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Full Length Research Paper

Freezing tolerance of wheat cultivars at the early growing season after winter

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Cold stress is a worldwide abiotic stress in temperate regions that affects plant development and yield of winter wheat (*Triticum aestivum* L.) cultivars and other winter crops. This study was conducted to evaluate the effect of freezing stress at the early growing season on survival and also the relationship between resistances to freezing stress at this stage with drought resistance after postanthesis in eight Iranian bread wheat cultivars. An experiment was laid out in a split–plot arranged in a randomized complete blocks design with three replications. Under control treatment among all cultivars, Marvdasht and Chamran cultivars had the lowest grain yield and biomass; except these two cultivars there were no significant differences between others. Postanthesis water stress caused more reduction in grain yield of Zarin, Phishgam and Marvdasht and biomass of Phishgam and Parsi cultivars, respectively. The lowest and highest LT50 temperatures of crowns at the early growing season were seen in Marvdasht (-4°C) and Pishtaz (-1.13°C) cultivars, respectively. Correlation coefficients of soluble and insoluble sugars concentration between crown survivals under freezing stress were, respectively positive and negative. Grain yield under postanthesis water stress had the highest correlation coefficient (R=-86.2%) with crown survival under freezing stress at the early growing season. Therefore, postanthesis water stress-sensitive cultivars were more resistant to freezing stress at the beginning of growing season after the cold of winter.

Key words: Wheat, cold stress, freezing stress, crown, soluble sugar.

INTRODUCTION

Cold stress is a worldwide abiotic stress that affects plant development and yield production of winter wheat cultivars (Chu et al., 2010) and other winter crops (Lang et al., 2005) in temperate regions. The yield loss due to occurrence of these stresses is very high in some years. Chilling injury (less than 20°C) and freezing injury (less than 0°C) are two kinds of cold stress. Currently, about 15% of yield loss in cereals crops is due to cold stress (Aniol, 2002). In temperate regions of the world such as Iran, cool season crops (for example, winter wheat) plants are often planted in the fall. These plants usually spent part of their growth period before winter (approximately 4 to 6 leaf stage in wheat). These plants acclimatize during autumn, during which their metabolism is redirected towards synthesis of protectant molecules such as soluble sugars (glucose, fructose, sucrose, starch and fructan), sugar alcohols (sorbitol, ribitol, inositol and manitol) and low-molecular weight nitrogenous compounds (proline and glycine betaine) (Vitamvas et al., 2007; Chen and Murata, 2002, 2008; Yuanyuan et al., 2010). Temperature increases in late February and in early March (early spring) led to fast loss of cold acclimation and increase in cold sensitivity and then sudden falling of temperature at this time brings serious effects on survival and yield production (Roustaei, 2008). Global warming also causes warmer winter and faster growth in winter in temperate plant such as winter wheat which makes plants more susceptible to cold stress (Chu et al., 2010).

Introduction of resistant wheat cultivars are highly

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effective in reducing damage caused by cold stress. Because of difficulties in selecting cold resistance in field, laboratory methods are used for this purpose (Levit, 1956; Harvey, 1998). Results of field and laboratory methods have been highly correlated (r=0.86) (Levit, 1956). In exposure with cold stress, the most critical part of cereals in the early stages of growth is crown. Regrowth of crown after application of cold stress has a vital role in cereals cold resistance (Ashraf and Taylor, 1974). In this relation, crown freezing is one of the most important laboratory methods, because resistance of crown against cold stress is a necessary prerequisite for cold resistance (Brule-Bable and Fowler, 1989). A sudden drop in air temperature at the early growing season (after severe cold in winter) is the most winter wheat growing areas and occurrence of drought stress at the final growth stage of wheat (post anthesis) is the two main abiotic stresses that almost caused significant reduction in grain yield production in winter wheat cultivars in temperate regions (Roustaei, 2008). Growth starting in late winter or early spring in winter wheat is associated with reduction in resistance to freezing and cold stress. Occurrence of chilling and freezing stresses in this condition ultimately lead to a significant reduction of live plants and hence significant reduction in grain yield production. So, the objectives of this study were to determine the resistance to the freezing stress in different wheat cultivars at the beginning of growing season and the relationship between freezing stress resistance characteristic with resistance to post anthesis water stress.

MATERIALS AND METHODS

Plant materials

Eight bread wheat cultivars (Bahar, Parsi, Pishtaze, Phishgam, Chamran, Zarine, Sivand and Marvdasht), were obtained from Agricultural Research Institute of Kermanshah in Iran.

