

## Wheat-Rye Translocation in Iranian Bread Wheat Cultivars and Their Ion Distribution in Response to Salinity Stress

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### ABSTRACT

The short arm of rye (*Secale cereale* L.) chromosome 1 (1RS), besides being part of the rye genome, is present in many hundred wheat cultivars as either 1RS.1BL or 1RS.1DL wheat-rye translocation. In this study, the distribution of the wheat-rye translocation was examined in 33 Iranian winter and spring wheat cultivars, nine of which had a known donor of 1RS.1BL translocation and the other 24 were randomly selected cultivars without a known source of 1RS.1BL in their pedigree. The presence of the translocation was verified in 4 cultivars, using genomic *in situ* hybridization analysis. We also compared the Na<sup>+</sup> exclusion and K<sup>+</sup>/Na<sup>+</sup> ratios in leaf and root of the identified 1RS.1BL translocations and in eight randomly selected non-translocated (NT) control cultivars grown in hydroponic solutions, containing 0 and 200 mM NaCl. Mean comparisons showed that the 1RS.1BL cultivars (Atrak, Dez, Falat, Rasul) had significantly lower rates of whole plant dry weight and root dry weight in the presence of 200 mM NaCl compared with NT control cultivars. No significant difference was observed between translocated and NT cultivars for Na<sup>+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> ratios in their leaves or roots. Although, there are many useful genes in 1RS arm, it has no substantial contribution to Na<sup>+</sup> exclusion in comparison with NT controls at seedling stage.

**Keywords:** Cytogenetics, Salt tolerance, *Secale cereale*, *Triticum aestivum*, 1RS.1BL Wheat-Rye translocation.

### INTRODUCTION

Rye (*Secale cereale*, R genome) offers the potential to introduce desirable genes for wheat (*Triticum aestivum* L., ABD genomes) improvement. Of special interest is the short arm of rye chromosome 1R (1RS), carrying genes in order to enhance yield, water use efficiency, and disease resistance (Mettin *et al.*, 1973; Zeller, 1973; Villareal *et al.*, 1998; Berzonsky and Francki, 1999; Ehdaie *et al.*, 2003). The short arm of Petkus rye chromosome 1R harbours a number of resistance genes including *Sr31* (stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici*), *Lr26* (leaf rust, *P. recondita* Rob. ex

Desm. f. sp. *tritici*), *Yr9* (stripe rust, *Puccinia striiformis* Westend f. sp. *tritici*), and *Pm8* (powdery mildew, *Blumeria graminis* f. sp. *tritici*). Many successful wheat cultivars containing 1RS translocation from two different rye genotypes have been released, including the Veery line developed at CIMMYT (Merker, 1982; Rajaram *et al.*, 1983). The Veery line was derived from crosses between a Mexican spring semi-dwarf and the Siberian winter bread wheat variety Kavkaz, which carries a 1RS.1BL translocation chromosome with the 1RS arm coming from Petkus rye (Zeller, 1973; Schlegel and Korzun, 1997).

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Various methods have been employed to detect the 1RS translocation, including conventional cytology (Zelle, 1973), electrophoretic analysis of seed storage proteins (Koeber and Shepherd, 1986; Landjeva *et al.*, 2006), chromosome N and, C-banding (Rayburn and Carver, 1988), PCR with specific primers (Koeber, 1995; Weng *et al.*, 2007) and PCR-ELISA (Zuniga *et al.*, 2008). Also, chromosome banding and the abundance of DNA markers in cereal genomes (Devos and Gale, 1992) provided means to study the presence of alien chromatin in wheat and genomic *in situ* hybridization (GISH) provided a powerful tool to detect or verify the rye chromatin in wheat backgrounds (Heslop-Harrison *et al.*, 1990; Anugrahwati *et al.*, 2008). GISH can identify either whole alien chromosome substitutions or whole-arm translocations in wheat (Miller *et al.*, 1995). Alien chromatins can be easily visualized by GISH, not only in metaphase spreads but also within interphase nuclei. In practice, the technologies chosen to characterize 1RS in wheat will depend on the breeding goals of a program and the resources available to that program. Biochemical and PCR technologies, adaptable to automation and rapid analysis of large samples, will be most effective for identifying lines and varieties with 1RS. RFLP analyses, chromosome banding, GISH, and FISH, which allow the detection of segmental introgressions, should be used in programs requiring a more thorough characterization of 1RS.

