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Molecular evaluation of 1RS-rye translocation distribution in the Iranian dryland wheat cultivars and elite promising lines

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ABSTRACT
The short arm of 1RS rye (*Secale cereal*e L.) chromosome carrying genes involved in resistance to biotic and abiotic stresses provided a valuable source in developing new bread wheat (*Triticum aestivium* L.) cultivars resilient to stressed environments. The 1BL.1RS and 1AL.1RS translocations have been utilized in wheat breeding programs on a global scale. In this study, three rye specific primers (RyeR3/F3, PAW161 and O-SEC5'-A/O-SEC3'-R) were applied to investigate the distribution of 1RS arms in 22 dryland promising wheat genotypes, 7 dryland commercial wheat cultivars (Azar2, Ohadi, Rasad, Sabalan, Karim, Rijaw and Homa) and three genotypes with international origin (Seri82, 21thFW-236, and 21thFW-244) along with positive and negative controls to verify the results. 1RS arm presence was confirmed in the 86% of Iranian dryland promising lines and wheat cultivars. Using O-SEC5'-A/O-SEC3'-R primer none of the examined Iranian dryland wheat lines and cultivars was found to carry 1AL.1RS translocation. In this study Rye specific markers proved effective in the successful detection of wheat cultivars carrying 1RS arms and application of MAS in identifying wheat genotypes tolerant to environmental stresses. A high frequency of 1BL.1RS translocation was detected in the dryland promising wheat lines as well as in the commercial dryland wheat cultivars. This finding suggested the need for diversifying resistance gene sources by introducing the 1AL.1RS translocation into Iranian wheat germplasm. Genetic diversity revealed here, may be explored for developing new wheat cultivars with enhanced level of adaptation to adverse environmental conditions.

Keywords: 1AL.1RS, 1BL.1RS, Specific primers, Stress tolerance.

شناسائی مولکولی جابجائی کروموزومی 1RS چاودار-گندم درارقام تجاری و ژنوتیپ‌های پیشرفته گندم سازگار برای کشت در شرایط دیم

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چکیده
بازوی کوتاه کروموزوم 1RS چاودار (*Secale cereal*) حامل ژن‌های مقاومت به تنش‌های زنده و غیر زنده می‌باشد و منبع با ارزشی برای اصلاح ارقام جدید گندم (*Triticum aestivium*) مقاوم به تنش‌های محیطی به‌شمار می‌رود. جابه‌جایی‌های 1BL.1RS و 1AL.1RS به‌طور وسیعی در جهان در برنامه‌های اصلاحی گندم مورد استفاده قرار گرفته است. در این تحقیق جهت بررسی توزیع بازوی 1RS در ارقام و لاین‌های امید بخش گندم دیم کشور از ۳ نشانگر اختصاصی چاودار (RyeR3/F3، O-SEC5/A و PAW161) استفاده شد. ارقام گندم استاندارد به عنوان کنترل مثبت و منفی جهت تائید نتایج مشاهده شده بکار گرفته شدند. نتایج حاصل حضور بازوی 1RS را در ۸۶ درصد از لاین‌های گندم امید بخش دیم کشور تائید نمود. جهت تمایز بین جابه‌جایی‌های 1BL.1RS و 1AL.1RS از نشانگر اختصاصی O-SEC5/A استفاده شد که بر این اساس حضور جابه‌جایی 1AL.1RS در هیچکدام از لاین‌های امید بخش و ارقام تجاری دیم کشور مشاهده نگردید. نتایج این تحقیق استفاده موفقیت آمیز نشانگرهای اختصاصی چاودار را در شناسایی بازوی 1RS در ارقام گندم تائید و نشان داد که این نشانگرها قادرند جهت شناسائی ژنوتیپ‌های گندم حامل ژن‌های موثر در تحمل به تنش‌های محیطی بکار گرفته شوند. با توجه به فراوانی بالای جابه‌جایی 1BL.1RS در ژنوم ارقام و لاین‌های گندم دیم، استفاده از ژنوتیپ‌های گندم حامل جابه‌جایی 1AL.1RS تنوع ژنتیکی بیشتری را از نظر منابع ژنی مقاومت به تنش‌ها فراهم خواهد کرد. اطلاعات حاصل از این تحقیق در مورد تنوع ژنتیکی جابه‌جایی‌های 1BL.1RS و 1AL.1RS در ارقام و لاین‌های امیدبخش گندم دیم کشور می‌تواند به منظور انتقال ژن‌های مفید و تولید ارقام جدید گندم متحمل به تنش‌های زنده و غیر زنده محیطی مورد بهره‌برداری قرار گیرد.