First experiment

The research was carried out in the wheat growth season of 2010 to 2011 at Agricultural Research Farm of Razi University, Kermanshah, Iran. These cultivars were chosen because they have the highest area under cultivation in the Kermanshah province and they are new modern cultivars with unknown physiological characteristics. The research was in a field where the previous crop was a corn. The soil was a clay loam (36.1% clay and 30.7% silt) and the experiment was laid out in a splitï plot arranged in a randomized complete blocks design with three replications. Irrigation regimes, normal and stress (stress treatment was applied from anthesis by stopping of irrigation until maturity whereas in control treatment soil moisture with regular irrigation was maintained approximately at field capacity) occupied the main plots, while cultivars were assigned to the sub-plots. Each plot included 40 rows 20 cm apart, 4 m long, 4 and 3 m distances were taken between test plots and replicates, respectively. Seeds were sown at a density of 400 seeds per square meter on 12 October. Based on soil analysis, nitrogenous fertilizer as urea ($CO(NH_2)_2$) was applied prior to planting, as topdressing at tillering stage and at flowering stage, 50 kg N/ ha^{-1} in each stage. Biomass and grain yield for each cultivar were measured by harvesting 2 m^2 of the central part of each plot at crop maturity. Harvest index was measured by dividing grain yield to biomass production.

Second experiment

In this experiment resistance to cold stress (chilling and freezing of wheat cultivars (Bahar, Parsi, Pishtaze, Pishgam, Chamran, Zarine, Sivand and Marvdasht) were studied at five temperatures that is, 3, -3, -5, -7 and -9°C with a thermogradient device. On 27th February 2011, at the beginning of growing season, 20 plants of each cultivar were harvested at 4 to 7 leaf stage seedlings and carefully cleaned the roots from the soil. Then, the plants were placed in moist papers and were transferred immediately to the laboratory. For preparation of samples, as much as two centimeters up and a centimeter down to the crowns were cut with scalpel and 10 crowns were chopped separately into powder. These powders were used for total soluble and insoluble sugar as described with AOAC (1995) and Zhu et al. (2009) with some modifications. Hundred milligrams (100 mg) of powder in each treatment was used for extraction of soluble sugars. Ethanol (80%) at 80°C for 60 min was used as the extraction buffer. Subsequently, samples were centrifuged at 14000 rpm for 15 min and supernatant was collected. This step was repeated three times. Supernatant was used for the determination of total soluble sugars and insoluble residues were used for the determination of starch (insoluble sugar). For freezing test, another ten crowns of each cultivar were used at each temperature (3, -3, -5, -7 and -9) in each replication.

For this purpose, the crowns were placed and attached to the wall of aluminum containers which were filled with wet sand. Samples were placed in a programmable device Thermogradian for 14 h at 3°C. Then temperature dropped to 2°C every 20 min. In order to balance ambient temperature, the environmental temperature of crown in each treatment was maintained for 1 h and then samples were harvested. To prevent rapid melting, freezed samples were kept in refrigerator (at 4 ± 1°C) for a day and night. Then samples of each treatment were transferred to the greenhouse and were planted in plastic pots with 15 cm height and 15 cm diameter containing 1:1 Humus and straw leaf (two third of crown put in the soil). Irrigation was regularly performed. After three weeks, live plants were counted. Symptoms of healthy plants were production of leaves and roots together. Then, the temperature at which 50% of each cultivar was lost (LT50) was determined using diagram. Mean, minimum and maximum of temperature were from 20 February till 19 March (A) and Amberothermic curve (B) in Kermanshah city are shown in Figure 1. Statistical analysis and graphs drawing were performed using MSTATC, SAS, Excel and Word software's.

RESULTS

In the control treatment, grain yield and biomass of Marvdasht and Chamran cultivars were significantly lower than other cultivars. In terms of grain yield and biomass productions, except Marvdasht and Chamran, there were no significant differences between other cultivars (Table 1). Postanthesis water stress significantly reduced grain yield of cultivars (Table 1). Greatest reduction in grain yield in Zarin, Marvdasht and Pishgam and greatest reduction in biomass in Parsi and Pishgam cultivars, respectively was seen. Lowest reduction in grain yield and biomass were in Marvdasht and Chamran cultivars,