Although there are many genes for useful quantitative and qualitative traits in 1RS arm,

little has been understood about the possible effect of this segment on salinity tolerance of wheat. Because of the complex nature of salinity tolerance, as well as the difficulties in maintaining long-term growth experiments, trait-based selection criteria are recommended for screening techniques (Noble and Rogers, 1992; Colmer *et al.*, 2005). Traits used for screening germplasm for salinity tolerance of wheat have included Na<sup>+</sup> exclusion (Garcia *et al.*, 1995; Poustini and Siosemardeh, 2004), and K<sup>+</sup>/Na<sup>+</sup> discrimination (Asch *et al.*, 2000). Glasshouse experiments have shown that landraces with low Na<sup>+</sup> accumulation yield better than high Na<sup>+</sup> genotypes at moderate salinity (Munns and James, 2003), therefore, Na<sup>+</sup> exclusion is a robust trait that probably reflects the salinity tolerance in the field.

This study aimed to identify the 1RS translocated chromosome in Iranian bread wheat cultivars. The identified 1RS translocations and some NT cultivars were also evaluated in a hydroponic culture in terms of their leaf and root Na<sup>+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> ratios.

## MATERIALS AND METHODS

### Plant Materials

Thirty three wheat cultivars were used, 9 of which had a known donor of 1RS.1BL translocation and 23 were randomly selected cultivars without a known source of 1RS in their pedigree (Table 1). The wheat variety Kavkaz was used as positive control in each

**Table 1.** Iranian wheat cultivars used in this study, distributed according to the 1RS.1BL donor in their pedigree.

1RS.1BL donor	Cultivar names and growth habits <sup>a</sup>
Kavkaz	Alamut (W), Falat (S), Hamun (S), Karaj 2 (F), Rasul (S), Shahriar (W), Zagros (S), Atrak (S), Dez (S), MV 17 (W)
without a known source of 1RS	Alborz (F), Alvand (F), Atila (S), Bulani (S), Darab 2 (S), Ghods (F), Inia (F), Kavir (S) Mahdavi (F), Mahuti (S), Navid (F), Niknejad (S), Omid (W), Pishtaz (S), Roushan (F), Sabalan (W), Sardari (W), Sholeh (S), Sorkh-Tokhm (S), Tabasi (S), Tajan (S), Tubari (S), Zarrin (F)

<sup>a</sup> Letters inside parenthesis show growth habit where S: Spring; W: Winter; F: Facultative.

round of *in situ* hybridization. The Hungarian IRS.1BL bread wheat cultivar MV 17 (Köszegi *et al.*, 2000) also was included to be verified as it is cultivated in Iran.

### Chromosome Staining

Chromosome preparation and squashing were done based on Mirzaghaderi (2010). Briefly, seeds were germinated in petridishes. Root tips were cut and pretreated in ice cold water for 24 h in order to arrest the cells in metaphase. Root tips were then fixed in ethanol:acetic acid (3:1, v/v) for 3 d at 4°C. Five seeds of each cultivar and 5-10 cells with well spread metaphase chromosomes from each seed were examined using hematoxylin or Feulgen staining method. Cultivars containing only two chromosomes with major secondary constrictions were suspected as those having 1RS.1BL translocation.