واژه‌های کلیدی: 1AL.1RS, 1BL.1RS، آغازگرهای اختصاصی، تحمل به تنش.

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Introduction

1RS rye translocations are known important sources of genes controlling resistance to biotic and abiotic stresses. Many wheat-rye translocations have been identified (Jiang *et al*., 1994; Friebe *et al*., 1996). However, the 1BL.1RS and 1AL.1RS, are the sources, used widely in wheat breeding programs (Lukaszewski, 1990; Rabinovich, 1998). Numerous commercial wheat cultivars carrying 1BL.1RS and 1AL.1RS wheat-rye translocations have been released globally (Rabinovich, 1998). 1BL.1RS translocation was first transferred from 'Petkus' rye to Russian wheat cultivars 'Kavkaz' and "Aurora. The wheat line GRS1201 and cultivar 'Amigo' both carry 1AL.1RS translocation originated from the rye variety 'Insave' (Porter *et al*., 1991; Sebesta *et al*., 1995).

Studies shown that 1BL.1RS translocation carries genes for resistance to important wheat diseases, including stripe rust (Yr9) (*Puccinia striiformis* Westend), stem rust (Sr31) (*Puccinia graminis*), leaf rust (Lr26), (*Puccinia recondita* Rob, ex Desm), and powdery mildew (Pm8 and pm17) (Rabinovich 1998). Furthermore, increased kernel weight and higher above-ground biomass are reported due to the 1BL.1RS transfer to wheat genetic background (Carver and Rayburn, 1994; Moreno-Sevilla *et al*., 1995). The 1AL.1RS translocation shown to carry genes that are responsible for resistance to brown rust (*Puccinia graminis* Pers), greenbug (*Schizaphis graminum* Rondani) and powdery mildew (*Erysiphe gramminis* DC) (Sebesta *et al*., 1995). Moreover, 1AL.1RS translocation has been found to effectively increase the yield (Lukaszewski 1990; William and Mujeeb-Kazi 1993). The 1RS arm enhances adaptation to adverse environmental conditions by increasing tolerance to salinity, drought, acid soils and improved water use efficiency (Merker, 1982). Furthermore, increased level of grain yield (Kim *et al*., 2004)**,** embryogenesis, callus growth and regeneration of microspore in tissue culture have been reported in cultivars carrying 1RS translocations (Graybosch, 2001).

A number of 330 wheat cultivars from 35 different countries are reported to carry wheat-rye translocations (Rabinovich, 1998). 1RS arms are traced in more than 50% of wheat varieties grown in North West and Northern China (Zhou *et al*., 2004). Reports shown that 53 percent of wheat varieties grown in Hungary over the past 20 years contained 1RS translocation (Hoffmann, 2008).

