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# Distribution of 1AL.1RS and 1BL.1RS Wheat-rye Translocations in Iranian Wheat, Using PCR Based Markers and SDS-PAGE

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Short arm of rye chromosome 1 (1RS) in wheat (*Triticum aestivum* L.) improvement is being widely utilized by many plant breeders. The 1BL.1RS translocation derived from the Russian wheat cultivar "Kavkaz", carring genes for major wheat diseases such as stem, strip and leaf rusts and powdery mildew resistance. The 1AL.1RS translocation derived from "Amigo", possessing resistance genes for stem rust, powdery mildew and greenbug. The distribution of the wheat-rye translocations 1BL.1RS and 1AL.1RS was studied in 44 Iranian wheat cultivars (29 bread wheat cultivars and 15 *durum* wheats). In this study the presence of the translocations was identified in 5 cultivars (Dez, Atrak, Rasul, Falat and Moghan3), using SDS-PAGE technique and 3 DNA markers based PCR. The both results of PCR based markers and SDS-PAGE showed that the frequency of the 1BL.1RS in Iranian bread wheat is very low (5 cultivars of bread wheat) and 1AL.1RS did not exist in Iranian wheat backgrounds. Such techniques are quick and reliable tools to recognize and to distinguish these two wheat-rye translocations in wheat genetic background.

Keywords: wheat-rye translocation, 1BL.1RS, 1AL.1RS, *Triticum aestivum, Secale cereale*, Iran, SDS-PAGE, DNA marker

# Introduction

Wheat lines with wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) translocations have been developed to increase genetic variation of wheat. More than 16 wheat-rye translocations have been recognized (Jiang et al. 1994; Friebe et al. 1996). Among which, the 1AL.1RS and the 1BL.1RS translocations have been utilized for hexaploid (*T. aestivum* L.) and tetraploid wheat (*T. durum* Desf.) breeding (Friebe et al. 1987; Lukaszewski 1990; Villareal et al. 1991). Translocation 1BL.1RS involving the short arm of rye chromosome 1R and the long arm of wheat chromosome 1B is accepted in wheat breeding programs. In researches from 1991 to 1995, between 505 commercial cultivars

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of T. aestivum from 17 countries, 45% carried 1BL.1RS translocation (Rabinovich 1998). A similar translocation common in wheats is 1AL.1RS. The 1BL.1RS translocation, at first derived from 'Petkus' rye has been introduced into wheat cultivars throughout the world by Russian wheat cultivar 'Kaykaz' and its derivatives (Rajaram et al. 1990; Rabinovich 1998). 1RS has been replaced in 1BL.1RS translocation carries genes for some wheat disease resistance such as stem rust (Sr31), stripe rust (Yr9) and leaf rust (Lr26) and powdery mildew (Pm8; Zeller 1973). This translocation has favorite effects on grain yield and biomass accumulation and adapt to stressful environmental conditions (Lukaszewski 1990; Villareal et al. 1991; Moreno-Sevilla et al. 1995; Shearman et al. 2005). The 1AL.1RS translocation, at first derived from 'Insave' rye, was primarily introduced into wheat cultivars by germplasm line 'Amigo' and has been used initially in North America and Australia (Lukaszewski 1990; Martin and Stewart 1990; Rabinovich 1998). This translocation possesses genes for stem rust, greenbug and powdery mildew resistance (Sebesta and Wood 1978). 1BL.1RS translocation has deleterious effects on dough quality characteristics such as reduced mixing tolerance, dough strength, intense dough stickiness and diminished loaf volume (Dhaliwal and MacRitchie 1990; Martin and Stewart 1990; Fenn et al. 1994; McKendry et al. 1996) 1AL/1RS translocation also decreased the end use quality of wheat but was less than 1BL.1RS (Graybosch et al. 1993). The reduction in dough strength is a result of the presence of  $\gamma$  secalins from rye and the replacement of glutenins and gliadins (Dhaliwal and MacRitchie 1990). In Secale cereale, storage proteins (secalins) classified into four fundamental groups. These four groups are high molecular weight secalins (HMW) and 75K  $\gamma$ -secalins,  $\omega$ -secalins, and 40K  $\gamma$ -secalins. (Shewry et al. 1994). The structural genes for the group of HMW secalins were located on the long arm of chromosome 1R (1RL; Lawrence and Shepherd 1981; Singh and Shepherd 1984; Shewry et al. 1984). The  $\omega$ -secalins and the 40K  $\gamma$ -secalins were located on the short arm of the same chromosome (1RS) (Shepherd 1968; Lawrence and Shepherd 1981). The name of locus on the long arm is Sec 3, and the locus on the short arm is Sec 1 (Shewry et al. 1984). Studying results shown that families of genes tightly linked into Sec 1 locus and also indicated Sec 3 and Sec 1 loci are loosely linked (Carrilo et al. 1990). Several techniques have been used for detected this arm of rye on wheat such as cytogenetic observations after N and C-banding of chromosomes (Rayburn and Carver 1988), high-performance liquid chromatography (HPLC; Lookhart et al. 1991) rye specific DNA sequences as primers for polymerase chain reactions (PCR; Koebner 1995; Katto et al. 2004; Weng et al. 2007), isozyme analysis or various methods based on the detection of rye grain storage proteins (Koebner and Shepherd 1986; Landjeva et al. 2006) and genomic in situ hybridization (Miller et al. 1995; Busch et al. 1995; Clarke et al. 1996). Recently, PCR based DNA markers were employed to identify wheat-rye chromosomal translocations (Koebner 1995; Shimizu et al. 1997; Nadella et al. 2002; Nagy and Lelley 2003; Weng et al. 2007).