### Genomic *in situ* hybridization (GISH)

For further analysis of the wheat GISH, the rye genomic DNA was labeled with biotin-16-dUTP using a nick translation kit (Roche). GISH protocol was based on Mirzaghaderi *et al.* (2010). For each cultivar, two slides from different seeds were examined. Slides were incubated in RNase A (10 µg ml<sup>-1</sup> in 2×SSC) for 1 h at 37°C and washed in 2×SSC for 5 min. Slides were hydrolyzed in 10 mM HCl for 5 min at RT and treated by pepsin (10 µg ml<sup>-1</sup> in 10 mM HCl) for 10 min at 37°C. After washing in 2×SSC, the slides were stabilized in 4% (w/v) paraformaldehyde in 1×PBS for 10 min at RT followed by washing for 2 × 5 min and dehydrating in ethanol series (70%, 90% and 100%). Slides were then denatured in 50% formamide in 2×SSC for 2.5 min at 70°C and dehydrated in cold ethanol series. The hybridization solution, containing 50% (v/v) formamide, 2×SSC, 10% (w/v) dextran sulfate, 0.3 mg ml<sup>-1</sup> of sheared salmon testes DNA, about 3 mg ml<sup>-1</sup> of labeled probes and 15 mg ml<sup>-1</sup> autoclaved genomic DNA of wheat was denatured in boiling water for 6 min.

After chilling on ice, 30 µl of the hybridization mixture was applied to each slide and covered with a coverslip. Slides were placed for 10 min at 80°C and left overnight at 37°C for hybridization in a closed humidified container. After removing the coverslips in 2×SSC, post-hybridization washing was performed in 50% (v/v) formamide in 2×SSC for 2 × 10 min at 42°C followed by rinsing in 2×SSC for 2 × 10 min at RT. The biotin-labeled probe was detected with 5 µg ml<sup>-1</sup> Fluorescein Avidin in blocking buffer (3% BSA in washing buffer). After incubation at 37°C for 30 min, the slides were washed in washing buffer (0.1 M sodium bicarbonate, 0.05% igeal CA 630, pH 8) for 4 × 5 min and amplified using 5 µg ml<sup>-1</sup> Fluorescein Anti-Avidin followed by a second round of washing. The slides were air-dried and mounted in 30 µl of mounting medium containing 1 µg ml<sup>-1</sup> propidium iodide or DAPI as counterstain. Slides were analyzed with a Zeiss Axioplan2 microscope and images were captured using a CCD camera in to the Isis software (Version 4.4.25).

Beside the GISH, CISH was applied on Atrak cultivar, in which a chromogenic detection kit (Fermentas) was used for detection. Briefly, after hybridization to the slides, biotin labeled probe was detected following incubation with alkaline phosphatase streptavidin according to the manufacturer's instructions. Alkaline phosphatase, which is conjugated to the streptavidin, cleaves a substrate to generate a colored precipitate. Slides were washed in washing buffer and analyzed with a light microscope.

### Physiological Analysis

Seeds were germinated for 4 d at RT on moist perlite in trays before being transferred to a supported hydroponic setup containing Hoagland solution with pH 6.7 (Hoagland and Arnon, 1938). After 4 d, seedlings were transferred to aerating, 20-litre boxes in a completely randomized design containing full strength nutrient solution in a glass house (25 °C ± 3 °C/15 °C ± 3 °C day/night). Each box



was considered as a block and contained 12 pots, each having 3 seedlings of a genotype.

Solution levels were maintained daily by addition of deionized distilled water and NaCl treatments were initiated when all plants were at the 2.0 to 2.2 Haun leaf stage. Two NaCl treatments of 0 mM (control) and 200 mM NaCl were reached through stepwise increments of 50 mM per day. The 4<sup>th</sup> leaf of each plant was harvested when elongation of this leaf was completed. Na<sup>+</sup> and K<sup>+</sup> contents were measured as described by Munns *et al.* (2010). Briefly, leaves were rinsed with deionized distilled water before being oven-dried at 70 °C and the dried tissue samples were ground and placed in 15 ml centrifuge tubes. Na<sup>+</sup> and K<sup>+</sup> were extracted from tissue samples by shaking approximately 30 mg of ground leaf material in 5 ml of 0.5 M HNO<sub>3</sub> for 2 d. Na<sup>+</sup> and K<sup>+</sup> were measured using a flame photometer (BWB-XP, UK) and the data were analyzed by analysis of variance.