Rapid and valid detection of 1AL.1RS and 1BL.1RS translocations in a breeding program will help to succeed in developing new tolerant wheat cultivars to cope with adverse climate changes. Some studies have used morphological traits such as percentage of germination, spike length and spike seed density to detect 1RS arms in wheat genotypes (Li *et al*., 2006). Others, have utilized metabolites and 1RS molecular markers to identify 1RS translocations (Zeller and Hsam, 1983). However, morphological traits and metabolites are subject to environmental variations and therefore are not suitable for tracing 1RS in wheat genome. C-banding, GISH, FISH (Rayburn and Carver, 1988; Heslop-Harrison *et al*., 1990; Miller *et al*., 1995; Anugrahwati *et al*., 2008; Mirzaghaderi *et al*., 2011), monoclonal antibody (skerritt and lew, 1990), ELISA assay (Zuniga *et al*., 2008), seed storage protein (Landjeva *et al*., 2006), high-performance liquid chromatography (Lookhart *et al*., 1991) are among various methods that have been used to detect wheat-rye 1RS translocation. These methods are time consuming, expensive and require difficult technical steps.

Molecular markers provided a fast and reliable tool for detecting 1RS

translocation and selection of wheat genotypes carrying resistant genes. Studies have used several PCR-based markers to identify1RS translocations in wheat (Shimizu *et al*., 1997; Nadella *et al*., 2002; Nagy and Lelley, 2003; Weng *et al*., 2007; Katto *et al*., 2004). In this research, three rye-specific primers pairs including RyeR3/F3, PAW161 and O-SEC5'-A/O-SEC3'-R were used to monitor the presence of 1BL.1RS and 1AL.1RS translocations in Iranian dryland wheat genotypes.

Material and Methods

Plant Material

The plant materials included 22 dryland wheat promising lines originated from the Elite Regional Wheat Yield Trial at Dryland Agricultural Research Institute (DARI, Maragheh, Iran), 7 commercial dryland wheat cultivars (Azar2, Ohadi, Rasad, Sabalan, Karim, Rijaw and Homa) and 3 selected cultivars or lines with international origin (Seri82, 21thFAWWON236 and 21thFAWWON-244), along with the cultivar Kavkaz as the positive control for 1BL.1RS, cultivars Tam107 and Amigo as the positive controls for 1AL.1RS and the standard Chinese Spring cultivar as the negative control for both 1BL.1RS and 1AL.1RS translocations (Table-2).

DNA extraction, primer design and PCR amplifications

Genomic DNA was extracted from young leaf tissues harvested from seedlings grown in greenhouse, following CTAB method (Doyle and Doyle, 1987). DNA quality and quantity were verified on 1% agarose gel and nanodrop spectrophotometer. Three rye-specific primers pairs including RyeR3/F3, PAW161 and O-SEC5'-A/O-SEC3'-R were used for PCR based amplifications of target DNA fragments (Table-1). RyeR3/F3 and PAW161 markers amplified 1400 and 366 bp fragments respectively, representing the presence of 1AL.1RS and 1BL.1RS translocations. O-SEC5'-A/O-SEC3'-R marker amplified three bands with 700, 1095 and 1530 bps length. Bands with 700 and 1530 bps indicated the presence of 1BL.1RS and bands with 1530 and 1095 bps represented the 1AL.1RS translocation.

PCR amplifications were carried out using a standard thermocycler (BioRAD, USA). PCR conditions for the primers started with the 5 min of initial denaturation at 95°C, followed by 35 cycles of 40 s at 95 °C, 40 s at 61 °C, 132 s at 72 °C, and 5 min final extension at 72 °C for RyeR3/F3 primer, 35 cycles of 40 s at 95 °C, 40 s at 61 °C, 40 s at 72 °C, and 5 min final extension at 72 °C for the PAW161 primer and 35 cycles of 40 s at 95 °C, 40 s at 52 °C, 156 s at 72 °C, and 5 min final extension at 72 °C for the O-SEC5'-A/O-SEC3'-R Primer. All amplifications were performed in a 50 µl volume reaction containing the final concentration of 0.02 units/µl of *Taq* DNA polymerase, 100 µM of dNTPs mix, 2.0 mM $MgCl_2$, 0.5 µM of each primer pairs, 5 µl of 10x PCR buffer and 50 ng of template DNA. Amplified fragments were analyzed through electrophoresis on 2% agarose gels in 0.5×TBE buffer, stained with ethidium bromide and visualized and recorded under ultraviolet light. DNA ladder DM2300 (SMOBIO) composed of 12 individual DNA fragments: 3k, 1.5k, 1k, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bps was used for easy referencing of amplified fragments.