The aim of present study was to provide an overview of Iranian bread and *durum* wheats, for 1AL.1RS and 1BL.1RS translocations in order to select beneficial genotype and cultivar for future wheat improvement programs.

No.	Cultivar	Pedigree	Year of introduction	Banding patern <sup>+</sup> (Glu-A1, Glu-B1, Glu-D1)	Quality score <sup>1,+</sup>
1	Atrak	Kauz"s"-CIMMYT, Mexico	1995	2*, 7+9, 5+10	3+2+4
2	Darab2	Maya"s"/Nac- CIMMYT, Mexico	1995	2*, 17+18, 2+12	3+3+2
3	Zarrin	RK-15841 CIMMYT-ICARDA, Turkey	1995	1, 7+8, 2+12	3+3+2
4	Sardari	Landrace from Kurdistan, Iran	Very old	2*, 7+8, 2+12	3+3+2
5	Azar2	Kaz/Tr71/3/Maya"s"//Bb/Inia/4/Sefid	1999	2*, 7+8, 2+12	3+3+2
6	Ghods	Rsh/5Wt/4/Nor10/K54*2//Fn/3/Ptr/6/omid//Kal/Bb	1989	2*, 7+8, 2+12	3+3+2
7	Omid	Landrace No.1-29-11085, Saveh, Iran	1956	N, 7+8, 2+12	1+3+2
8	Tajan	Bow"s"/Nkt"s"(CM67428-GM-LR-5M-3R-LB-Y), CIMMYT, Mexico	1995	2*, 13+16, 5+10	3+3+4
9	Chamran	CIMMYT-Mexico Attila,(CM85836-50Y-OM-OY-3M,-OY)	1997	2*, 7, 5+10	3+1+4
10	Rasul	Veery"s"=Kvz/Buho"s"//Kal/Bb	1992	1, 7+8, 2+12	3+3+2
1	Falat	KVZ/Buho"s"//Kal/Bb=seri82	1990	1, 7+9, 5+10	3+2+4
2	Dez	Kauz*2/Opta/Kauz CRG-737-1y-O10M-OY	2002	2*, 17+18, 2+12	3+3+2
3	Golestan	Alondra"s"-Me	1986	N, 17+18, 5+10	1+3+4
4	Alvand	1-27-6275/CF1770, Karaj, Iran	1995	1, 7+8, 2+12	3+3+2
5	Moghan3	(LR-N10B)*An3E-Mexico	1973	2*, 7+8, 2+12	3+3+2
6	Shirudi	Attila, CM85836-4Y-OM-OY-8M-OY-OPZ	1997	1, 7+9, 5+10	3+2+4
7	Kavir	Stm/3/Kal//V534/Jit716	1997	2*, 17+18, 2***+12	3+3+nd
8	Sardari	Landrace from Kurdistan, Iran	Very old	2*, 7+8, 2+12	3+3+2
9	Omid	Landrace from Saveh, Iran	1956	N, 7+8, 2+12	1+3+2
0	Roshan	Landrace from Esfahan, Iran	1958	N, 7+8, 2+12	1+3+2
1	Mahdavi	TI/PCH/5MT48/3/WT*//Nar59/TOTA634/MUS	1995	1, 17+18, 2+12	3+3+2
22	Niknejad	F13471/Crow "S"	1995	2*, 7+9, 5+10	3+2+4
23	Marv dasht	Hd2172/Bloudan//Azadi	1999	2*, 7+8, 2+12	3+3+2
24	Pishtaz	Alv and//Aldan"S"/Las58-40072-48	2002	2*, 7+8, 2+12	3+3+2
5	Shiraz	Gv/D630//Ald"S"/3Azd	2002	2*, 7+8, 2+12	3+3+2
26	Azadi	(4820*J-32-25409)*Mxp	1980	2*, 7+8, 2+12	3+3+2
27	Hirmand	Byt/4/Jar//Cfn/Sr70/3Jup"S"	1991	2*, 17+18, 2+12	3+3+2
28	Navid	(Kirkpinar 79)63-112/66-2*7C	1990	2*, 7, 5+10	3+1+4
29	Alborz	I R642-Son64)CM-218 Fn-Md*K1174/cofn2(Son64-k1 Rend/cn	1978	2* 17+18 2+12	3+3+2