## RESULTS AND DISCUSSION

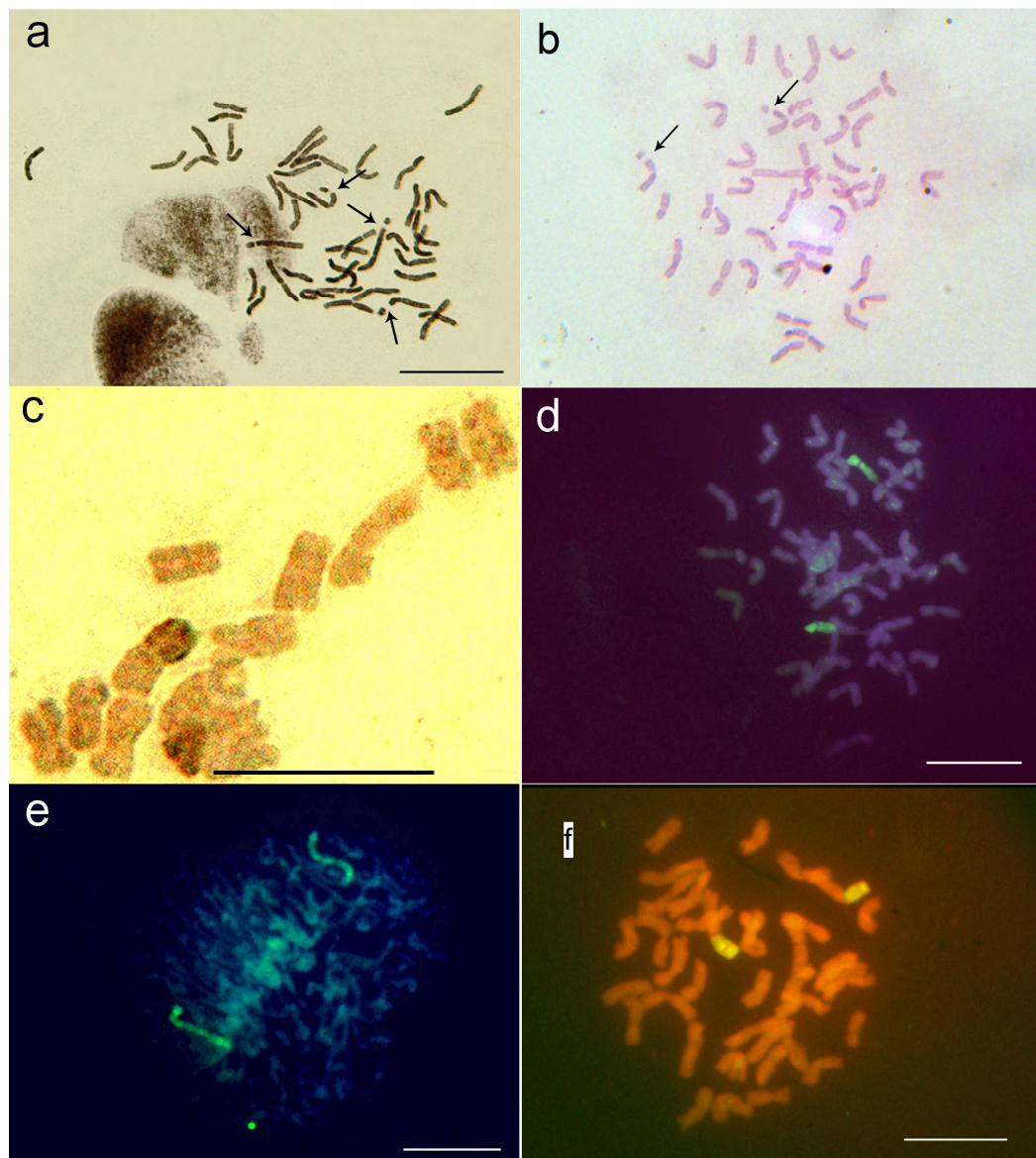
The translocation was found in 4 of the 9 cultivars (45%), having a known source of 1RS.1BL in the pedigree (Table 1, Figure 1). Conventional staining revealed that these 4 cultivars (Atrak, Dez, Falat, Rasul) were 1RS.1BL translocation as well. 1RS was not detected in the other 23 cultivars that lacked a known source of 1RS in their pedigree. No heterogeneity was found for 1RS translocation in plant materials.

