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Evaluation of vernalization requirement in wheat inbred lines and cultivars under controlled conditions

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Abstract

An understanding of vernalization requirement is a prerequisite for the development of cold tolerant cultivars for high stress regions. Vernalization requirement in winter wheat (*Triticum aestivum* L.) has not been adequately addressed. Therefore, the objective of the present study was to understand how the vernalization dates are related to cold tolerance, phenological development and photosynthesis in four inbred lines (inbred line 1, 2, 3 and 4) and two wheat cultivars (Mironovskaya-808 and Pishtaz). These genotypes were subjected to vernalization temperature (5° C) on 30.11.2012, 17.12.2012, 09.01.2013, 13.02.2013 and 08.03.2013 as different vernalization dates. Control plants were grown under 25/20° C, day/night condition. Final leaf number was determined at intervals throughout the growth period to measure vernalization status. Number of days until heading was registered and lethal temperature (LT_{50}) was determined. Photosynthesis rate was measured at the end of winter and flowering stages. According to the results the individual effect of genotype and vernalization date was significant on final leaf number, number of days until flowering and LT_{50}. However, photosynthesis rate was just affected by vernalization date. In addition, interaction between vernalization date and genotype was significant on final leaf number, number of days until flowering and LT_{50}. These results support the hypothesis that vernalization responses regulate phenological growth and affect cold tolerance through their influence on the rate of plant development.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the major crops in Iran and world-wide. It is grown across a wide range of environments and is considered to have the broadest adaptation of all cereal crop species (Briggle and Curtis, 1987). Winter wheat varieties are planted in the fall and, if they have adequate tolerance to survive winter freezing temperatures, usually have higher yield potential than spring varieties planted later in the spring because of their longer growing period (Galiba *et al.,* 2009). Generally, cold tolerance in wheat should refer to performance at temperatures lower than the optimum for growth. In winter cereals, cold tolerance is associated with the occurrence of cold-hardening or cold acclimation, which is triggered by induction of cold responsive genes after exposure of plants to low but non-freezing temperature for certain periods of time (Winfield *et al.,* 2010; Armonienė *et al.,* 2013). Low-temperature tolerance is determined by a highly integrated system of structural and developmental genes that are regulated by environmentally responsive, complex pathways (Fowler and Limin, 2007). Vernalization is the acceleration of flowering by a cold treatment (Chouard, 1960).Vernalization requirement has a major influence on plant development. Cereals are normally long day plants (Thomas and Vince-Prue, 1997) in which day length affects apical morphogenesis, leaf production and other developmental processes (Kirby, 1969). Short day conditions extend the length of the vegetative phase by increasing the number of leaves and delaying the reproductive phase visualized by double ridge formation (Mahfoozi *et al.,* 2001). Exposure to temperature in the vernalization range shortens the vegetative phase by decreasing the number of leaves in cereals with a vernalization requirement (Wang *et al.,* 1995). It has been reported that the ability of wheat plants to maintain cold tolerance decreases after the vegetative/reproductive transition (Mahfoozi *et al.,* 2001), but mechanisms exist which slow down the rate of phenological development and extend the vegetative phase (Prasil *et al.,* 2004). Winter wheat varieties usually have a greater vernalization requirement and a higher sensitivity to short days than spring wheat varieties, which enable them to remain in the vegetative phase during winter. Spring wheat varieties have no, or a very limited, need for vernalization. A vernalization requirement enables winter wheat to maintain the expression of low temperature genes at higher intensity and for a longer period of time than in spring wheat (Prasil *et al.,* 2004). A decrease in tolerance after a long period of cold acclimation has been shown in by Mahfoozi *et al.* (2001). There is evidence that in most circumstances non-vernalized wheat plants have little response to photoperiod, although the temperature response of vernalization may change in long days as compared with short days (Brooking and Jamieson, 2002). The results from these two studies strongly suggest that photoperiod and vernalization factors should be applied either simultaneously, as multiplicative factors, or sequentially (Mcmaster *et al.,* 2008).

The objective of the present project was to understand how the vernalization requirements are related to cold tolerance, phenological development and photosynthesis in four inbred lines and two wheat cultivars.

Materials and methods

Plant materials

Wheat cultivars, Mironovskaya-808, cold resistant winter wheat and Pishtaz, semi-resistant spring wheat and four inbred lines (8th generation) were obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran. The inbred lines were produced by simple crossing between above motioned cultivars, which differ in terms of vernalization requirement.