<sup>1</sup> Quality score assigned for HMW-G subunits according to Payne et al. (1987); <sup>+</sup> According to Bahraii (2003); N: null; nd: not identified

### **Materials and Methods**

# Plant materials, primers and PCR analysis

A total of 44 wheat cultivars, comprising 29 bread wheat cultivars (*Triticum aestivum*, 2n = 6x = 42; listed in Table 1) and 15 *durum* wheat cultivars (*T. turgidum*, 2n = 4x = 28; listed in Table 2), Chinese Spring as a negative control for 1AL.1RS and 1BL.1RS translocations, Kavkaz as a positive control for 1BL.1RS translocation and TAM107 as a positive control for 1AL.1RS translocation, were included in this study. Total genomic DNA was isolated from fresh leaves using Saghai-Maroof et al. (1984) method. Rye specific PCR based molecular markers were "PAW161", "Rye R3/F3" and "O-SEC5'-A/O-SEC3'-R" that details listed in Table 3. PCR conditions for PAW161 and Rye R3/F3 primers were programmed at 95°C for 3 min, 94°C for 45 s, 62°C for 60 s, and 72°C for 90 s for

Table 2. Local name, type and glutenin subunits information of Iranian *durum* wheat cultivars used in this study

No.	Scientific name	Location of collect	Туре	Banding patern (Glu-A1, Glu-B1)	Quality score <sup>1</sup>
1	T. durum apulicum	Behbahan	Domestic	2*, 7	3+1
2	T. durum pininum	Shiraz	Domestic	2*, 20	3+1
3	T. durum leucomelon	Khoramshahr	Domestic	2*, 17+18	3+3
4	T. durum erthromelon	Behbahan	Domestic	2*, 7+8	3+3
5	T. durum hordeiform	Ahvaz	Domestic	2*, 17+18	3+3
6	T. durum melanopus	Kermanshah	Domestic	N, 7	1+1
7	T. durum	Khoi	Selective line	N, 7	1+1
8	T. durum	Oroomieh	Selective line	N, 20	1+1
9	T. durum	Kerman	Selective line	N, 20	1+1
10	T. durum	Hamedan	Selective line	2*, 20	3+1
11	T. durum	Kermanshah	Selective line	N, 7	1+1
12	T. durum	Ilam	Selective line	N, 20	1+1
13	T. durum	Iran	Domestic	1, 7	3+1
14	T. durum	Iran	Domestic	N, 7	1+1
15	T. durum provinicial	Ardebil	Domestic	N, 20	1 + 1

<sup>1</sup> Quality score assigned for HMW-G subunits according to Payne et al. (1987); N: null

Table 3. Details of 3 specific primers used for wheat-rye translocations

Markers	Primer sequence $(5' \rightarrow 3')$	References Guidet et al. 1991; Weng et al. 2007; Yediav and Baoch 2010		
PAW161	F: TGAGGGCCCAGACGGCCCTTTTTG			
	R: TTATCGCAATTACAACTCAAATTT	2		
RyeR3/F3	F: GATCGCCTCTTTTGCCAAGA	Katto et al. 2004; Weng et al. 2007; Yediay and Baoch 2010		
	R: TCACTGATCACAAGAGCTTG			
O-SEC5'/A O-SEC3'/R	F: CTATTAGTTCGAAAAGCTTATGA R: GCATATGACTCAAATTATTTTT	Shimizu et al. 1997; Yediay and Baoch 2010		