Results and Discussion

Rye-specific primers were used to investigate the presence of 1AL.1RS and 1BL.1RS translocations in the Iranian dryland wheat cultivars and elite promising lines. RyeR3/F3 primers amplified a 1400 bps fragment in Kavkaz (positive control for 1BL.1RS), Tam107

and Amigo (positive controls for 1AL.1RS), 19 (86%) of dryland promising lines (DARI-3 -4 -5 -6 -7 -8 -9 -10 -12 -14 -15 -16 -17 -18 -19 -20 -21 -23 -24), all of dryland commercial cultivars (Azari2, Ohadi, Karaj, Sabalan, Rasad, Rijaw and Homa) and the three selected genotypes with international origin (Seri82, 21thFW-236, and 21thFW-244). However, no fragment was amplified by RyeR3/F3 primer in the Chines Spring cultivar (negative control for both 1BL.1RS and 1AL.1RS translocations) and in 3 (13%) of dryland promising lines (DARI-11, 13 and 22) (Figure 1).

RyeR3/F3, designed by Katto *et al.*, (2004), has been related to Ty3/gypsy-like retro-transposons sequence, which is located on the rye chromosome 1 centromere region. Reverse transcriptase genes, integrase enzyme, RNaseH, long terminal repeat, primer-binding site and poly purine rich sequences are the most important regions of the Ty3/gypsy-like retro-transposons (Kovalchuk *et al*., 2005).

PAW161 primers amplified a 366 bps fragment in Kavkaz (positive control for 1BL.1RS), Tam107 and Amigo (positive controls for 1AL.1RS), 19 (86%) of dryland promising *wheat lines* (DARI-3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -18 -19 -20-22 -23 -24), all of examined dryland cultivars as well as all genotypes with international origin. On the other hand PAW161 primers did not amplify any fragment in the Chines Spring cultivar and three (13%) of dryland wheat promising *lines* (DARI-16, 17 and 21) (Figure-2). The PAW161 marker was designed based on rye chromosome telomeric regions (Weng et al., 2007).

O-SEC5'-A/O-SEC3' marker used to distinguish between 1AL.1RS and 1BL.1RS translocations, resulted in the amplification of three bands with different lengths of 700, 1095 and 1530 bps. O-SEC5'-A/O-SEC3' primers amplified two different fragments with 700 and 1530 bps in Kavkaz (positive control for 1BL.1RS), 7 (31%) of dryland wheat promising lines (DARI-4 -5 -6 -7 -9 -20 -24), 2 (28%) of commercial dryland wheat cultivars (Rasad, Rijaw) and two of the examined international wheat genotypes (Seri82 and 21FW-236). However, the PCR reactions for O-SEC5'-A/O-SEC3' marker amplified two fragments with 1530 and 1095 bps in Tam107 and Amigo (the positive controls for 1AL.1RS) and in one line with international origin (21thFAWWON-244). No amplification was detected in the Chines Spring cultivar, the negative control for both 1AL.1RS and 1BL.1RS translocations (Figure 3).

Table 1. Primers sequence and annealing temperature used in this study

Primers	Sequence (5´-3´)	Band size (bps)	Annealing temperature	Reference
RyeR3/F3	F: GATCGCCTCTTTTGCCAAGA R: TCACTGATCACAAGAGCTTG	1400	61°C	Katto *et al*., 2004
O-SEC5'-A/O-SEC3'	F: CTATTAGTTCGAAAAGCTTATGA R:GCATATGACTCAAATTATTTTTT	700, 1530, 1095	52°C	Shimizu *et al*., 1997
PAW161	F: TGAGGGCCCAGACGGCCCTTTTTG R: TTATCGCAATTACAACTCAAATTT	366	61°C	Weng *et al*., 2007

