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Comparative Study of some Characteristics in Leaves and Roots of two Canola Genotypes under Lead Stress

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Abstract
The effects of lead on the proline content and dry weight of leaves and roots were investigated in two canola cultivars (*Brassica napus L.*) grown in the Hoagland solution. The growth of treated plants was inhibited under lead stress. Lead induced differential accumulation of proline in canola grown in solution with the addition of 0, 100 and 200 mgL^{-1} of Pb. Hyola308 cultivar showed low biomass reduction under stress condition (lead-tolerant genotype). The younger leaf (second leaf) showed low reduction in dry weight under stress and root growth decreased progressively with increasing concentration of Pb. This reduction was remarkable in the Sarigol cultivar. There was a low Pb accumulation in the lead-tolerant genotype (Hyola 308). Canola had the ability to accumulate Pb primarily in its roots (especially in the case of Hyola308 0) and accumulated it in the shoots in much lesser concentrations. For the younger leaf increment in proline content was about two-fold. Proline content in roots was found to be lower than that of leaves under non-stress condition. Although there was linear dose dependent increase in the proline accumulation in roots, yet their magnitude was lower than the related values for leaves. However, this trend was reversed under high stress level. Under this condition, proline accumulation was consistently higher in the younger leaf. Furthermore, proline content in the roots of lead-susceptible cultivar was higher than the second and third leaf.

Key words: *Brassica napus*; Canola; Lead stress; Proline

Introduction

Some heavy metals such as lead exist in both natural and agricultural soils as a result of environmental pollution (Steffens 1994). Heavy metals make a significant contribution to environmental pollution and emanate mostly from various industrial effluents, mining and smelting of metalliferous ores, sewage sludge, etc. (Nedel - Koska and Doran 2000). Pollution due to heavy metal is a matter of growing concern because of their toxicity to all forms of life. Heavy metals accumulate in soil and hence get maximum exposure. Heavy metals such as zinc, copper and magnesium are vital for plant growth since they are components of many enzymes. Some metals such as lead, mercury, cadmium, nickel, arsenic, chromium, etc., have no known biological functions and are toxic to life even at very low concentration (Salt *et al.* 1995). High concentrations of essential (and also nonessential) heavy metals in the growth medium lead to growth inhibition in plants (Hall 2002). In addition, a heavy metal excess may stimulate the formation of reactive oxygen species and free radicals, resulting in oxidative stress (Dietz *et al.* 1999). Heavy metals are not bio degradable. They keep on accumulating in

soil and water and hence they are a major and far reaching threat. Therefore, study of plants exposure to heavy metals particularly at the biochemical level deserves priority.

One of the most common stress responses in plants is overproduction of different types of compatible organic solutes such as proline and glycine betaine (GB) (Serraj and Sinclair 2002). The organic solutes have been proven to be helpful in osmo regulation (Rhodes and Hanson 1993), enzyme activity (Mansour 2000), detoxification of reactive oxygen species (Greenway and Munns 1980; Ashraf 1994a, 1994b), and protection of membrane integrity (Bohnert and Jensen 1996). Proline has been reported to accumulate in tissues and/or organs of plants subjected to drought, salt, temperature and heavy metal stress, or infected by some pathogens in plants (Arora and Saradhi 2002). Proline accumulation in plant tissues has been suggested to result from (a) a decrease in-proline degradation, (b) an increase in proline biosynthesis, (c)a decrease in protein synthesis or proline utilization and (d)hydrolysis of proteins (Charest and Phan 1990). There are evidences that plants such as tomato (De and Mukherjee 1998), *Vigna unguiculata* (L) Walp (Bhattacharjee and Mukherjee1994) respond to heavy metal stress through accumulation of proline.

Aghaz*et al.* (2012, 2013) showed significant differences between the lead and cadmium treatments for proline accumulation. However, there was no significant difference among the ecotypes. Proline content increased in the leaves under both stress conditions. Accumulation of proline under heavy metal stress seems to be widespread among plants (Costa and Morel 1994; Chen *et al*. 2001;Zengin and Munzuroglu 2005; Kuzenetsov and Shevyakova 1997; Radic*et al*. 2010). Free proline accumulation may be a response to leaf damage (Posmyk*et al*. 2009) or may be a symptom of stress (Yang *et al*. 2011) when exposed to high lead concentration and that a higher level of proline is associated with lead sensitive plants. This study was conducted to examin the effects of lead on the proline content and dry weight of different tissues in two canola cultivars.

Materials and Methods

The experiment was conducted in the hydroponic culture system under greenhouse condition. Two canola cultivars (*Brassica napus L.*), Sarigol (salt- sensitive) and Hyola 308 (salt tolerant) were subjected to 0, 100 and 200mgL^{-1}Pb concentrations using a split plot design with three replications. These two cultivars were evaluated previously under salinity stress (Bandehagh *et al.* 2008; Bandehagh *et al.* 2011).

Seeds were sterilized and germinated in Petri dishes and seven-day-old seedlings of uniform size were transferred into large sand-

tanks housed within an environmentally-controlled greenhouse (14 h daily light, 600-800 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), thermoperiod 25 oC\17oC (day\night), relative humidity 50 percent\60 percent (day\night). The PVC tanks contained washed silica sand (99% pure) having an average bulk density of 1.5 Mg m^{-3}. The tanks were sub-irrigated and flushed four times daily with a modified Hoagland nutrient solution (Figure 1). Lead stress was imposed in the $PbCl_2$ form gradually to seven-day old seedlings.

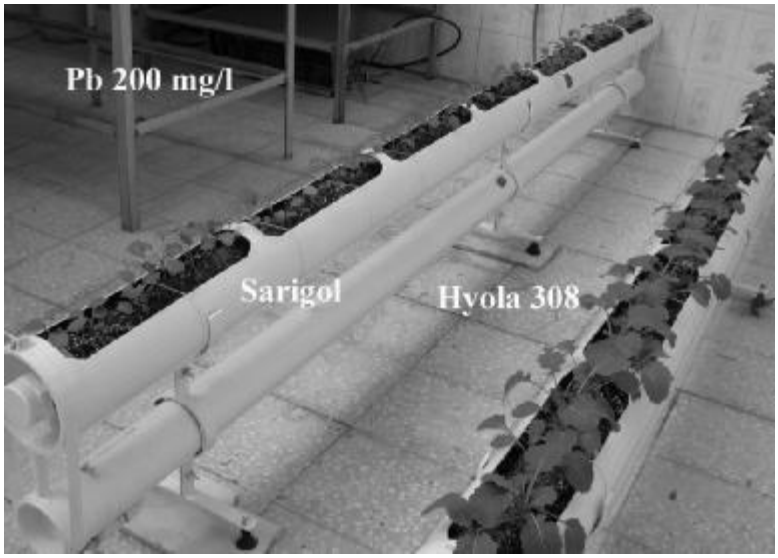

Figure 1. Sarigol and Hyola308 plants under lead treatment. Plants were grown in sand and irrigated with Hogland's solution. Two canola cultivars (*Brassica napus L.*) were subjected to 0, 100 and 200 mgL^{-1} Pb concentrations using a split plot design with three replications. Plants were treated for three weeks.

Three weeks after imposing lead stress, plants were harvested for measuring roots and shoots.. After separation of shoots, the roots were carefully removed from the sand and washed with distilled water to remove any additional salt surface contamination and dried on absorbing paper. Fresh and dry weight was measured on a sample of 50 plants. Fresh weights were measured immediately after plant harvesting. Total dry weight of second and third leaves and roots were determined after drying the samples for 48 h in an oven at 70°C.

The amount of lead in the medium in which the experimental and control plants were grown was determined by atomic mass spectrometry. Quantification of proline was made in the leaf and root samples. Free proline was measured using ninhydrin reagent (Bates *et al.* 1973).

Data were subjected to analysis of variance based on the statistical model of the split plot design and means were compared using Duncan's multiple range test.

Results

Effect of lead stress on growth

Lead treatments changed some morphological attributes of plants and decreased the total dry matter (Figure 2). The mean reduction in total dry weight of the two canola cultivars under exposure to 100 mgL^{-1} Pb was 57%. Exposure to 200 mgL^{-1} Pb led to a75% reduction in total dry weight (Figure2). The relative reduction in total dry weight in the Sarigol cultivar was greater than that in the Hyola308 cultivar. Therefore, this cultivar was a lead tolerant genotype as compared to Hoyola308.

In order to study the detailed differences between the sensitive and tolerant cultivars, the roots and the second and third leaves (from top) were selected for further analysis. The difference between these two genotypes for the relative reduction in root weight (expressed as a percentage of control plants) was significant. The relative reduction in root dry matter was higher in the salt sensitive cultivar, Sarigol, compared with the salt tolerant cultivar, Hyola308 (Figure3). Under the high stress treatment, the root dry weight of Sarigol cultivar was 20% of the control, but in the Hyola308 cultivar, the dry weight was reduced to 50% of the control.