30 cycles and for O-SEC5'-A/O-SEC3'-R primer was programmed 95°C for 5 min; 94°C for 60 s; 50°C for 120 s, and 72°C for 180 s for 35 cycles. Diagnostic markers to study wheat–rye translocation in wheat background listed in Table 4. PCR amplification was carried out in a gradient thermocycler (BioRad). PCR conditions were performed same for each primer. The reactions were in a 25  $\mu$ l volume, containing 50 ng of template DNA, 1 unit of *Taq* DNA polymerase, 2 nmol of dNTP and 75 mM Tris-HCl, pH 8.8, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.0 mM MgCl<sub>2</sub>, 0.1% Tween 20, 0.2  $\mu$ M primer pair, 100  $\mu$ M each of dATP, dGTP, dCTP and dTTP. PCR products were separated on 2% Agaros gels. Total proteins were separated from one half of grains after crushing with dissolving buffer and separated by sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) using 12% acrylamide gels in the discontinuous system of Laemmli (1970). The bands were visible after staining with Coomassie blue. Sec-1 band was identified by comparison with Chinese Spring and in almost 40 kDa regions.

Table 4. Diagnostic markers to study wheat-rye translocation in wheat background

Markers	Diagnostic bands (bp)	1AL.1RS TAM107	1BL.1RS Kavkaz	Chinese Spring	References
PAW161	350	+	+	_	Weng et al. 2007
RyeR3/F3	1400	+	+	_	Weng et al. 2007
	1,530	+	+		
O-SEC5'/A	1,095	+	+	_	Yediay and Baoch 2010
O-SEC3'/R	700				

### Results

All three rye-specific primer pairs amplified rye fragment in Dez, Atrak, Rasul, Falat and Moghan3 cultivars. The "Rye R3/F3" and "PAW161" primers amplified, respectively, 1.4 kb and 366 bp bands, in TAM 107 (positive control for 1AL.1RS translocation) and Kavkaz (positive control for 1BL.1RS translocation) and also in Dez, Atrak, Rasul, Falat and Moghan3 (Fig. 1). No amplification existed in non-1RS wheat (Chinese Spring) and 24 bread wheat cultivars and 15 durum wheat cultivars with this primer pairs. The "O-SEC5'-A/O-SEC3'-R" was used to clean between wheat cultivars with 1AL.1RS and 1BL.1RS translocations. These primer pairs amplified two different PCR products of 1,530 and 1,095 bp only for TAM107 and two PCR products of 1,530 and 700 bp for Kavkaz and Dez, Atrak, Rasul, Falat and Moghan3. No PCR product could be amplified in Chinese Spring wheat (negative control for both translocations) and other bread wheat cultivars and whole durum wheat cultivars with these primer pairs. In all 1AL.1RS and 1BL.1RS wheat-rye translocation cultivars was observed the 1,530 bp band with "O-SEC5'-A/O-SEC3'-R" primer (Fig. 1). On SDS-PAGE gel, Sec-1 band was identified by comparison with Chinese Spring and in almost 40 KDa region (Afshari 2006; Anugrahwati et al. 2008) in Kavkaz, Tam107, Dez, Atrak, Rasul, Falat and Moghan3 among Iranian bread wheat and no band appeared in Iranian durum wheat in that position and size (Fig. 2).



Figure 1. Identification of wheat-rye translocation in Iranian wheat by rye-specific DNA markers.
A RyeR3/F3, 1) Chinese Spring, 2) Kavkaz, 3) TAM107, 4) Dez, 5) Falat, 6) Tajan, 7) Atrak, 8) Rasul,
9) Moghan3, 10) Omid. B PAW161, 1) Kavkaz, 2) TAM107, 3) Rasul, 4) Atrak, 5) Falat, 6) Dez, 7) Pishtaz,
8) Marvdasht, 9) Moghan3, 10) Alvand, 11) Chinese Spring. C O-SEC5'-A/O-SEC3'-R, 1) Kavkaz,
2) Moghan3, 3) Atrak, 4) Dez, 5) Rasul, 6) Falat, 7) Chinese Spring