Growth conditions

The seed were soaked in tap water for 24 h and then six seeds were sown in each pot on 5th November 2012 in 20-cm pots containing a clay-loam soil at 2.5 cm depth. The pots were moved to a growth chamber at a temperature of 25/20° C, day/night, with a long day photoperiod of 16/8 h, irradiance of 400 µmol m^{-2} s^{-1}, and relative humidity of 65%. After seedling establishment, vernalization was induced by exposure plants to low temperature (5° C) on 30.11.2012, 17.12.2012, 09.01.2013, 13.02.2013 and 08.03.2013 as different vernalization date. Control plants were grown under 25/20°C, day/night condition.

Data collecting

Final leaf number was recorded by counting the leaves on main tiller until flag leaf was emerged. In addition, number of days from seed sowing to heading was recorded for each treatment. The data were collected from two replications.

Apical meristem development was evaluated and vegetative stages such as single ridge and double ridge stage were photographed.

In order to determined lethal 50 percent temperature (LT_{50}) plant samples were collected and transplanted into aluminium pots containing moist soft sand and placed in an adjustable freezers at -3° C for 12 h. Afterwards, the descending temperature gradient was 2° C per hour until -17° C and then 3° C per hour until -25° C. Finally the plants were taken out form the freezer and placed at 4° C for 12 h before transferring into growth chamber at 18±2° C, long day photoperiod and 350 µmol m^{-2} s^{-1} photosynthetic photon flux density. After three weeks, dead plants were counted and LT_{50} was calculated. The lethal temperature was defined as that temperature at which 50% of the samples die and 50% survive. Data were analysed with three replications. Net photosynthesis was measured with a portable photosynthesis system (LICOR Biosciences, 6400). All measurements were made at flowering stage during the late of winter on the same leaves at 9-10° C with three replications.

Statistical analysis

The data were analysed in a completely randomized block design arranged in a factorial arrangement. Data were analyzed with ANOVA following the GLM procedures in SAS. Differences among the means of treatments were compared by Duncan's Multiple Range Test at the 0.05 confidence level.

Results and discussion

Analysis of variance indicated that final leaf number and days until heading were affected by vernalization date and genotype as well as interaction between them (Table 1).

Table 1. Analysis of variance on final leaf number and days until heading affected by vernalization date and genotype.

S.O.V	d.f	Final leaf number	Days until heading
Block	1	0.08	4.60
Date	5	17.36**	17583.60**
Genotype	5	26.32**	812.05**
Date × genotype	25	5.18**	414.38**
Error	35	0.58	6.80
C.V (%)		7.42	4.11

*, ** and ns: significant at 0.05, 0.01 and no significant, respectively.

Table 2. Analysis of variance on lethal temperature and photosynthesis affected by vernalization date and genotype.

S.O.V	d.f	LT_{50}	Photosynthesis
Block	2	0.05	13.63
Date	3	330.74**	3455.44**
Genotype	5	191.42**	17.94ns
Date × genotype	15	36.42**	14.25ns
Error	46	1.09	40.78
C.V (%)		16.69	11.73

*, ** and ns: significant at 0.05, 0.01 and no significant, respectively.

Similarly lethal 50 percent temperature was affected by vernalization date and genotype as well as interaction these factors (Table 2). The results showed that photosynthesis was affected just by vernalization date (Table 2). According to figure 1, the highest leaf number was obtained from inbred line 4 and mironovskaya-808 cultivar in control treatment. Delay in vernalization decreased leaf number in all genotypes, however this reduction was more pronounced in inbred line 4. There was no significant difference between control treatment and the first vernalization date in terms of final leaf number in Pishtaz cultivar (Figure 1). The highest leaf number in Pishtaz cultivar was observed when plants were subjected to vernalization temperature on 09.01.2013. The final leaf number decreased with delay in vernalization in this cultivar (Figure 1). In case of mironovskaya-808 cultivar the results were more erratic, however the lowest final leaf number was observed on 09.01.2013 vernalization date (Figure 1).

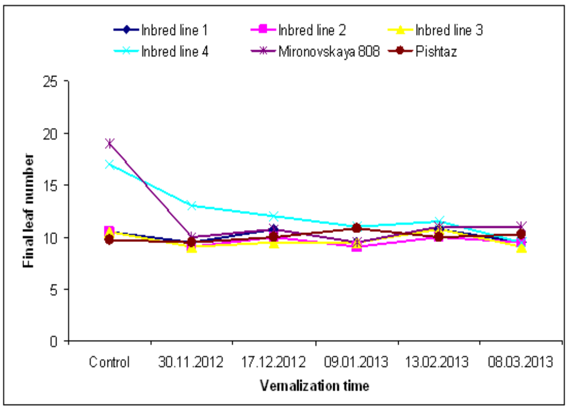

Fig. 1. The effect of different vernalization date on final leaf number.