The relative reduction in leaf dry weight (expressed as a percentage of the control plants) of these two leaves was significantly different(Figure3). The relative reduction in the dry matter of the second leaf was approximately 23% and 47% following low and high Pb treatments, respectively. This reduction for the third leaf was 44% and 65%.The reductions in the fresh and dry weight of the two leaves were greater in Sarigol cultivar as compared to Hyola308. The maximum reduction was observed in the third leaf of the Sarigol cultivar (Figure 3). Under high Pb stress, the leaf dry weight of the Sarigol cultivar was one third of the control, but in Hyola308, the dry weight was 50% of the control.

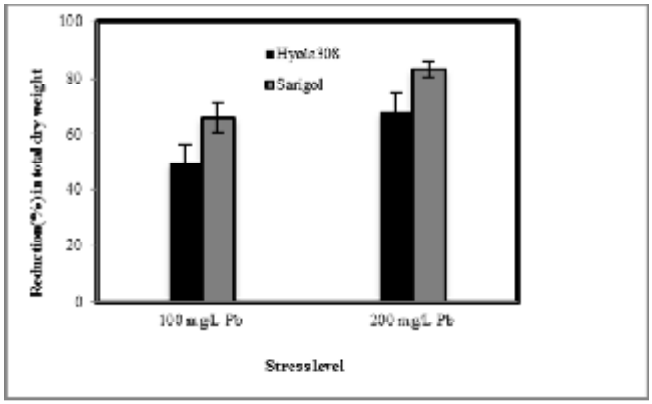

Figure 2. Effect of lead treatments on reduction (as a percent of the control) in total dry weight in two canola cultivars.

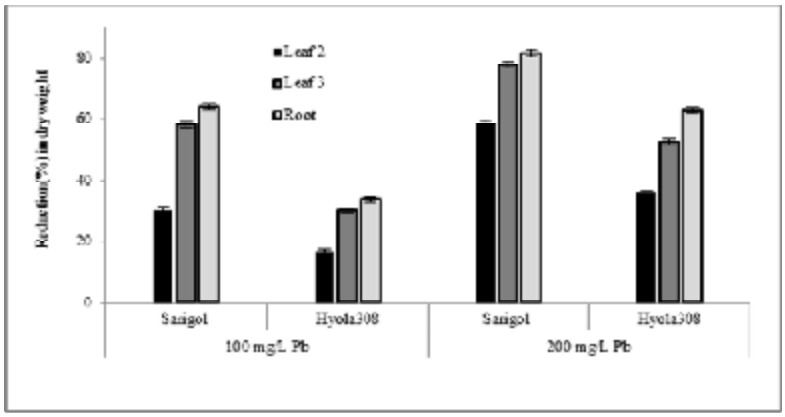

Figure 3. Relative reduction (as a percent of the control) in dry weight of the leaves and roots in two canola cultivars under lead stress.

Lead content of roots and leaves

Lead stress had significant effect on the Pb content of roots in the two cultivars. As expected, the greatest effect was observed under200 mgL^{-1}Pb. The lead stress increased the Pb content of roots in both cultivars, but to a lesser extent in Hyola308 (Figure4). Furthermore, lead treatments had significant effect on the Pb content of leaves and cultivars. The greatest effect was observed at 200 mgL^{-1} Pb (Figure 4). Lead treatments increased the Pb content of leaves in both cultivars, but to a more extent in Sarigol (Figure4). Significant difference was also observed between the two leaves for Pb concentration. The Pb content of the third leaf was significantly greater than that of the second leaf under lead stress conditions. Moreover, increment in Pb content of the third leaf was significantly greater than that of the second leaf and roots.

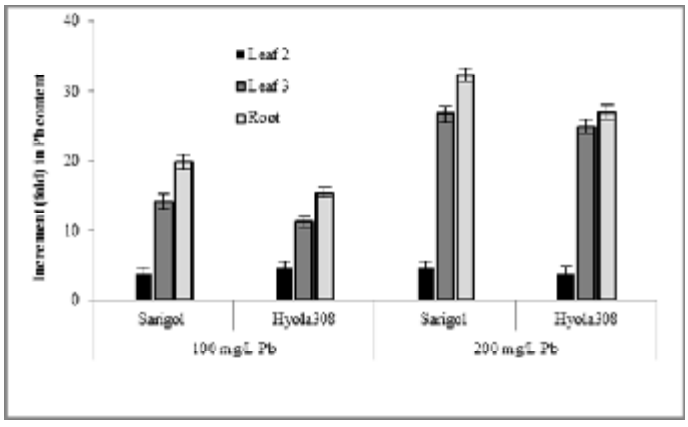

Figure 4. Effect of lead treatments on of increment of Pb content inleaves3 and roots of two canola cultivars.

Effect of lead stress on proline content of roots and Leaves

Lead stress levels had significant effects on the proline content of different tissues in two cultivars. Free proline content in the leaves and roots increased with increasing Pb concentrations

(Figure 5), although varying in organs and among the cultivars. The greatest effect was observed at the 200 mgL^{-1}Pb treatment. In plants under low stress level, the proline increase was higher in the second leaf and under high stress the roots showed the highest increment in proline content (Figure 5). Furthermore, there was a significant difference for proline contents (absolute and relative values) between two cultivars. Proline content was the greatest in Hyola308 by taking in consideration the absolute values but, by taking the relative value (as a percent of the control),Sarigol was better than Hyola308 (Figure 6).On average, the proline content of the second leaf increased 13-fold at 100 mgL^{-1}Pb and this increment for root proline content was about 38-fold (in comparison with the control) at 200 mgL^{-1}Pb(Figure5) .However, at all levels of stress, the second leaf had the highest absolute concentration of proline.

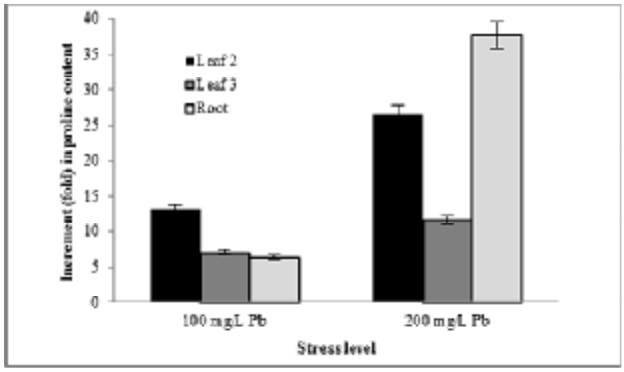

Figure 5. Increment in proline content of leaves and roots under lead stress in canola.

Absolute concentration of proline was greater in Hyola308 than Sarigol on the average of control and lead stress levels (Figure 6). However, the increase in proline content of Hyola308 was 10-fold as compared with a seven fold increase in Sarigol at low lead stress. In contrast, free proline accumulation in Hyola308 was markedly lower (23-fold) than in Sarigol (28-fold) at high lead stress (Figure7). Proline content in different tissues increased with increasing Pb concentrations, but the response varied between the cultivars. The lead sensitive cultivar (Sarigol) had the highest amount of root proline (30-fold as compared with the control) at all levels of Pb in comparison with the second and third leaves and the lead tolerant cultivar (Hyola308) had the highest amount of proline in the second leaf (24-fold as compared with the control) at all levels of Pb (Figure 8).The third leaf of the two cultivars had the lowest increment in proline content at all levels of Pb as compared with the control.

Discussion

The results in the present investigation indicated that lead stress obviously inhibits the root and shoot growth of studied cultivars and the extent of reduction was different among genotypes. Sarigol cultivar showed higher growth reduction under stress while this was lower in Hyola308. Lead exposure resulted in a decline in dry matter accumulation in the root and leaf

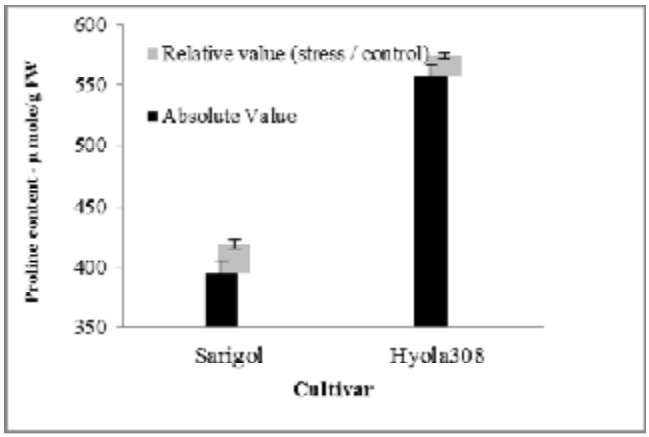

Figure 6. Proline content of two canola cultivars under Pb-stress conditions (black: absolute value and white: relative value, as % of the control)

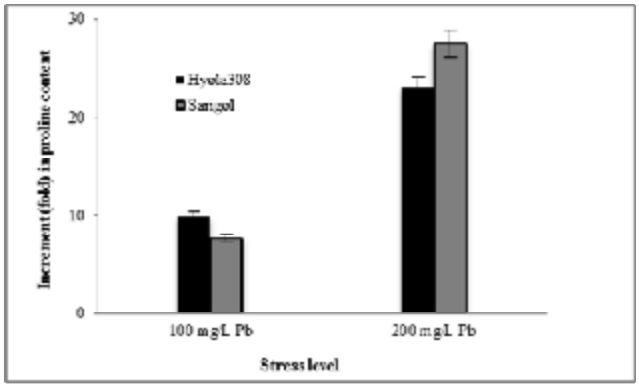

Figure 7. Increment in proline content of two canola cultivars under Pb-stress conditions.