The satellite or secondary constriction of 1RS is not expressed in a wheat genetic background (Merker, 1982). In lines lacking a 1BL.1RS translocation, somatic cells usually have four chromosomes with prominent satellites associated with their short arms, the two 1B and two 6B chromosomes. Because 1RS replaces 1BS in a Robertsonian 1RS.1BL translocation, the detection of only two satellited chromosomes in somatic cells gives a quick and initial indication of the 1RS.1BL translocation. This is only useful when 1RS replaces 1BS, not 1AS or 1DS. In several studies, counting the number of satellited

chromosomes in somatic cells led to the initial identification of 1BL.1RS translocations (Berzonsky *et al.*, 1991; Zeller, 1973). We also found such a mitotic analysis an easy and inexpensive assay in detecting the 1RS.1BL translocations. Genomic *in situ* hybridization is more accurate than conventional staining, which may be affected by the quality of the preparations. GISH can also detect other types of 1RS translocation such as 1RS.1AL, 1RS.1DL and any other rye translocation as well, but these types of translocations were not present among the studied cultivars. CISH was also as sensitive as GISH in detecting 1RS translocation (Figure 1c). 1RS rye segment was reliably recognized by light microscopy followed by CISH. This technique has advantages over FISH, because it uses conventional peroxidase reactions that can be observed under light microscopy at the sites where the probe is hybridized, thus allowing preservation of the test slides (Walter *et al.*, 2011).

There are some reports about the presence of rye chromatin among Iranian bread wheat. Previously, two Iranian bread wheat cultivars have been reported as 1B (Waines and Ehdaie, 2007). In another report Atrak and Mahdavi were distinguished as 1RS.1BL translocation based on their seed storage protein pattern in SDS-PAGE (Afshari, 2006), but conventional staining and GISH did not detect 1RS segment in Mahdavi cultivar in the present study.

The pedigree of the translocated lines shows that the introduction of the 1RS.1BL translocation into Iranian bread wheat cultivars is a result of the utilization of the CIMMYT 1RS.1BL wheat in their pedigree during the hybridization programs. It seems that the wheat-rye translocation for the short arm of chromosome 1 (1RS) has not been employed by Iranian breeders as much as many other breeders in countries such as USA, Australia, China, Mexico, India, and many European countries that have widely used 1RS.1BL translocated wheat cultivars in their breeding programs (Berzonsky and Francki, 1999). In a similar study, Yediay *et al.* (2010) found low frequency (4%) of 1RS.1BL translocation among Turkish wheat cultivars and landraces



**Figure 1.** Detection of the *IRS.1BL* translocation in Iranian wheat cultivars: (a) Hematoxylin staining of metaphase chromosomes of Hamun showing 4 chromosomes with secondary constriction; (b) Feulgen staining of metaphase chromosomes of Atrak, showing only 2 chromosomes with secondary constriction; (c) CISH on a partial metaphase chromosome spread of Atrak using rye genomic DNA probe showing that it is *IRS.1BL*, (d-f) GISH on metaphase and prophase chromosome spreads of Dez, Falat and Rasul respectively, using rye genomic DNA probe showing translocated rye chromosome arm (Scale bar= 20  $\mu$ m).

as well and 1AL.1RS translocation was not detected.