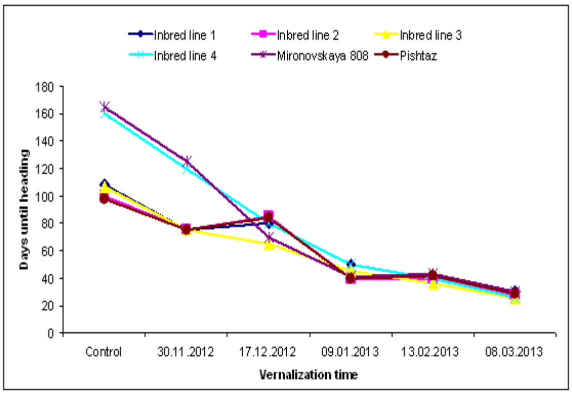

Fig. 2. The effect of different vernalization date on days until heading.

Leaf appearance rate is controlled mostly by the temperature of the apical meristem and leaf expansion zones (McMaster and Wilhelm, 2003). Final leaf number is largely controlled by responses to vernalization and photoperiod (Brooking and Jamieson, 2002). Similar results have been found by Wang *et al.* (1995) who stated that low temperature shortens the vegetative phase by decreasing the number of leaves in cereals. Final leaf number is determined by the number of primordia initiated up to the attainment of floral transition (Robertson *et al.,* 1996). The longest growth period was obtained from mironovskaya-808 cultivar and inbred line 4 (Figure 2). Generally the number of days until heading decreased with delay in vernalization (Figure 2). Days until heading decreased in mironovskaya-808 and Pishtaz cultivar, however an increase was observed in days until heading in Pishtaz cultivar when the plants were subjected to vernalization temperatures on 17.12.2012 (Figure 2). Irrespective if genotypes, the shortest growth period was observed in plants which were vernalized on 08.03.2013 (Figure 2). It should be noted that vernalization on 17.12.2012 increased the number of days until heading compared with other vernalization dates (Figure 2). Reproductive meristems are more sensitive to frost damage than vegetative meristems and therefore, small differences in developmental stages can affect plant survival to freezing temperatures. It is well known that vernalization is cultivar-specific (Mc Master *et al.,* 2008; White *et al.,* 2008) and that its major effects are the reduction of the duration from the end of the low temperature treatment to flowering and to shorten the duration of phenological phases (Levy and Peterson, 1972; Robertson *et al.,* 1996). Heading and flowering dates are affected by genetics and environmental conditions (Salazar-Gutierrez *et al.,* 2013). A strong interaction between genetics and the temperature occurs when the crop is exposed to low temperatures during vegetative and reproductive development and which is affected by sowing date, seeding rate, fertilizer applications, irrigation and fluctuations in short-term weather and long-term climate conditions (Hu *et al.,* 2005). In addition, it has been reported that the heading or flowering date of winter wheat is mainly a function of spring temperatures (Xue *et al.,* 2004).

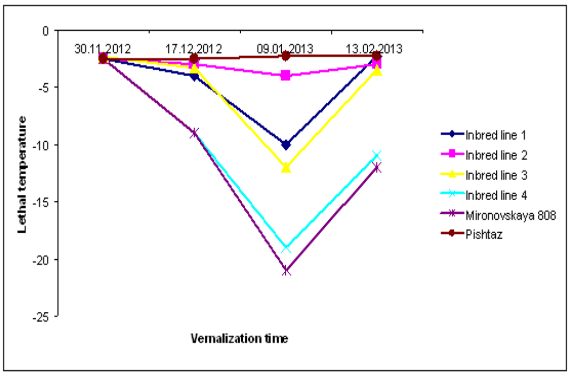

Fig. 3. The effect of different vernalization date on lethal temperature.

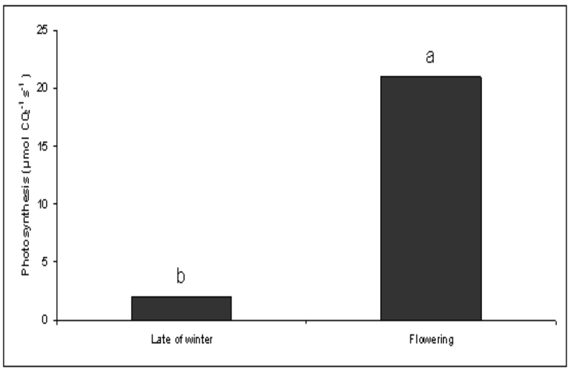

Fig. 4. Photosynthesis rate affected by date.