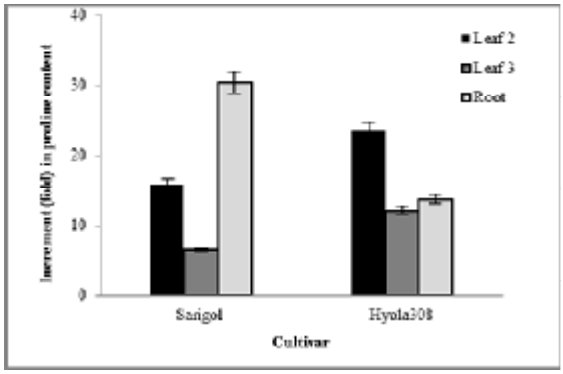

Figure 8. Increment in proline content of leaves and roots in two canola cultivars under lead stress.

tissues of both cultivars, but the Sarigol cultivar exhibited the greatest decline in dry matter in response to stress. The second leaf (the younger leaf) showed lower reduction in dry weight at the two stress levels and root growth decreased progressively with increasing concentration of Pb (Figure3). This reduction was remarkable in high Pb concentration and also in Sarigol. In this research, the second leaf was lead-tolerant with a much higher degree of tolerance in Hyola308 and the roots were lead-sensitive with lower degree of sensitivity in Hyola308.In the previous study on these two genotypes (Bandehagh *et al.* 2011), the number of salt, proline and lead-responsive proteins identified was greatest in leaves of the Hyola308 cultivar, and most of the responsive proteins were found in the second leaf of both genotypes across all salinity levels. Bandehagh *et al.* (2011) reported that the second leaf has a discrimination role between two genotypes under salinity condition.

Canola has the ability to accumulate Pb primarily in its roots (especially in the case of Sarigol plants) and transport and concentrate it in its shoots in much lesser concentrations. These differences in root and shoot uptake can possibly be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot response depends on the root response (Salt *et al.* 1997). The results in the present investigation were similar to those observed by Nanda Kumar *et al.* (1995) and Dushenkov *et al.* (1995) for the use of plants to remove heavy metals from aqueous streams and soils. Godbold and Kettner (1991) in the study on seedlings of spruce (*Piceaabies*) grown in solutions containing Pb, showed that growth of primary, secondary and tertiary roots was reduced, and that the initiation of lateral roots was more sensitive to lead than the growth of already established older roots.

Lead content of the third leaf was greater than the second leaf. Therefore, the second leaf could maintain the ionic balance and thus its growth rate in comparison with the third leaf, especially in the stressed Hyola 308 plants (Figure3).Based on data from Salt's laboratory, the phytochelatins are produced in roots of *Brassica juncea* exposed to Pb, suggesting that phytochelatins are involved in Pb detoxification (Salt *et al.* 1995).The uptake and accumulation of Pb in roots treated with Pb may be explained by the findings of Dushenkov*et al.* (1995). They indicated that at higher concentrations, more Pb was removed from the solution than accumulated in the roots, as a result of the formation of an amorphous white precipitate on the walls and at the bottom of the hydroponic container.

The results showed that Pb induced proline accumulation. Increased lead concentrations significantly enhanced proline accumulation in the canola plants. Plant physiologists have studied the accumulation of proline in a number of species subjected to abiotic stresses. Accumulation of proline in response to heavy metal exposure seems widespread among plants (Costa and Morel 1994). It was observed that under stress circumstances, proline level in the younger leaf (the second leaf) was higher than the older leaf (the third leaf). For the younger leaf, the

increment in proline content was two times more than the older leaf. This difference was large in the Hyola308 cultivar and also with the high dose of Pb. Generally the younger leaf (especially in lead-tolerant cultivar) is metabolically more active where proline is actively synthesized and this appears to be the reason for this difference. Proline increases the stress tolerance of plants through such mechanisms as osmoregulation and stabilization of protein synthesis (Kuznetsov and Shevyakova1997). Accumulation of proline in response to some heavy metals was determined in non-tolerant and metal-tolerant *Silene vulgaris* (Moench) Garcke; the constitutive proline concentration in leaves was 5 to 6 times higher in the metal-tolerant ecotype than in the non-tolerant ecotype (Schat*et al.* 1997). Proline content in roots under non-stress condition was lower than that of the leaves. Although there was linear dose dependent increase in proline accumulation in the roots, yet their magnitude was lower than the related values for the leaves. However, this trend was reversed at the high stress level. For instance, following treatment with lead, proline accumulation were 6 to 38 times higher than that of control for 100 and 200 mgL^{-1}Pb concentration. respectively. These values were 13 to 26 times higher than that of the control for the lowest and highest doses, respectively in the second leaf and 7 to 12 times in the third leaf (Figure5). Handique and Handique (2009) working with lemongrass showed that increase in proline accumulation in the roots for cadmium, mercury and lead metals was lower than the corresponding values for the leaves. In contrast with this report, in *Vigna unguiculata*, the proline accumulation in roots was found to be higher than that of the leaves following exposure to lead and cadmium (Bhattacharjee and Mukherjee 1994). Since roots are in direct and constant contact with the metal amended soil it was expected that the proline accumulation in roots would be very high. In the present study, proline content in the roots of Sarigol (lead-susceptible cultivar) was found to be higher, compared to that of leaves at the highest dose (Figure8). On the other hand, in Hyola308 (lead-tolerant cultivar) proline content in the second leaf was found to be higher, compared to that of the third leaf and also roots at the same dose. One reason may be that in leaves, particularly younger leaves, the proline level was higher because it is actively synthesized there. The second reason may be the photo activation of key enzymes involved in proline synthesis in the leaves (Arora and Saradhi 1995). The present findings are in corroboration with the report of Saradhi and Saradhi (1991). They reported that heavy metal induced proline accumulation can be used as marker of heavy metal pollution. Based onFigure6, Hola308 was regarded as a lead-tolerant cultivar. It was shown that Hyola308 had the largest absolute value for the proline content but, Sarigol had the highest relative value. The relationship between proline level and Pb accumulation revealed that the accumulation of free proline corresponds to the uptake of the lead by canola genotypes. Proline accumulation may play a role in heavy metal detoxication (Costa and Morel 1994).Proline could be involved in the metal chelation in the cytoplasm (Farago and Mullen 1979).

Conclusion

Phytoremediation as an environmental remediation technology is a fascinating area of research. Aquacultured seedlings of canola appear to have the potential to provide a novel method for the removal of leadand probably other heavy metals from contaminated waters of various sources. The present study showed that proline accumulation can be used as a biochemical indicator of heavy metal stress in canola. In the present study it appears that the younger leaf and roots are the ideal organs to assess proline accumulation.

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Allelic Variation of *VRN-1* Locus in Iranian Wheat Landraces

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Abstract
Wheat is a crop with spring and winter types and wide adaptability to different climate conditions. The wide adaptability of wheat is mainly controlled by three groups of genetic factors and among them vernalization (*VRN*) genes play pivotal role in determining spring and winter types. In this study, 395 Iranian wheat landraces were characterized with specific primer pairs designed based on *VRN-1* promoter and intron regions. Using the specific primers for *Vrn-A1c* allele, two fragments were amplified in 35 genotypes. Based on MADS-Box and promoter regions of *VRN-1* gene specific primers, two new fragments were amplified in Iranian wheat landraces which has not been reported previously. *Vrn-A1b* allele determining spring habit was the most frequent allele, whereas *Vrn-A1c* showed less frequency. Frequency of dominant allele *Vrn-A1b*, in winter genotypes was higher than that of spring type. It supports the presence of other regulatory sites outside of the *VRN* promoter region.

Keywords: Earliness *per se* genes; Landraces; Photoperiod; Spring and winter growth habit

Introduction
Wheat landraces represent an important source of genetic variation that can be used to improve commercial varieties by means of introducing new alleles or combination of genes (Ciaffi *et al.* 1992). Primary habitats of wheat ancestors are situated in the northern and eastern parts of the Fertile Crescent and modern wheat cultivars were evolved from their ancestors which mostly were distributed in these areas (Harlan and Zohari 1996).