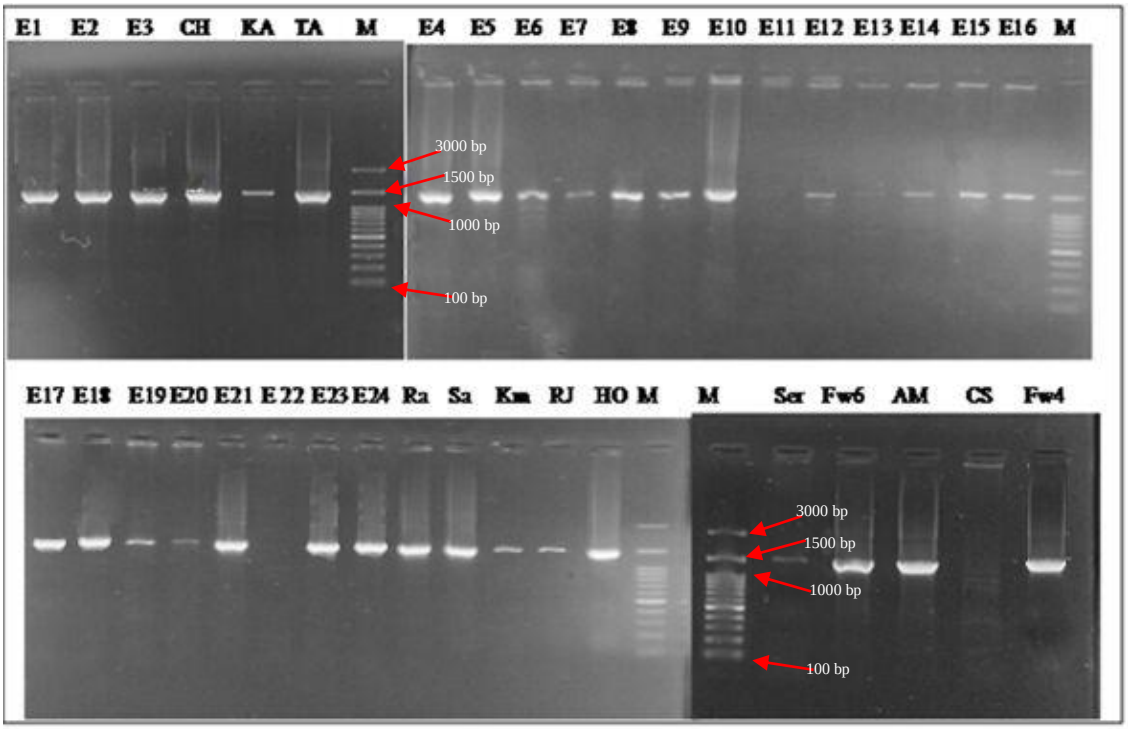

Figure 1. Fragment amplified by RyeR3/F3 markers in the examined genotypes. (E1: Azar2, E2: Ohadi, E3–E24: Iranian dryland wheat promising lines DARI-3–24 , Ra: Rasad, Sa: Sabalan, RJ: Rijaw, HO: Homa, M: DNA ladder, Ser: Seri82, Fw6: 21thFAWWON-236, AM: Amigo, CS: Chiness Spring, FW4: 21thFAWWON-244, KA: kavkaz, TA: Tam107.