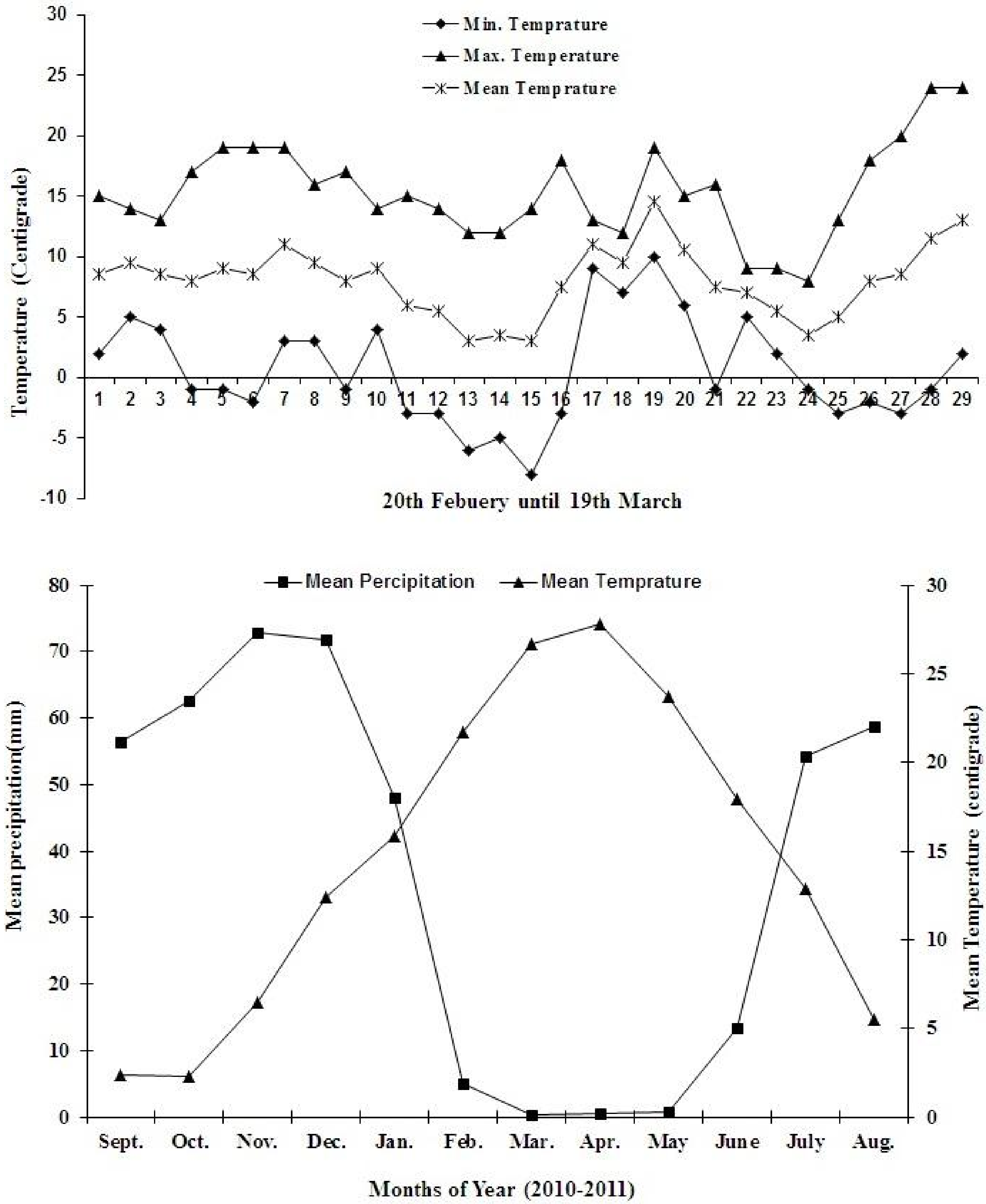

Figure 1. Mean, minimum and maximum temperatures (ºC) from 20 February till 19 March (A) and Amberothermic curve (B) of Kermanshah city in Iran for agricultural year 2010 to 2011.

respectively. In the control treatment, Pishgam cultivar had the highest harvest index (Table 1). Except for Chamran cultivar, postanthesis water stress caused significant reduction in harvest index of other cultivars with the highest reduction in harvest index in Marvdasht cultivar (23/4%). Before the application of freezing stress

Table 1. Mean comparison of grain yield, biomass and harvest index of different wheat cultivars under post anthesis water stress.

Cultivar	Biomass (g/m^2)		Relative decrease (%)	yield (g/m^2)		Relative decrease (%)	Harvest index		Relative decrease (%)
	Control	Water stress		Control	Water stress		Control	Water stress	
Bahar	1604±92	1244±82	22.4	543±19	356±27	34.4	33.9±0.8	28.6±1.4	15.6
Parsi	1640±107	1173±104	28.5	519±28	328±57	36.8	31.7±0.4	27.5±2.4	13.2
Pishtaz	1596±85	1244±100	22.0	529±30	373±40	29.5	33.2±1.1	29.8±1.1	10.2
Pishgam	1493±38	1053±142	29.5	538±23	334±47	37.9	36.0±0.6	31.8±1.3	11.8
Chamran	1356±92	1067±115	21.3	421±33	335±26	20.4	31.0±0.7	31.7±1.4	-2.1
Zarin	1671±99	1302±92	22.1	543±27	336±37	38.2	32.5±0.4	25.7±1.6	21.1
Sivand	1649±109	1307±103	20.8	587±62	372±52	36.6	35.4±1.4	28.2±2.0	20.3
Marvdasht	1418±44	1160±68	18.2	492±15	308±19	37.4	34.7±0.1	26.6±0.9	23.4

Values are presented as mean ± standard error (n=4).

in the early growing season, the crown of Parsi cultivar and Marvdsht had the lowest and the highest concentration of soluble sugars, respectively (Figure 2). In the early growing season, the crown of Bahar, Zarin and Chamran cultivars had the highest and lowest concentration of starch, respectively.