In the next experiment, we compared the ion distribution and salt tolerance of 1RS.1BL cultivars versus a random sample of NT ones at seedling stage. The first mechanism that we used as a basis for salt tolerance was  $\text{Na}^+$  exclusion, as genetic differences in  $\text{Na}^+$

exclusion are highly correlated with differences in salinity tolerance between tetraploid and hexaploid wheat (Francois *et al.*, 1986; Gorham *et al.*, 1987).

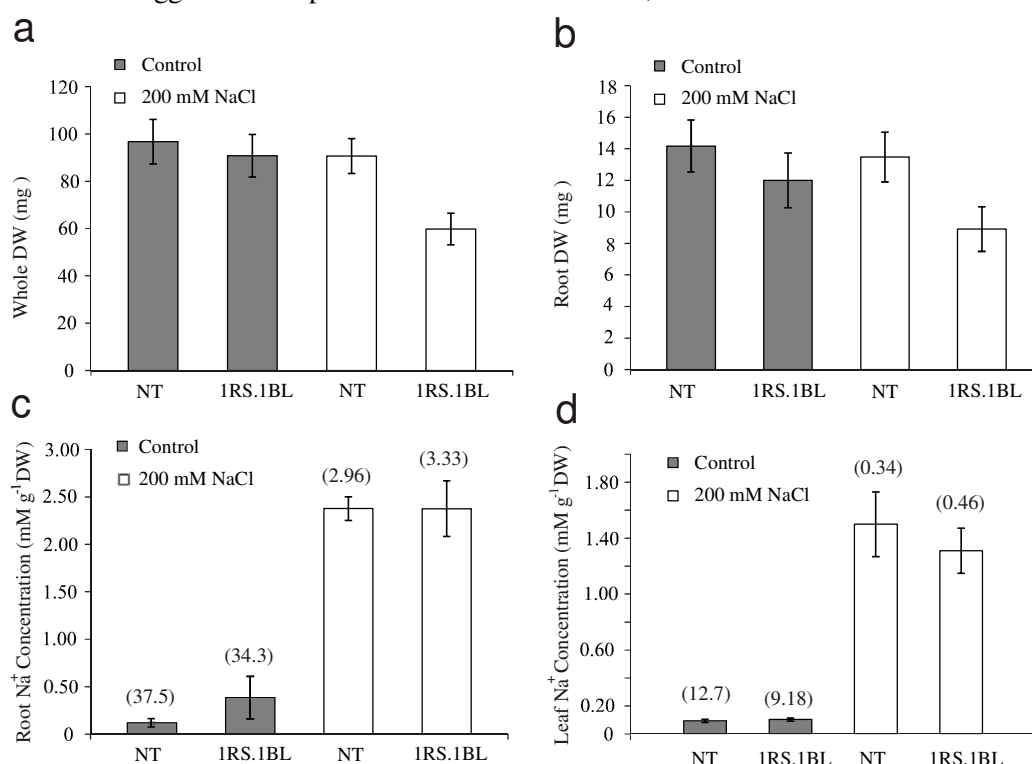
Mean comparisons showed that the 1RS.1BL cultivars (Atrak, Dez, Falat, Rasul) had significantly lower rates of whole plant dry weight and root dry weight in the presence