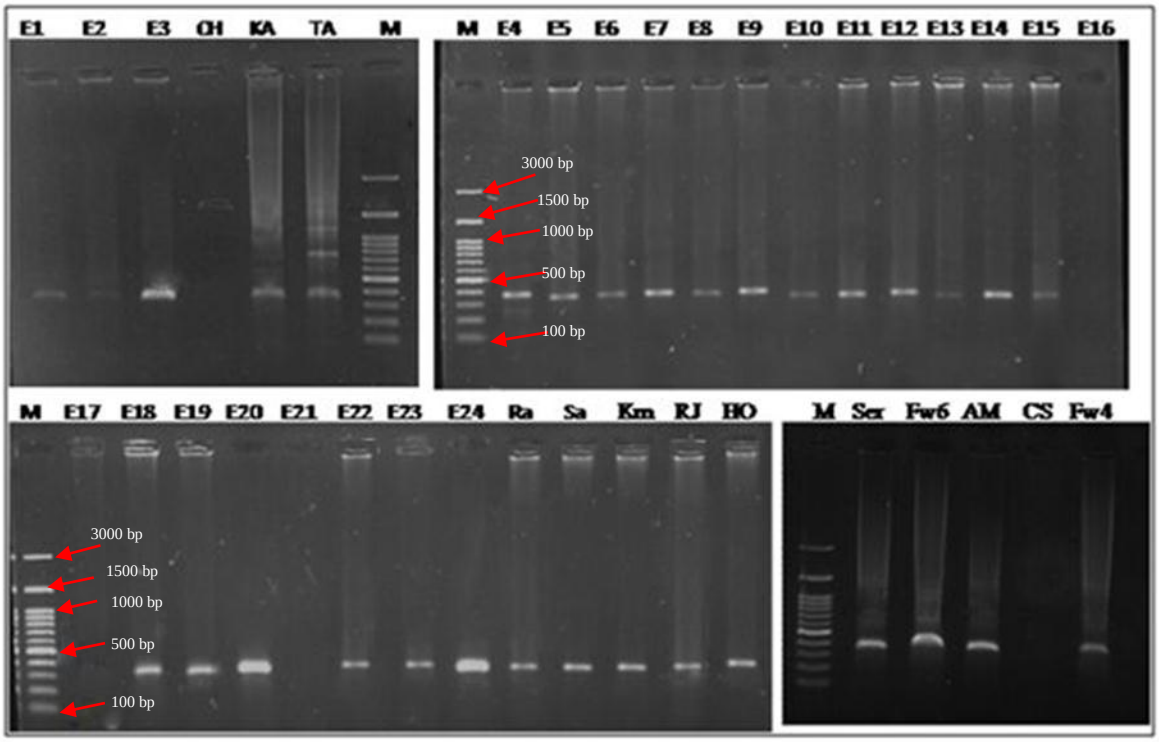

Figure 2. Fragment amplified by PAW161 marker in the examined wheat genotypes. Brevity used to distinguish different genotypes are similar to those used in the Figure (1) caption.