The adaptability of common wheat to wide range of environments and climate conditions is due to variation in vernalization requirement genes and day length for the control of ear emergence (Yan *et al*. 2004a). Based on vernalization requirement, wheat genotypes are classified into winter and spring types. In hexaploid wheat, vernalization requirement is primarily controlled by three orthologous of *VRN-1* genes, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, which are located on the long arms of chromosomes 5A, 5B, and 5D, respectively (Law *et al*. 1976; Worland 1996; Dubcovsky *et al*. 1998; Barrett *et al*. 2002; Iwaki *et al*. 2002; Yan *et al*. 2003). In the spring wheat different dominant *Vrn* alleles have differential effects on flowering time. Goncharov (2004) reported that wheat genotypes with dominant *Vrn-A1* allele flower earlier, whereas presence of dominant *Vrn-D1*, *Vrn-D5* and/or *Vrn-B1* results in late flowering under non-vernalization condition. It was found that altering the flowering time and different combinations of dominant *Vrn* alleles in wheat may cause variation

in plant height and yield components (Stelmakh 1992; Stelmakh 1998).

Different mutations in the *VRN-1* locus caused expression of the dominant spring growth habit. For example, dominant *Vrn-A1* allele conferring spring growth habit originated from mutations either in the promoter or intron region of recessive *vrn-A1* allele which control winter growth habit in diploid, tetraploid and hexaploid wheat (Yan *et al*. 2004b; Fu *et al*. 2005; Dubcovsky *et al*. 2006; Pidal *et al*. 2009). In *Triticum monococcum*, the promoter region of *Vrn-A^{m}1*, (*Vrn-A^{m}1a*, *Vrn-A^{m}1b*, *Vrn-A^{m}1g*) have different length of deletions, and also one bp deletion at the CArG-Box region of *Vrn-A^{m}1f* allele was identified (Yan *et al*. 2003; Dubcovsky *et al*. 2006; Pidal *et al*. 2009). In addition to similar deletions in CarG-box region of *Vrn-A1d*, and *Vrn-A1e* alleles, a deletion in VRN-box *Vrn-A1b* was reported in tetraploid wheat (Yan *et al*. 2004b; Pidal *et al*. 2009). Yan *et al*. (2004a) found an insertion of a fold back repetitive element and a duplicated region in the promoter of dominant *Vrn-A1a*. They demonstrated that *Vrn-A1a* allele differed from the recessive *vrn-A1* allele in isoline Triple Dirk-C by the insertion of a 222-bp fold back element in the larger fragment and a 131-bp fold back element in the smaller fragment. Their findings suggest that the duplication of the promoter region occurred after the insertion of the fold back element. The *Vrn-A1b* allele has several single nucleotide polymorphisms and deletions in the promoter region. The *Vrn-A1c* allele was reported from IL369 wheat genotype from Afghanistan, IL162 from Egypt (Yan *et al*. 2004a) and Pavon-76 and NR-287 from Pakistan (Iqbal *et al*. 2011). This rare allele shows a large deletion in the first intron (Fu *et al*. 2005). Iqbal *et al*. (2011) in the study of wheat genotypes from Pakistan could identify *Vrn-A1c* allele, but they did not find any deletion in the first intron of *Vrn-A1* in the two genotypes which *Vrn-A1c* allele was detected. Fu *et al*. (2005) used primer pair Intr1/A/F2 and Intr1/A/R3 to detect deletion in the first intron of *VRN-A1* and primer pair Intr1/C/F and Intr1/AB/R as a positive control to identify genotypes lacking this deletion. Using these primer pairs, they could identify both presence and absence of first intron deletion in Afghanian landrace IL369. They also confirmed the presence of eight unique SNPs, five unique one-bp indels in promoter, introns 1, 2, 4 and, 6 as well as exon 7 regions, and one large 5504-bp deletion in the first intron of dominant *Vrn-A1* allele from IL369.

Yan *et al*. (2003) reported that deletions in the *VRN-A^{m}1* promoter of diploid wheat were associated with the spring growth habit. Yan *et al*. (2004a) and Fu *et al*. (2005) in analysis of the dominant *Vrn-A1* alleles from the hexaploid landrace IL369 and tetraploid cultivar Langdon did not identify any variation in the promoter region of the gene compared with its respective recessive alleles.

Tranquilli and Dubcovsky (2000) reported that vernalization requirement in wheat and barley is controlled by the epistatic interaction between *VRN-1* and *VRN-2* loci. In the winter genotypes, vernalization up-regulates *VRN-1* gene which is dominant for spring growth habit (Danyluk *et al*. 2003; Trevaskis *et al*. 2003; Yan *et al*. 2003), whereas vernalization process decreases the abundance of the *VRN-2* product (Yan *et al*.

2004a). Based on this molecular model the *VRN-2* transcription product is a repressor for the *VRN-1*. A single functional copy of *VRN-2* product is sufficient to stop flowering (Yan *et al*. 2003, 2004b). However mutation in the VRN-2 protein causes an inactive repressor, and also mutations that alter the *VRN-1* recognition site for VRN-2 repressor are associated with the dominant spring growth habit in *VRN-1* locus. Consequently, transcription of *VRN-1* gradually increases, leading to competence to flower.

In our best knowledge, no study has been performed to analyze the allelic variation at the vernalization requirement genes on Iranian wheat landraces. In view of the lack of information on the occurrence of *Vrn* alleles in Iranian wheat landraces, here we examined the *VRN-1* genotypes of 395 wheat landraces collected from various regions of Iran.

Materials and Methods

Plant material

The plant materials consisted of 395 Iranian wheat landraces, including 154 spring, 193 winter, 46 with unknown growth habit and two facultative genotypes as well as two standard cultivars, Chinese Spring and Thatcher. Seeds of the plant materials were obtained from gene bank of International Maize and Wheat Improvement Center (CIMMYT).

DNA marker analysis

Leaf tissues from 10 greenhouse grown seedlings per genotype were pooled and genomic DNA was isolated using the CTAB method (Saghai-Maroof *et al*. 1984). We used *Vrn-A1* allele-specific markers based on promoter or intron 1 mutations (Table 1) described by Yan *et al*. (2004a), Fu *et al*. (2005) and Golovnina *et al*. (2010). PCR was performed in a 10 µl volume in a BioRad thermocycler containing 0.6 µl of each of the 5 µmol/l forward and reverse primers, 4 µl PCR ready MasterMix (Amplicon), 3 µl sterile water, 2.8 µl template DNA. PCR programs for each primer pair is given in Table 1. PCR products were separated on 2% agarose gel at 100V, stained with ethidium bromide and subsequently visualized using UV light. For detecting the exact size of DNA bands, we used 50/100 bp plus ladder (Fermentas). In addition, 4% polyacrylamide gel was used to determine exact size of *Vrn-A1b* allele. Amplification experiments were repeated to confirm allelic composition result.

Results and Discussion

VRN-1 promoter region marker

Allelic variation at the promoter region of *VRN-1* gene in 395 Iranian wheat landraces were tested with primers VRN1AF and VRN1R. Amplification of genomic DNA from the promoter region of the landraces using these primers showed the presence of PCR products with the length of 480, 650 and 750-bp (Figure 1) which were also reported by Yan *et al*. (2004a). Amplification of two 650 and 750-bp fragments in 16 genotypes including 10 winter, five spring and one facultative genotypes confirmed the occurrence of the dominant *Vrn-A1a* allele in these landraces. Thatcher and nine spring, five

Table1. Primer sequences, annealing temperatures and expected PCR product sizes for detecting alleles at the *VRN1* loci in wheat

Marker	Primer	Sequence5-3	Expected size (bp)	Annealing temperature	PCR profile*
VRN-A1 Promoter region	VRN1AF	GAAAGGAAAAATTCTGCTCG	500	55	Touch down
	VRN1-R	TGCACCTTCCC(C/G)CGCCCCAT			
IL 369 *VRN-A1* Deletion	Intr1/A/F2	AGCCTCCACGGTTTGAAAGTAA	1170	57.2	57.2 Ramp
	Intr1/A/R3	AAGTAAGACAACACGAATGTGAGA			
VRN-A1 Non-deletion	Intr1/C/F	GCACTCCTAACCCACTAACC	1068	62	62 Ramp
	Intr1/AB/R	TCATCCATCATCAAGGCAAA			
	AP1_ProDel_F1	ACAGCGGCTATGCTCCAG	152		Touch down
	AP1_ProDel_R1	TATCAGGTGGTTGGGTGAGG			
	AP1_2F	CTGTGGTGTGTGTTTGTGGCGAGAG	200		Touch down
	AP1_2R	ACCCTACGCCCCTACCCTCCAACAC			

*Touch down: 1, 95℃, 5 min; 2, 96℃, 1 min; 3, 68℃, 5 min, -2.0℃/cycle; 4, 72℃, 1 min; 5, go to step 2, 4 more times; 6, 96℃, 1 min; 7, 58℃, 2 min, -2.0℃/cycle; 8, 72℃, 1 min; 9, go to step 6, 4 more times; 10, 96℃, 1 min; 11, 50℃, 1 min; 12, 72℃, 1 min; 13, go to step 10, 24 more times; 14, 72℃, 5 min; 15, 4℃, 5 min.