of 200 mM NaCl compared to other NT cultivars (Alamut, Bulani, Hamun, Mahdavi, Niknejad, Pishtaz, Sabalan, Shahriar) (Figure 2a and b). There was no significant difference between 1RS.1BL translocated and NT control cultivars from the view point of  $\text{Na}^+$  concentrations and  $\text{K}^+/\text{Na}^+$  ratios in their leaves or roots (Figure 2c and d). However, the  $\text{Na}^+$  concentration in the leaves of 1RS.1BL translocations was generally higher ( $P > 0.05$ ) than that in the leaves of NT control cultivars in the presence of 200 mM NaCl (Figure 2d). The relationship between salinity tolerance and  $\text{K}^+/\text{Na}^+$  discrimination was also considered as shown in Figure 2, because  $\text{K}^+/\text{Na}^+$  rather than  $\text{Na}^+$  alone was used as an index of salinity tolerance for cultivar comparisons in wheat (Chhipa and Lal, 1995; Dvorak et al., 1994) and rice (Asch et al., 2000; Zhu et al., 2001).

Although the translocation is associated with increased resistance to a range of fungal diseases and is suggested to impart a selective

advantage, no correlation between the presence of the translocation and  $\text{Na}^+$  exclusion was found among the studied cultivars. In this report, the presence of the 1RS rye genome in wheat background as 1RS.1BL did not significantly affect ion concentrations within the leaves at high salt concentrations (200 mM NaCl). It even generally dropped the dry weight of the seedlings significantly ( $P < 0.05$ ) at the presence of 200 mM NaCl (Figure 2). This result is in consistence with other reports where the presence of the rye genome did not significantly affect ion concentrations of the leaves (Gorham, 1990). Other works have shown that the most salt tolerant Iranian cultivars are Alvand, Roushan, Sorkh-tokhm, Sholeh, Tabasi, Kavir, Mahoti, Mahdavi (Poustini and Siosemardeh, 2004). None of these cultivars have 1RS segment in their genome. On the other hand, the cultivar Ghods, known as a salt sensitive cultivar, is a



**Figure 2.** Dry Weight (DW) and  $\text{Na}^+$  concentrations of non-translocated (NT) wheats (cvs. Alamut, Bulani, Hamun, Mahdavi, Niknejad, Pishtaz, Sabalan, Shahriar) and 1RS.1BL translocations (Atrak, Dez, Falat, Rasul) grown at 0 and 200 mM NaCl, each line in 3 replications: (a) Whole plant dry weight; (b) Root dry weight; (c)  $\text{Na}^+$  concentration for root, (d)  $\text{Na}^+$  concentration of 4<sup>th</sup> leaf in which mean of  $\text{K}^+/\text{Na}^+$  ratios are given in parentheses. Bars represent means  $\pm$  standard error

NT cultivar as well (Poustini and Siosemardeh, 2004). These results indicate that 1RS segment may have negative effect, or no positive effect, on wheat salinity tolerance. If so, the cultivar differences in salinity tolerance would be more effective regardless of the presence or absence of 1RS arm. A wider range of cultivars or 1RS near isogenic lines may need to be investigated to achieve better verification of the relationship between the presence or absence of 1RS and salinity tolerance.

1RS arm has increased root biomass and

branching in cultivars that contain it (Waines and Ehdaie, 2007). Therefore, it may increase grain yield significantly in irrigated and rain-fed conditions. Plant breeders may use more 1RS translocated wheat cultivars in Iran and integrate root characters into components of yield analysis in wheat characters. On the other hand, the situations may be different in salinity affected areas and root biomass of 1RS translocated lines may be affected by salt more than normal wheat genotypes. 1RS.1BL is also associated with detrimental effects on dough quality due to the replacement of some