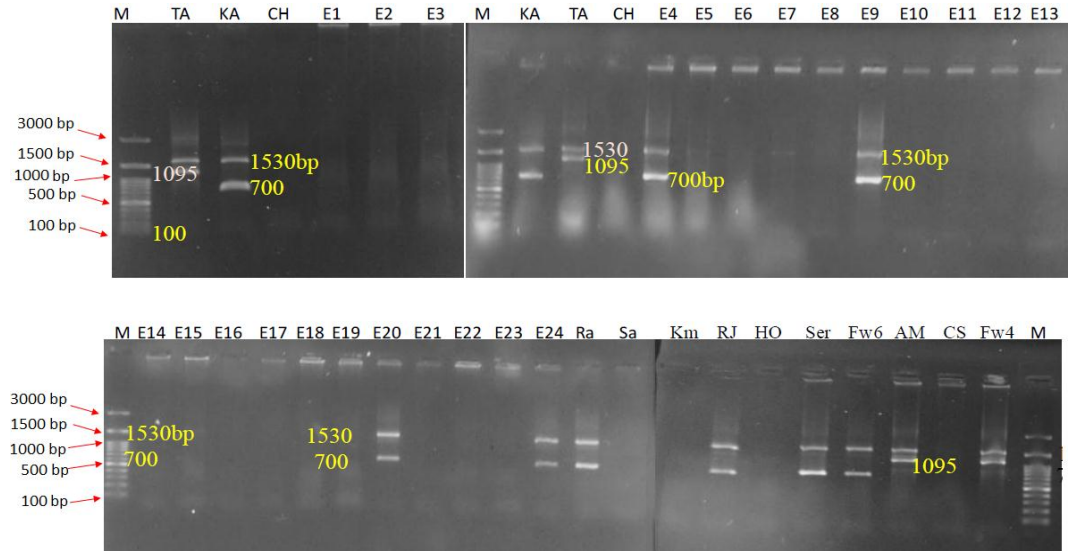

Figure 3. Fragments amplified by O-SEC5'-A/O-SEC3' in the examined wheat genotypes. Brevity used to distinguish different genotypes are similar to those used in the Figure-1 caption.

Utilization of 1RS-rye translocations in wheat breeding programs have led to increased tolerance to biotic and abiotic stresses in the developed cultivars, enhancing the yield potential under stressed environments (Villareal *et al*., 1991). It is therefore essential to know the abundance and distribution of these translocations in the breeding germplasm. Many studies have tried to identify 1RS arms in wheat genotypes using different methods (Gill and Kimber, 1977; Weng *et al*., 2007. Zuniga *et al*., 2008). 1RS arm was identified in 50% of the bread wheat cultivars from China using SDS-PAGE method (Zhou *et al*., 2004). Application of SDS-PAGE, Giemsa-C banding and GISH methods, have confirmed the presence of 1BL.1RS translocation in 35 (53%) of Hungarian bread wheat cultivars (Hoffmann 2008). In addition, N-banding, storage proteins and DNA markers analysis have shown that 17 (55%) of bread wheat cultivars in Bulgaria carry wheat-rye 1BL.1RS translocation (Landjeva *et al*., 2006). Yediay *et al*., (2010) examined nine rye specific primers to screen Turkish durum and bread wheat cultivars for wheat-rye translocations. They identified 4 bread wheat cultivars carrying 1BL.1RS translocation, but no cultivar was found with 1AL.1RS translocation. Tabibzadeh *et al*., (2013) studied the distribution of the wheat-rye translocations 1BL.1RS and 1AL.1RS in 29 Iranian bread wheat cultivars and 15 Iranian durum wheat cultivars using SDS-PAGE and PCR-based DNA markers. They found 1BL.1RS translocation in 5 (17%) of the examined bread wheat cultivars, but no presence of 1AL.1RS translocation was reported. Mirzaghaderi *et al*., (2011) confirmed the presence of 1BL.1RS in some cultivars using GISH techniques. In a different study 1BL.1RS translocation was identified in 14 out of 66 (21%) examined wheat cultivars and 1AL.1RS translocation was detected in the wheat cultivar Sholeh, the variety that is known for high tolerance to salinity (Bagherikia *et al*., 2014).

The cultivar Kavkaz has been used as a source of 1BL.1RS translocation to develop new wheat cultivars in many countries (Trubacheeva *et al*., 2011). Previously using RyeR3/F3 primer (Tabibzadeh *et al*., 2013; Bagherikia *et al*., 2014) and PAW161 primer (Tabibzadeh *et al*., 2013) presence of

1RS arm was reported in in 17% of Iranian wheat cultivars. In this study, a higher percentage of the elite promising dryland bread wheat lines and cultivars carrying 1BL.1RS translocation was detected. We identified the 1RS arm in 86% of the examined dryland bread wheat genotypes using RyeR3/F3 and PAW161 markers (Table 2). Wheat grown under dryland conditions are exposed to drought stress at different stages of crop development, which retards the formation of yield component and restricts grain yield by lowering both kernel number and kernel weight. Clearly an improved level of stress tolerance is required for wheat lines and cultivars developed to grow under dryland conditions. The higher frequency of 1RS arm detected in the Iranian dryland wheat cultivars and the elite promising lines in this study, may be explained by the higher level of stress tolerance required for the adaptation to dryland conditions due to the genes that are transferred through wheat-rye translocation.