As can be seen from figure 3, vernalization on 30.11.2012 caused the lowest cold resistance in all genotypes, in other words when plants were vernalized on this day, at -2.5° C half of the plants died (Figure 3). The highest cold resistance was observed in inbred line 4 and mironovskaya-808 cultivar when these genotypes were vernalized on 09.01.2013 (Figure 3). The obtained results indicated that when inbred line 4 and mironovskaya-808 cultivar were vernalized on 09.01.2013, lethal temperature decreased to -17 and -22° C, respectively (Figure 3). According to the results, there was no significant difference between vernalization in Pishtaz cultivar in terms of lethal temperature (Figure 3). Similarly, increase in cold resistance was observed in inbred line 1 and inbred line 3 when these genotypes were vernalized on 09.01.2013 (Figure 3). In general, vernalization on 09.01.2013 increased cold resistance in wheat genotypes compared with other vernalization dates (Figure 3). Similar reaction has been reported in previous experiments, having a very high survival rate after controlled freezing of plants cold-acclimated in the field (Rapacz *et al.,* 2008).

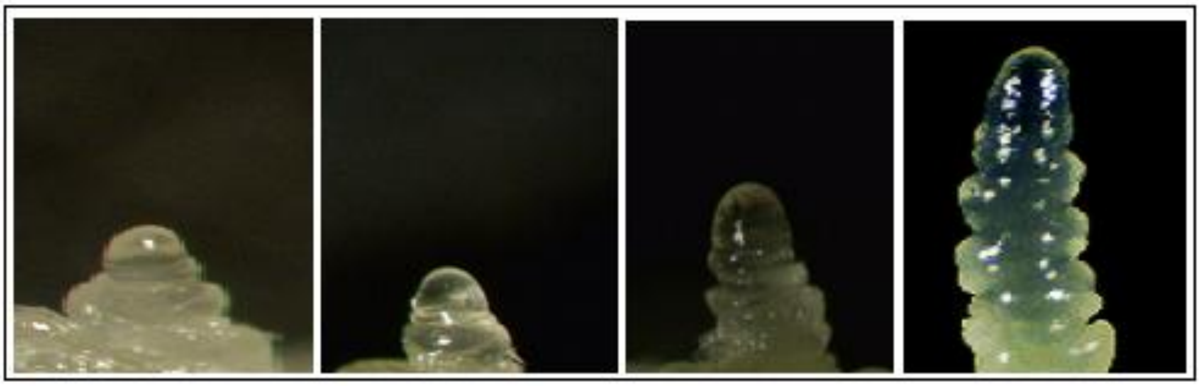

Fig. 5. Phenological growth stage of mironovskaya-808 cultivar affected by vernalization dates (left to tight: 30.11.2012, 17.12.2012, 09.01.2013 and double ridge stage).

Based on the results photosynthesis rate at flowering stage was more than photosynthesis rate at the end of winter (Figure 4). Many physiological processes and also photosynthesis are sensitive to cold stress which is a main proof for the decline of growth and productivity in plants under low temperature conditions (Liang *et al.,* 2007). One of the most important chloroplast components for photosynthesis is chlorophyll and it has a positive relationship with photosynthetic rate (Guo *et al.,* 2008). It has been stated that chlorophyll content decreases under low temperature conditions (Liang *et al.,* 2007). Phenological growth stages of wheat genotypes are shown in figure 5, 6, 7 and 8. The development of wheat can be followed by observing the differentiation of the main stem shoot apex. Mahfoozi *et al.* (2001) have shown that decrease in frost tolerance preceded the double-ridge stage in cold-acclimated winter wheat plants. If there is a developmental stage in spring wheat (without any vernalization requirement) after which plants gradually lose their frost tolerance this has not been observed.

Fig. 6. Phenological growth stage of inbred line 4 affected by vernalization dates (left to tight: 30.11.2012, 17.12.2012, 09.01.2013).

Fig. 7. Phenological growth stage of Pishtaz cultivar affected by vernalization dates (left to tight: 30.11.2012, 17.12.2012, 09.01.2013).

Fig. 8. Phenological growth stage of inbred lines 1, 2 and 3 affected by vernalization dates (left to tight: 30.11.2012, 17.12.2012, 09.01.2013).

Conclusion

According the results, delay in vernalization significantly decreases final leaf number and number of days until heading. Furthermore, the results confirmed that vernalization on 09.01.2013 increases cold resistance in wheat cultivars. In general, these findings prove that vernalization regulates phenological growth period and affect cold tolerance through their influence on the rate of plant development.

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