Ramp: 1, 94℃, 5 min; 2, 94℃, 30 s; 3, 0.5℃/s to annealing TM; 4, annealing TM 30 s; 5, 0.2℃/s to 72℃; 6, 72℃, 30s; 7, go to step 2, 39 more times; 8, 72℃, 5 min; 9, 4℃, 5 mim.

winter, and three unknown genotypes showed only 750-bp fragment and in 28 landraces including 25 spring and three winter genotypes a 650-bp fragment was only amplified. Amplification of 480-bp fragment in 334 genotypes consisted of 176 winter, 117 spring, 40 unknown and one facultative genotypes demonstrated that they carried dominant spring habit *Vrn-A1b* allele. *Vrn-A1b* indicates promoter deletions (no intron deletion) (Fu *et al*., 2005). In 13 genotypes consisted of 11 spring and two winter landraces both 480 and 650-bp bands were observed which was not reported in the previous studies. In addition, three winter and one spring genotypes were heterozygote for 480 and 750-bp fragments. The recessive *vrn-A1* allele was not amplified in any of the 395 examined Iranian wheat landraces.

Yan *et al*. (2003) classified the presence of insertions or deletions in the *VRN-A1* promoter as dominant *Vrn-A1* and their absence as recessive *vrn-A1*. Yan *et al*. (2004a) characterized the allelic variation at prompter region in the polyploid wheat and reported amplification of 650 and 750-bp fragments in wheat genotypes carrying dominant *Vrn-A1a* allele. They found that dominant *Vrn-A1a* allele differ from the recessive *vrn-A1* allele by insertion of a 222-bp foldback element in the large fragment and a 131-bp foldback in the smaller fragment.

IL 369 *VRN-A1* Deletion

To identify *VRN-A1* intron 1 deletion, we used the primer pair Intr/A/F2 and Intr/A/R3. This primer pair amplified PCR products of 1170-bp in 21 genotypes consisted of 18 spring, two winter, and one unknown growth habit. In addition, a new allele of 710-bp was detected in 11 spring, two winter, and one genotype with unknown growth habit (Figure 2).

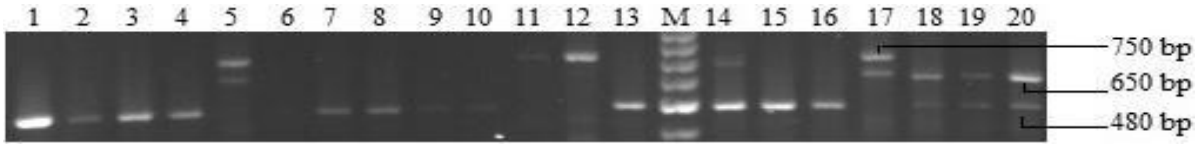

Figure 1. Banding pattern of *Vrn-A1* locus in some Iranian wheat landraces based on primer pair VRN1AF and VRN1R. *Vrn-A1a*: 650 bp +750 bp, *Vrn-A1b*: 480 bp, *Vrn-A1j*: 650 bp, *Vrn-A1k*: 750 bp. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

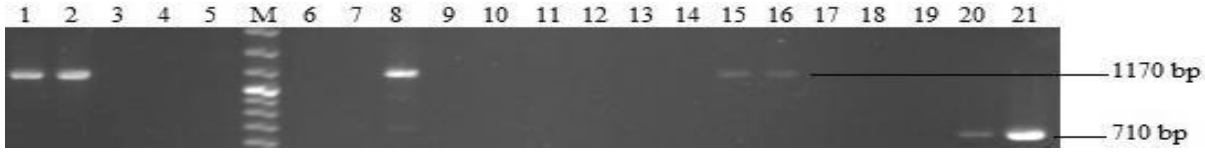

Figure 2. Banding pattern of *Vrn-A1c* locus in some Iranian wheat landraces based on primer pair Intr1/A/F2 and Intr1/A/R3. A new allele *Vrn-A1cb* was detected. *Vrn-A1c*: 1170 bp, *Vrn-A1cb*: 710 bp. M: GeneRuler 100 bp plus DNA ladder marker (Fermentas)

Yan *et al*. (2004a) in the analysis of allelic variation at the *VRN-1* promoter region in the polyploid wheat, in addition to the *Vrn-A1a* and *Vrn-A1b* alleles, identified a new allele named *Vrn-A1c* with size 1170-bp in IL369 and IL162, landraces from Afghanistan and Egypt, respectively. They reported that IL369 has a dominant *Vrn-A1* allele with an identical promoter region to the recessive *vrn-A1* allele. Iqbal *et al*. (2011) by analyzing allelic variation at the *Vrn-A1* locus of 59 Pakistani spring wheat cultivars amplified 1170-bp allele in the advanced breeding lines of NR-287 and Pavon-76 only. Zhang *et al*. (2008) reported that *Vrn-A1c* allele is common among Chinese tetraploid spring genotypes. Santra *et al*. (2009) by genetic and molecular characterization of vernalization genes *Vrn-A1* in spring wheat germplasm from the Pacific Northwest region of the USA did not observe *Vrn-A1c* allele in any of the 117 genotypes.

Vrn-A1 non-deletion marker

The primer pair Intr1/C/F and Intr1/AB/R was used to amplifiy non-deletion *Vrn-A1* marker in Iranian wheat landraces. Using this primer pair, a 1068-bp fragment was amplified in 389 genotypes including 153 spring, Chinese Spring cv., 189 winter, 45 unknown and two landraces with facultative growth habit. The result indicates that all the Iranian landraces carry recessive *vrn-A1* allele (Figure 3).

Zhang *et al*. (2008) in the analysis of allelic variation at the vernalization gene *Vrn-A1* in Chinese wheat cultivars used two primer pairs Intr1/A/F2 and Intr1/A/R3, and Intr1/C/F and Intr1/AB/R, for the *Vrn-A1* first intron to distinguish between two alleles of *Vrn-A1* gene. They reported amplification of a 1068-bp fragment in all cultivars tested using the primer pair Intr1/C/F and Intr1/AB/R, whereas no PCR product was produced using primer pair Intr1/A/F2 and Intr1/A/R3. These results indicate that the large intron 1 deletion (*Vrn-A1c* allele) was not present in the Chinese cultivars. Iqbal *et al*. (2007) reported that in Canadian spring wheat cultivars, *Vrn-A1b* and *vrn-A1* (500-bp) alleles differ in 20 bp. Nowak and Kowalczyk (2010) also confirmed the presence of recessive *vrn-A1* allele in all of the examined winter wheat cultivars from the Polish register. Golovnina *et al*. (2010)

with molecular characterization of vernalization loci *VRN1* in the wild and cultivated wheats found that the majority of the wild wheats have a winter growth habit, suggesting that the recessive *vrn-A1* allele with an intact *VRN1* promoter is the ancestral character.

Allelic variation at the *VRN*1 promoter region

PCR screening of *VRN1* promoter region of Iranian wheat landraces was provided with primer pairs AP1_ProDel_F1/AP1_ProDel_R1 and AP1_2F/AP1_2R. The first primer pair amplified the region flanking the 48-bp deletion. The expected PCR product size for the *vrn-Am1b* allele carrying the 48-bp deletion is 104 bp, whereas for *Vrn-Am1f* and the wild-type *vrn-Am1* alleles are 151 bp and 152 bp, respectively (Yan *et al*. 2003; Pidal *et al*. 2009). Using primer pair AP1_ProDel_F1 and AP1_ProDel_R1, PCR product of 152 bp was observed in 134 spring, Chinese Spring cv., 189 winter, 41 unknown and one facultative accession. In addition, we could amplify a novel 400 bp in 18 spring, eight winter and four unknown genotypes which may be due to large insertion in this region (Figure 4). Seven winter accession (Ardabil2, Saghez1, Saghez2, Ghazvin7, Kermanshah3, Sabzvar8, Torbat-Heidarieh3), and one spring genotype (Mashhad6) were heterozygote for these fragments.