Table 2. Distribution of 1RS translocations in the examined wheat genotypes

Cultivars and genotypes	Loci		
	O-SEC5'-A/O-SEC3' 1AL.1RL	PAW161	Rye R3/F3
DARI-3	-	+	+
DARI-4	-	+	+
DARI-5	-	+	+
DARI-6	-	+	+
DARI-7	-	+	+
DARI-8	-	+	+
DARI-9	-	+	+
DARI-10	-	+	+
DARI-11	-	+	-
DARI-12	-	+	+
DARI-13	-	+	-
DARI-14	-	+	+
DARI-15	-	+	+
DARI-16	-	-	+
DARI-17	-	-	+
DARI-18	-	+	+
DARI-19	-	+	+
DARI-20	-	+	+
DARI-21	-	-	+
DARI-22	-	+	-
DARI-23	-	+	+
DARI-24	-	+	+
Azar2	-	+	+
Ohadi	-	+	+
Rasad	-	+	+
Sabalan	-	+	+
Karim	-	+	+
Rijaw	-	+	+
Homa	-	+	+
Seri82	-	+	+
21FW-236	-	+	+
21FW-244	+	+	+
Kavkaz	-	+	+
TAM107	+	+	+
Amigo	+	+	+
Chiness Spring	-	-	-

O-SEC5'-A/O-SEC3' primers used to distinguish between 1AL.1RS and 1BL.1RS translocations, was designed based on omega-Secaline gene that is located on Sec-1 locus (Shimizu *et al*., 1997) within satellite regions of 1RS chromosome. Translocation of rye omega-Secaline genes with wheat Omega-gliaidin gene has been confirmed in a number of Iranian wheat cultivars using SDS-PAGE gel electrophoresis (Afshari, 2006; Tabibzadeh *et al*., 2013). In this study 1AL.1RS translocation was not detected in any of dryland wheat promising lines and cultivars. So far, the presence of 1AL.1RS translocation have only been reported in the Iranian wheat cultivar Sholeh (Bagherikia *et al*., 2014). The variety that is known for high tolerance to salinity and recommended for growing in regions under saline stress conditions. In general, 1RS arm studies in wheat germplasm indicated a higher frequency of 1BL.1RS compared to the 1AL.1RS translocation (Yediay *et al*., 2010; Landjeva *et al*., 2006; Hoffmann, 2008). This may in part be due to the late introduction of 1AL.1RS source to wheat genetic background that has led to lower frequency of 1AL.1RS translocation presence in wheat germplasm compared to the 1BL.1RS translocation (Rabinovich, 1998; Sebesta *et al*., 1995).

This study was the first attempt to specifically investigate 1RS arm in dryland wheat cultivars and in the elite wheat promising lines developed to grow under Iranian dryland conditions. Wheat genotypes with confirmed 1BL.1RS and 1AL.1RS translocations in this study, may be explored as sources of stress tolerance genes in wheat breeding programs. Our findings illustrated the successful use of rye-specific markers in the identification of 1RS arm in wheat genome. Based on the information provided in this study marker assisted selection (MAS) of wheat cultivars and lines carrying 1RS arm may be employed for identifying genotypes with an improved level of tolerance to environmental stresses. A high frequency of 1BL.1RS translocation presence in dryland wheat lines and cultivars detected in this study suggested the need for diversifying sources of stress resistance genes by introduction of 1AL.1RS translocation to adapted Iranian dryland wheat germplasm.

Gens transferred through 1AL.1RS translocation improve wheat tolerance to biotic and abiotic stresses, while having less negative impact on the end use quality of developed bread wheat cultivars (Sebesta *et al*., 1995; Merker, 1982). Our findings revealed the absence of 1AL.1RS translocation in the examined Iranian dryland wheat genotypes. Therefore, introducing the 1AL.1RS translocations to adapted wheat genetic background will further diversify genetic sources of genes responsible for wheat tolerance to environmental stresses. This will facilitate development of new wheat cultivars that are more resilient to adverse climatic changes and have higher end use quality at the same time.

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