resistant/susceptible varieties, it might be striking that the least similar varieties Harmankaya99, İzgi01 and Sönmez2001 lack the down-stream region of the second exon. Detailed expression analyses would be helpful for the determination of most important nucleotide changes whether there is a relation with the constitutive expression of the *Yr10* gene in these plants. We are planning to test these molecular data in F_2 generation to find out the relations with resistant geno-types selected in the field. It is thought that the results of this work will contribute to determine the divergence bet-

ween resistant and susceptible varieties and will be helpful to breeding applications.

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