Golovnina *et al*. (2010) by molecular characterization of *VRN1* locus in 27 accessions belonging to four diploid wheat species (*T. urartu*, *T. boeoticum*, *T. monococcum* and *T. sinskajae*), seven goatgrass accessions belonging to *Aegilops speltoides* and *Ae. squarrosa* (syn. *Ae. tauschii*) together with 17 accessions of seven polyploid species belonging to three known sections (*Dicoccoides*, *Triticum*, *Timopheevii*) using primer pair AP1_ProDel_F1/ AP1_ProDel_R1 amplified the expected size of 152 bp in the majority of the studied wheat accessions and in one goatgrass species, *Ae. Speltoides*. No PCR products was found in *Ae. squarrosa* accessions. Out of 27 wheat accessions, 10 showed PCR products of the lower size, which can be explained by deletions in the promoter region. Pidal *et al*. (2009) reported that primer pair AP1_ProDel_F1 and AP1_ProDel_R1 in diploid wheat (*T. monococcum*) amplified the region flanked by 48-bp deletion in *VRN1* promoter. They identified a 104-bp fragment for *vrn-Am1b* with 48-bp deletion as well as 151 and 152-bp fragments for *Vrn-Am1f* and wild type *vrn-Am1* alleles, respectively.

Golovnina *et al*. (2010) extracted all available *VRN1* promoter sequences belonging to different wheat genomes (A, B, D) from GenBank and aligned together with primer sequences. They found a 17-bp deletion in D genome near the region complementary to the reverse primer (AP1_ProDel_R1), and a duplicated fragment (CCTCAC) near this region in A genome. Therefore, they developed a new primer (AP1_2F/AP1_2R) for amplification of D genome. In our study, a PCR product of 400 bp was amplified in 375 Iranian wheat landraces including 141 spring, 187 winter, 45 unknown, and two facultative growth habits using primer pair AP1_2F and AP1_2R (Figure 5).

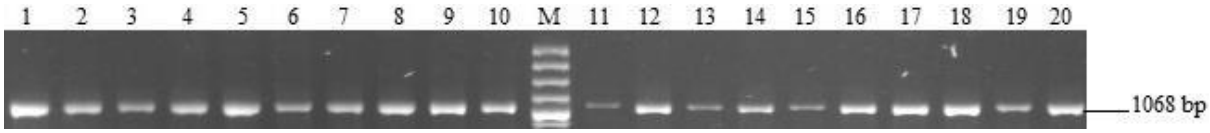

Figure 3. Banding pattern of non-deletion *Vrn-A1* locus in some Iranian wheat landraces based on primer pair Intr1/C/F and Intr1/AB/R. M: GeneRuler 100 bp plus DNA ladder marker (Fermentas)

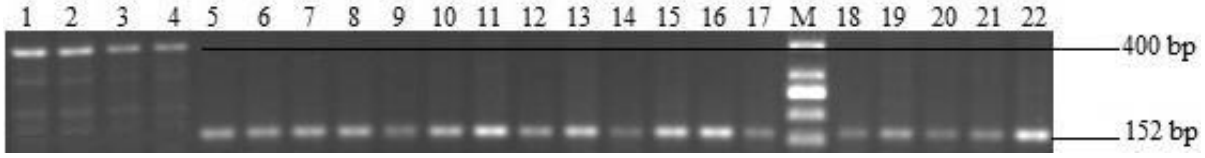

Figure 4. Banding pattern of *Vrn-1* promoter region in some Iranian wheat landraces based on primer pair AP1_ProDel_F1 and AP1_ProDel_R1. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

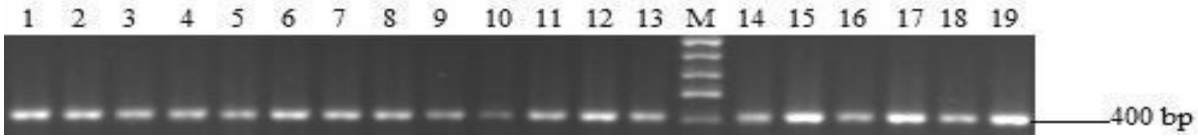

Figure 5. Banding pattern of *Vrn-1* promoter region in some Iranian wheat landraces based on primer pair AP1_2F and AP1_2R. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

Distribution of *VRN-1* locus alleles in Iranian wheat landraces

Among the detected *VRN-1* alleles, *Vrn-A1b* allele was the most frequent allele (84.56%) and combination of *Vrn-A1j/Vrn-A1cb* was the least frequent (0.25%) in the Iranian wheat landraces (Tables 2 and 3). The frequency of dominant allele *Vrn-A1b* in the spring and winter genotypes were 35.03% and 52.70%, respectively. Fifteen spring and two winter accessions carried both *Vrn-A1b* and *Vrn-A1c* alleles. Most of these accessions (15) are from east and southeast of Iran. *Vrn-A1b* along with the novel *Vrn-A1cb* allele were amplified in 11 spring and two winter genotypes. These findings show their strength in fulfillment of spring growth habit in Iranian wheat landraces. In our study, the presence of some allelic combination in the winter and spring wheat landraces was not in agreement with those of reported in previous studies. This indicates accurate field and greenhouse evaluations is necessary for determination of growth habit.

Iwaki *et al*. (2001) by studying 272 wheat cultivars from different geographical regions demonstrated that the dominant *Vrn-A1* allele in the European common wheat cultivars is the most frequent. Iqbal *et al*. (2007) in the analysis of 40 spring wheat cultivars from Canada confirmed the presence of *Vrn-A1a* allele in 34 spring wheats. The *Vrn-A1b* allele was found in the Rescue cv. and two of its substitution lines RC5D and CR5A. Four of their examined cultivars carried winter habit *vrn-A1* allele.

Table 2. Distribution of *VRN-1* alleles in wheat landraces with different growth habit

		Growth habit			
		Spring	Winter	Facultative	Unknown
Allelic combination	Total	No.	No.	No.	No.
Vrn-A1a	16	5	10	1	0
Vrn-A1b	334	117	176	1	40
Vrn-A1c	21	18	2	0	1
Vrn-A1cb	14	11	2	0	1
Vrn-A1j	28	25	3	0	0
Vrn-A1k	18	9	5	0	3
Vrn-A1b Vrn-A1c	18	15	2	0	1
Vrn-A1b Vrn-A1cb	14	11	2	0	1
Vrn-A1b Vrn-A1j	13	11	2	0	0
Vrn-A1b Vrn-A1k	4	1	3	0	0
Vrn-A1c Vrn-A1j	3	3	0	0	0
Vrn-A1j Vrn-A1cb	1	1	0	0	0

Table 3. Allelic variation at *VRN-A1* locus in Iranian wheat landraces

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Iran1	*Vrn-A1b*	Birjand1-w	*Vrn-A1b*	Kerman2-w	*Vrn-A1b*
Urmia1-w	*Vrn-A1b*	Bojnourd2-w	*Vrn-A1b*	Sirjan1-w	*Vrn-A1b*
Iran2	*Vrn-A1b*	Torbat-Heidar1-w	*Vrn-A1b*	Kerman3-w	*Vrn-A1b*
Iran3	*Vrn-A1b*	Bojnourd3-s	*Vrn-A1b*	Kerman4-w	*Vrn-A1b Vrn-A1c*
Iran4	*Vrn-A1b*	Feridan1-s	-	Shahreza7-w	*Vrn-A1b*
Malayer1-w	*Vrn-A1b*	Borujen1-w	*Vrn-A1b*	Shiraz6-w	*Vrn-A1b*
Arak1-w	*Vrn-A1b*	Yazd1-w	*Vrn-A1b*	Moghan (Garmi)1-w	*Vrn-A1b*
Iran5	*Vrn-A1b*	Yazd2-w	*Vrn-A1b*	Urmia5-w	*Vrn-A1b*
Iran6	*Vrn-A1k*	Shahre-Kord1-w	*Vrn-A1b*	Ardabil2-w	*Vrn-A1j*
Sanandaj1-s	*Vrn-A1k*	Shahreza1-w	*Vrn-A1b*	Tabriz1-w	*Vrn-A1a*
Dareh-Gaz1-w	*Vrn-A1b*	Shahreza2-w	*Vrn-A1b*	Mianeh1-w	*Vrn-A1b*
Kermanshah1-s	*Vrn-A1b*	Shirvan1-w	*Vrn-A1k*	Bandar-Abbas1-w	*Vrn-A1b*
Gazvin1-s	*Vrn-A1a*	Iran8	*Vrn-A1b*	Shiraz7-s	-
Shah-Abad1-s	*Vrn-A1b, Vrn-A1cb*	Shahreza3-w	-	Lenjan1-w	*Vrn-A1b*
Kerend1-s	*Vrn-A1b, Vrn-A1cb*	Borujen3-w	*Vrn-A1b*	Esfahan3-w	*Vrn-A1b*
Saveh1-s	*Vrn-A1b*	Borujen4-w	*Vrn-A1b*	Urmia6-w	*Vrn-A1b*
Gazvin2-s	*Vrn-A1a*	Semirom1-s	*Vrn-A1b*	Urmia7-w	*Vrn-A1b*
Gazvin3-w	*Vrn-A1b*	Ghoochan2-s	*Vrn-A1b*	Ghoochan3-f	*Vrn-A1b*
Gilane-Gharb1-w	*Vrn-A1b*	Birjand3-s	*Vrn-A1b*	Iran10	*Vrn-A1b*
Gilane-Gharb2-w	*Vrn-A1b*	Yazd3-w	*Vrn-A1b*	Lenjan2-w	*Vrn-A1b*
Ilam1-w	*Vrn-A1b*	Yazd4-w	*Vrn-A1b*	Esfahan4-w	*Vrn-A1b*
Ilam2-w	*Vrn-A1b*	Shahreza4-w	*Vrn-A1b*	Esfahan5-w	*Vrn-A1b*
Malayer2-w	*Vrn-A1b*	Birjand4-w	*Vrn-A1b*	Esfahan6-w	*Vrn-A1b*
Hamedan1-s	*Vrn-A1b*	Varamin1-w	*Vrn-A1b*	Mashhad1-w	*Vrn-A1b*
Gorgan1-s	*Vrn-A1b, Vrn-A1cb*	Semirom2-w	*Vrn-A1b*	Ghoochan4-w	*Vrn-A1b*
Kashmar1-w	*Vrn-A1b*	Shahreza5-w	*Vrn-A1b*	Mashhad2-s	*Vrn-A1b*
Kashmar2-w	*Vrn-A1b*	Shahreza6-w	*Vrn-A1b*	Najaf-Abad1-w	*Vrn-A1b*
Sabzvar1-w	*Vrn-A1b*	Shiraz1-w	*Vrn-A1b*	Torbat-Jam2-s	*Vrn-A1b*
Sabzvar2-w	*Vrn-A1b*	Shiraz2-s	*Vrn-A1b, Vrn-A1c*	Torbat-Jam3-w	*Vrn-A1b*