**Table 2.** Presence of the *1RS.1BL* translocation in Iranian wheat cultivars.

Cultivar	Mitotic analysis <sup>a</sup>	Genomic <i>in situ</i> hybridization	Pedigree
Alamut	-	-	Kauz/ Ti71/3 Maya"s"/Bb//Inia/4/Kj2/5/Anza
Falat	+	+	Kavkaz/(Sib)Buho//Kalyansona/Bluebird=Ser82
Hamun	-	-	Falat*Roushan
Karaj 2	-	-	(Falat*Th-Mt)Omid
Rasul	+	+	Veery"s"= Kavkaz/(Sib)
Shahriar	-	-	Buho//Kalyansona/Bluebird
Zagros	-	-	Kvz/Ti71/3/Maya"s"/Bb/
Atrak	+	+	Inia/4/Kj2/5/Anza/3/Pi/Nar//Hys
Dez	+	+	Tan"s"/Vee"s"/Oyata
MV17	+	+	Kauz"s"
Alborz	-	-	Kauz*2/Oyata//Kauz
Alvand	-	-	-
Atila	+	-	-
Bulani	-	-	-
Darab2	-	-	-
Ghods	-	-	-
Kavir	-	-	-
Inia	-	-	-
Mahdavi	-	-	-
Mahuti	-	-	-
Navid	-	-	-
Niknejad	-	-	-
Omid	-	-	-
Pishtaz	-	-	-
Roushan	-	-	-
Sabalan	+	-	-
Sardari	-	-	-
Sholeh	-	-	-
Sorkh-Tokhm	-	-	-
Tabasi	-	-	-
Tajan	+	-	-
Tubari	-	-	-
Zarrin	-	-	-

<sup>a</sup> Observation of only 2 chromosomes with major secondary constrictions.





gliadins, encoded by genes on 1BS, with secalins encoded by genes on 1RS and to the loss of genes controlling low molecular weights (LMW) subunits of glutenins from 1BS. For these reasons, breeding for high bread-making quality would favor the preferential transmission of the normal 1B chromosome or produce recombinant lines in order to break the linkage between secalin and other useful genes in 1RS arm.

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## جابجایی گندم-چاودار در واریته‌های گندم نان ایرانی و بررسی توزیع یونی آنها در پاسخ به تنش شوری

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### چکیده

بازوی کوتاه کروموزوم شماره ۱ چاودار (*Secale cereal* L.) علاوه بر اینکه بخشی از ژنوم چاودار است، در چند صد واریته گندم به صورت جابجایی 1RS.1BL، 1RS.1AL یا 1RS.1DL در دنیا وجود دارد. در این تحقیق وجود جابجایی گندم-چاودار در ۳۳ واریته زراعی گندم نان ایرانی بررسی شد. تعداد ۹ رقم از این ۳۳ رقم در شجره خود دارای یک جد از نوع 1RS.1BL بوده و بقیه فاقد یک جد مشخص دارای جابجایی 1RS بودند. وجود جابجایی مربوطه در ۴ واریته با استفاده از روش هیبریداسیون DNA ژنومی در محل (GISH) تأیید گردید. همچنین قابلیت دفع سدیم و نسبت پتاسیم به سدیم در ریشه و برگ این چهار واریته در محیط کشت هیدروپونیک در حضور صفر و ۲۰۰ میلی‌مولار NaCl بررسی و با یک نمونه تصادفی متشکل از هشت رقم فاقد جابجایی چاودار مقایسه گردید. مقایسه میانگین‌ها نشان داد که وزن خشک کل و همچنین وزن خشک ریشه در ارقام دارای جابجایی 1RS.1BL (اترک، دز، فلات و رسول) در حضور ۲۰۰ mM نمک طعام به طور معنی‌داری کمتر از ارقام فاقد جابجایی در مرحله گیاهچه بود تفاوت معنی‌داری بین این دو گروه از نظر میزان Na<sup>+</sup> و نسبت K<sup>+</sup>/Na<sup>+</sup> در برگ‌ها و ریشه وجود نداشت. با وجود اینکه ژن‌های مفید زیادی در بازوی کروموزومی 1RS وجود دارد ولی این بازو اثر معنی‌داری در دفع سدیم در مقایسه با ارقام فاقد جابجایی نداشت.