*Figure 2.* SDS-PAGE analysis of seed protein extracts from wheat. M) protein marker, 1) Kavkaz, 2) Chinese Spring, 3) Alvand, 4) Zarrin, 5) Golestan, 6) Shirudi, 7) Ghods, 8) Atrak, 9) Rasul, 10) Sardari, 11) Azadi, 12) Alborz, 13) Dez

#### Discussion

Recently detection of 1RS chromosome arm is one of the most intensively studied on wheat. Numerous markers based PCR for characterizing 1RS have been reported (Shimizu et al. 1997; Nadella et al. 2002; Nagy and Lelley 2003; Weng et al. 2007). Katto et al. (2004) reported a PCR-based marker which could detect segments of rye chromosome into wheat. Newly, the wheat-rye lines with 1RS were detected by C-banding, GISH, FISH (Rayburn and Carver 1988; Heslop-Harrison et al. 1990; Miller et al. 1995; Anugrahwati et al. 2008). These processes are time consuming and also need difficult technical to use. The molecular markers are cheaper, faster and easier than C-banding. GISH and FISH. At present PCR-based molecular markers are practical and beneficial tool in plant breeding. The 3 markers specific to 1RS chromosome tested in this study on 34 Iranian wheat cultivars that could be rapidly and easily screened of materials containing 1RS. The PCR experiments with two markers Rye R3/F3 and PAW161 demonstrated that the 1RS fragment is present only in the cultivars Dez, Atrak, Rasul, Falat and Moghan3 and with O-SEC5'-A/O-SEC3'-R primer was represented that 1RS in these cultivars is in the form of 1BL.1RS wheat-rye translocation. The "O-SEC5'-A/O-SEC3'-R" was used for clean between wheats with 1AL.1RS and 1BL.1RS translocations. Yediay and Baoch (2010) evaluated nine rye specific marker and they used six out of nine (PAW161 and Rye R3/F3 and O-SEC5'-A/O-SEC3'-R were used) to screen these translocation in bread and *durum* wheat cultivars from Turkey. In this country that is neighbor of Iran only 4 cultivars of bread wheat have 1BL.1RS translocation and 1AL.1RS translocation did not exist in none of them. Also, two markers PAW161 and Rye  $R_3/F_3$  with another 6 markers were employed by Weng et al. (2007) for evaluating them in detecting wheat-rye translocations in wheat genetic backgrounds. PAW161 and Rye R3/F3 able to detect 1RS in wheat backgrounds with these translocations.

PAGE or polyacrylamide gel electrophoresis can be used for identification and characterization of 1RS with separation of secalins, gliadins and glutenins. Different banding patterns associated the presence or absence of secalins can illustrate the presence or the absence short arm of 1R chromosome (Berzonsky and Francki 1999). Gupta and Shepherd (1992) differentiate between wheat lines with a 1AL.1RS, a 1BL.1RS, a 1DL.1RS translocation, and a 1R (1B) chromosome substitution by electrophoresis mobility on PAGE with the absence of group-1S gliadin bands, the presence of 1RS secalin bands, and the presence of 1BL glutenin bands. The SDS-PAGE results showed that among the 29 Iranian bread wheat cultivars and *durum* wheats, Dez, Atrak, Rasul, Falat and Moghan3 carried the 1BL.1RS translocation and the Sec-1 band did not present in other the 24 Iranian cultivars and all of *durum* cultivars. Sec-1 is complex locus that encoding two types of rye storage proteins,  $\gamma$ -secalins (with Mrs range from 36000 to 40000) and  $\omega$ -secalins (with Mrs range from 40000 to 51000) (Graybosch 2001). In 1AL.1RS, 1BL.1RS and 1DL.1RS loci encoding wheat low-molecular-weight (LMW) glutenin subunits and gliadins are lost from the 1AS, 1BS and 1DS, respectively and Sec-1 locus was replaced (Graybosch 2001). So, the results of our findings with DNA markers based PCR and SDS-PAGE technique represent that frequency of the 1BL.1RS in Iranian bread wheat is very low (5 cultivars of bread wheat) and 1AL.1RS did not exist in Iranian wheat backgrounds. These cultivars can contribute to the creation of new Iranian wheat populations with a larger genetic diversity.

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