Table3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Ardakan1-w	*Vrn-A1b*	Shiraz3-s	*Vrn-A1b, Vrn-A1c*	Torbat-Jam4-w	*Vrn-A1b*
Iran7	-	Iran9	*Vrn-A1b*	Damghan1-w	*Vrn-A1b*
Sabzvar3-w	*Vrn-A1b*	Fasa1-s	*Vrn-A1b*	Shah-Abad2-w	*Vrn-A1b*
Torbat-Jam1-w	*Vrn-A1b*	Niriz1-w	*Vrn-A1b*	Sanandaj2-w	*Vrn-A1b*
Ghoochan1-w	*Vrn-A1b*	Shiraz4-w	*Vrn-A1b*	Zanjan1-w	*Vrn-A1b*
Esfahan1-w	*Vrn-A1b*	Shiraz5-s	*Vrn-A1b, Vrn-A1c*	Zanjan2-s	*Vrn-A1b*
Ardakan2-w	*Vrn-A1b*	Hasht-Rood1-w	*Vrn-A1a*	Mashhad3-s	*Vrn-A1b*
Neishabour1-w	*Vrn-A1b*	Kerman1-w	*Vrn-A1b*	Esfahan7-w	*Vrn-A1b*
Neishabour2-s	*Vrn-A1b*	Ardabil1-s	*Vrn-A1a*	Sanandaj3-s	*Vrn-A1k*
Dastjerd1-s	*Vrn-A1b*	Urmia2-f	*Vrn-A1a*	Iran11	*Vrn-A1b*
Esfahan2-w	*Vrn-A1b, Vrn-A1k*	Urmia3-w	*Vrn-A1b*	Khonsar1-w	*Vrn-A1b*
Bojnourd1-w	*Vrn-A1b, Vrn-A1j*	Urmia4-w	*Vrn-A1b, Vrn-A1c*	Damghan2-w	*Vrn-A1b*
Torbat-Jam5-v	*Vrn-A1b*	Shah-Abad4-s	*Vrn-A1b*	Toyserkan1-w	*Vrn-A1a*
Naghadeh1-s	*Vrn-A1k*	Gazvin5-w	*Vrn-A1b*	Toyserkan2-s	*Vrn-A1k*
Iran12	-	Gazvin6-s	*Vrn-A1b*	Torbat-Heidari2-s	*Vrn-A1b*
Esfahan8-w	*Vrn-A1b*	Gazvin7-w	*Vrn-A1a*	Hamedan3-w	*Vrn-A1b*
Esfahan9-w	*Vrn-A1a*	Saghez2-w	*Vrn-A1a*	Iran14	*Vrn-A1b*
Borujerd1-w	*Vrn-A1b*	Shah-Abad5-w	-	Sabzvar5-w	*Vrn-A1b*
Borujerd2-s	*Vrn-A1b*	Sabzvar4-s	*Vrn-A1b*	Iran15	*Vrn-A1b*
Urmia8-w	*Vrn-A1b*	Ghoochan9-s	*Vrn-A1b, Vrn-A1c*	Sabzvar6-s	*Vrn-A1b*
Mahabad1-s	*Vrn-A1b, Vrn-A1k*	Torbat-Jam6-s	*Vrn-A1b*	Sabzvar7-s	*Vrn-A1b*
Mahabad2-s	*Vrn-A1b*	Birjand8-w	*Vrn-A1b*	Iran16	*Vrn-A1k*
Ghoochan5-s	*Vrn-A1b*	Birjand9-s	*Vrn-A1b*	Sabzvar8-w	*Vrn-A1b, Vrn-A1k*
Ghoochan6-s	*Vrn-A1a*	Semirom3-w	*Vrn-A1b*	Iran17	*Vrn-A1k*
Mashhad4-s	*Vrn-A1b, Vrn-A1j*	Ardestan1-w	*Vrn-A1b*	Sabzvar9-s	*Vrn-A1b*
Mashhad5-w	*Vrn-A1b*	Rafsanjan1-w	-	Bojnourd6-s	*Vrn-A1b*
Fooman1-s	*Vrn-A1b, Vrn-A1j*	Torbat-Jam7-w	*Vrn-A1b*	Iran18	*Vrn-A1b*
Birjand5-w	*Vrn-A1b*	Neishabour3-w	*Vrn-A1b*	Iran19	*Vrn-A1b*
Birjand6-w	*Vrn-A1b*	Shirvan2-w	*Vrn-A1b*	Sabzvar10-w	*Vrn-A1b, Vrn-A1j*
Birjand7-w	*Vrn-A1b*	Iran13	-	Kashmar3-s	*Vrn-A1b, Vrn-A1j*
Feridan2-w	*Vrn-A1b*	Arak2-s	*Vrn-A1b*	Yazd5-s	*Vrn-A1b, Vrn-A1j*
Bojnourd4-s	*Vrn-A1b, Vrn-A1j*	Ghasre-Shirin1-w	*Vrn-A1b*	Iran20	*Vrn-A1b*
Bojnourd5-s	-	Ghasre-Shirin2-w	*Vrn-A1b*	Yazd6-w	*Vrn-A1b*
Dareh-Gaz2-s	-	Gilane-Gharb3-w	*Vrn-A1b*	Sabzvar11-w	*Vrn-A1b*
Ghoochan7-s	*Vrn-A1b*	Gilane-Gharb4-s	*Vrn-A1b*	Iran21	*Vrn-A1b, Vrn-A1cb*
Sarakhs1-s	*Vrn-A1k*	Gazvin8-s	*Vrn-A1b*	Iran22	*Vrn-A1b*
Shahrud1-s	*Vrn-A1b*	Mahidasht1-w	*Vrn-A1b*	Sabzvar12-w	*Vrn-A1b*
Tabas1-w	*Vrn-A1b*	Gorgan2-s	*Vrn-A1b*	Sabzvar13-s	*Vrn-A1b, Vrn-A1j, Vrn-A1cb*
Meimeh1-w	*Vrn-A1b*	Kermanshah2-w	*Vrn-A1b*	Feridan3-w	*Vrn-A1b*
Meimeh2-w	*Vrn-A1b*	Sanandaj4-s	*Vrn-A1b*	Sabzvar14-s	*Vrn-A1b, Vrn-A1j*
Ghoochan8-s	*Vrn-A1b*	Shah-Abad-Gharb1-v	-	Iran23	*Vrn-A1b*
Esfahan10-w	*Vrn-A1b*	Saveh2-w	*Vrn-A1b*	Ardakan3-s	*Vrn-A1b*
Shahrud2-s	*Vrn-A1b, Vrn-A1cb*	Hamedan2-w	*Vrn-A1b*	Iran24	*Vrn-A1b*
Meimeh3-w	*Vrn-A1b*	Sanandaj5-s	*Vrn-A1b, Vrn-A1c*	Mashhad7-s	*Vrn-A1b*
Esfahan11-w	*Vrn-A1b*	Mahidasht2-s	*Vrn-A1b*	Najaf-Abad4-w	*Vrn-A1b*
Shahrud3-s	*Vrn-A1b*	Kermanshah3-w	*Vrn-A1a*	Iran25	*Vrn-A1b*
Semnan1-w	*Vrn-A1b*	Sanandaj6-s	-	Iran26	*Vrn-A1b*
Najaf-Abad2-s	*Vrn-A1b*	Maragheh1-w	*Vrn-A1b*	Iran27	*Vrn-A1b*
Najaf-Abad3-v	*Vrn-A1b*	Kermanshah4-w	*Vrn-A1b*	Ghoochan10-w	*Vrn-A1b*

Table 3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Shah-Abad3-s	*Vrn-A1b*	Sanjabi1-w	*Vrn-A1b*	Esfahan12-w	*Vrn-A1b, Vrn-A1c*
Mashhad6-s	*Vrn-A1a*	Divan-Dareh1-w	*Vrn-A1b*	Iran28	*Vrn-A1b*
Saghez1-w	*Vrn-A1a*	Malayer3-s	*Vrn-A1b*	Iran29	*Vrn-A1b*
Gazvin4-w	*Vrn-A1b*	Nahavand1-w	*Vrn-A1b*	Ardakan4-w	*Vrn-A1b*
Mashhad8-w	*Vrn-A1b*	Astara1-w	*Vrn-A1b*	Yazd7-s	*Vrn-A1b*
Mashhad9-w	*Vrn-A1b, Vrn-A1c*	Shahi1-w	*Vrn-A1b*	Ghoochan13-s	*Vrn-A1b*
Mashhad10-s	-	Esfahan14-w	*Vrn-A1a*	Tabas4-s	*Vrn-A1b, Vrn-A1*
Sabzvar15-s	*Vrn-A1b*	Torbat-Jam8-s	-	Iran41	*Vrn-A1b*
Sabzvar16-w	*Vrn-A1b*	Fariman1-w	*Vrn-A1b*	Hamedan7-w	*Vrn-A1b*
Mashhad11-w	*Vrn-A1b*	Gonabad1-w	*Vrn-A1b*	Tabas5-s	*Vrn-A1j, Vrn-A1*
Iran30	*Vrn-A1b*	Gorgan3-s	-	Esfahan16-s	*Vrn-A1j*
Mashhad12-w	*Vrn-A1b*	Semnan2-s	-	Saghez3-s	*Vrn-A1j*
Ghoochan11-w	*Vrn-A1b*	Shah-Abad6-w	*Vrn-A1a*	Fariman2-w	*Vrn-A1b*
Iran31	*Vrn-A1b*	Mashhad13-s	*Vrn-A1b*	Iran42	*Vrn-A1b*
Iran32	*Vrn-A1b*	Gazvin9-w	*Vrn-A1b*	Bojnourd13-w	*Vrn-A1b*
Neishabour4-w	*Vrn-A1b*	Sabzvar17-w	*Vrn-A1b*	Sabzvar19-s	*Vrn-A1b*
Bojnourd7-w	*Vrn-A1b*	Ardakan5-w	*Vrn-A1b*	Iran43	*Vrn-A1b*
Iran33	*Vrn-A1b*	Bojnourd11-w	*Vrn-A1b, Vrn-A1*	Niriz4-w	*Vrn-A1b*
Shahre-Kord3-w	*Vrn-A1b*	Shahre-Kord5-w	*Vrn-A1b*	Shiraz8-s	*Vrn-A1b, Vrn-A1c*
Neishabour5-w	*Vrn-A1b*	Torbat-Heidar4-w	*Vrn-A1b*	Shiraz9-s	*Vrn-A1b, Vrn-A1c*
Neishabour6-w	*Vrn-A1b*	Naein1-w	*Vrn-A1b*	Maragheh2-s	*Vrn-A1b, Vrn-A1*
Bojnourd8-s	*Vrn-A1b*	Shahre-Kord6-w	*Vrn-A1b*	Iran44	*Vrn-A1b*
Bojnourd9-w	*Vrn-A1b*	Semirom4-w	*Vrn-A1b*	Urmia9-w	*Vrn-A1b*
Bojnourd10-s	*Vrn-A1b*	Shirvan3-s	*Vrn-A1b, Vrn-A1*	Babol1-w	*Vrn-A1b*
Neishabour7-w	*Vrn-A1b*	Dareh-Gaz3-s	*Vrn-A1b, Vrn-A1*	Esfahan17-w	*Vrn-A1b*
Iran34	*Vrn-A1b*	Ghoochan12-s	*Vrn-A1j*	Damghan3-w	*Vrn-A1b*
Hamedan4-s	*Vrn-A1j*	Ghasre-Shirin3-s	*Vrn-A1j*	Iran45	*Vrn-A1b*
Iran35	*Vrn-A1b*	Malayer4-s	*Vrn-A1j, Vrn-A1*	Gazvin12-w	*Vrn-A1b*
Iran36	*Vrn-A1b*	Mahi-Dasht3-s	*Vrn-A1j, Vrn-A1*	Iran46-s	*Vrn-A1b*
Iran37	*Vrn-A1b, Vrn-A1c*	Kermanshah5-w	*Vrn-A1b*	Iran47-s	*Vrn-A1k*
Iran38	*Vrn-A1b*	Gazvin10-s	*Vrn-A1j*	Hamedan8-w	*Vrn-A1b*
Tabas2	*Vrn-A1b, Vrn-A1c*	Varamin2-s	*Vrn-A1k*	Iran48	*Vrn-A1b*
Iran39	*Vrn-A1b*	Iran40	*Vrn-A1b*	Gazvin13-w	*Vrn-A1b*
Shahre-Kord4-s	*Vrn-A1b*	Gilane-Gharb5-s	*Vrn-A1b*	Iran49-s	*Vrn-A1b*
Niriz2-w	*Vrn-A1b*	Hamedan6-s	*Vrn-A1k*	Iran50-s	*Vrn-A1b*
Shah-Roud4-w	*Vrn-A1b*	Esfahan15-s	*Vrn-A1b*	Hamadan9-w	*Vrn-A1b*
Hasht-Rood2-s	*Vrn-A1j*	Sanjabi2-w	*Vrn-A1b*	Tehran1-s	*Vrn-A1b*
Arak3-s	*Vrn-A1j*	Neishabour8-s	*Vrn-A1b, Vrn-A1*	Birjand11-s	*Vrn-A1b, Vrn-A1*
Sanandaj7-w	*Vrn-A1b*	Birjand10-w	*Vrn-A1b*	Sarakhs2-s	*Vrn-A1b*
Hamedan5-s	*Vrn-A1j*	Ghasre-Shirin4-s	*Vrn-A1b*	Iran51-s	*Vrn-A1b, Vrn-A1*
Tabas3-s	-	Shah-Abad7-s	*Vrn-A1b*	Iran52-s	*Vrn-A1j*
Esfahan13-w	*Vrn-A1b*	Bojnourd12-w	*Vrn-A1b*	Zanjan3-s	*Vrn-A1j*
Borujen5-w	*Vrn-A1b*	Kashmar4-s	*Vrn-A1b*	Shahrood5-s	*Vrn-A1b*
Torbat-Heidar3-w	*Vrn-A1k*	Kashmar5-w	*Vrn-A1b*	Semnan3-s	*Vrn-A1b*
Borujen6-w	*Vrn-A1b*	Sabzvar18-s	*Vrn-A1b*	Kerman5-s	*Vrn-A1b, Vrn-A1*
Zahedan1-s	*Vrn-A1b, Vrn-A1c*	Mashhad14-s	*Vrn-A1b*	Kerman10-s	*Vrn-A1b*
Zahedan2-s	*Vrn-A1b, Vrn-A1c*	Shahre-Kord8-s	*Vrn-A1b*	Kerman11-s	*Vrn-A1b*
Zahedan3-s	*Vrn-A1b, Vrn-A1c*	Mashhad15-s	*Vrn-A1b, Vrn-A1*	Esfahan23-s	*Vrn-A1b*
Zahedan4-s	*Vrn-A1b, Vrn-A1c*	Mashhad16-s	*Vrn-A1b, Vrn-A1*	Esfahan24-s	*Vrn-A1b*
Esfahan18-s	*Vrn-A1b*	Mashhad17-s	*Vrn-A1b, Vrn-A1*	Yazd8-s	*Vrn-A1b*

Table 3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Esfahan19-s	*Vrn-A1b*	Mashhad18-s	*Vrn-A1b, Vrn-A1c*	Tehran2-s	*Vrn-A1b*
Esfahan20-s	*Vrn-A1b*	Mashhad19-s	*Vrn-A1b, Vrn-A1c*	Chinese spring	-
Esfahan21-s	*Vrn-A1b*	Kerman7-s	*Vrn-A1b*	Thatcher	*Vrn-A1k*
Esfahan22-s	*Vrn-A1b*	Kerman8-s	*Vrn-A1b*		
Shahre-Kord7-	*Vrn-A1b*	Kerman9-s	*Vrn-A1b*		

In this study the frequencies of *Vrn-A1* alleles differed from those obtained for wheat cultivars from Europe, America and even Asia. Complementary studies are necessary to investigate the role of other genetic systems, especially earliness *per se*, and *VRN2* in determination of flowering time and adaptation in Iranian wheat landraces.

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