Under water and non-water stress conditions, the correlation coefficient between concentration of soluble and insoluble sugars with biomass and grain yield was not significant (Table 3). The correlation coefficient between insoluble sugars with grain yield and biomass in contrast to soluble sugars was positive. Evaluation of crown survival percentage of different cultivars in the freezing stress conditions showed that (Table 2), in the temperature of -3°C, Marvdasht cultivar had the highest crown survival (80%) and Pishtaz cultivar had the lowest crown survival (20%). Further reduction in freezing temperatures caused more significant reduction in crown survival of different cultivars. In the temperature of -5°C, the Pishtaz cultivars lost the entire of its crowns viability. Marvdasht and Pishgam cultivars at -9°C had the highest survival percentage (Table 2). Among different cultivars, Marvdasht and Pishtaz had the lowest and highest slope of decline in crown survival, respectively. The lowest and highest LT50 temperature of crowns were related to Marvdasht (-4°C) and Pishtaz (-1.13°C), respectively (Figure 3). Under the freezing stress, correlation coefficients of grain yield and biomass (in control and postanthesis water stress) with crown survival under freezing stress at the early growing season were negatively associated (Table 3). Grain yield under post anthesis water stress had higher correlation coefficients (R=-86.2%) with crown survival under freezing stress at the early growing season. Correlation coefficients of soluble and insoluble sugars concentration with crown survival were positive and negative, respectively.

DISCUSSION

The lowest grain yield of Marvdasht and Chamran cultivars under control treatment indicated that these two cultivars had lower yield potential than other cultivars and they also had lower ability in the use of appropriate environmental conditions, particularly soil moisture for grain yield production. As defined by both Hossein et al. (1990) and Clarck et al. (1992), wheat cultivars with lowest reduction in grain yield at water stress conditions were considered as resistant ones. Thus, Chamran cultivar because of having the lowest and Zarin, Pishgam and Marvdasht because of having the highest reduction in grain yield under post anthesis water stress (20.2%) are considered as resistant and sensitive cultivars, respectively. The harvest index result of interactions is between genetic, environmental and agronomic factors (Sidlauskas and Bernotas, 2003). In post anthesis water stress, harvest index sharply declined in all except in Chamran cultivar. In contrast, Yang et al. (2002), Xang et al. (1998) and Yang et al. (2000) showed that water stress caused significant increase of harvest index in wheat cultivars. If moisture stress during grain filling can be controlled in a way that plants can absorbs water during the night and photosynthesis not reduced and also carbohydrate remobilization, especially from stems increases (Yang and Zhang, 2005).

Probably the severity of water stress was so much that plants could not compensate the day water loses during the night. Lower reduction in harvest index of Chamran cultivar in water stress

Table 2. Percent of survival under different freezing temperatures in different wheat cultivars.

Cultivar	Different freezing temperatures (ºC)								
	+3	-3	Relative decrease (%)	-5	Relative decrease (%)	-7	Relative decrease (%)	-9	Relative decrease (%)
Bahar	100	50.0±5.7	50	40.0±11.5	60	13.3±6.6	87	0.0±0.0	100
Parsi	100	66.7±17.6	33	26.7±7.6	73	6.7±6.6	93	0.0±0.0	100
Pishtaz	100	20.0±5.5	80	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
Pishgam	100	46.7±6.6	53	26.7±6.6	73	20.0±5.5	80	6.7±2.6	92
Chamran	100	60.0±10.0	40	26.7±6.6	73	20.0±1.0	80	0.0±0.0	100
Zarin	100	60.0±2.0	40	20.0±2.0	80	13.3±6.6	87	0.0±0.0	100
Sivand	100	46.7±7.6	53	13.3±6.6	87	0.0±0.0	100	0.0±0.0	100
Marvdasht	100	80.0±10.0	20	40.0±7.7	60	13.3±6.6	87	13.3±3.3	87

Values are presented as mean ± standard error (n=4).

Table 3. Correlation coefficient between soluble and insoluble sugars and survival at -3 and -5°C in freezing stress at early growing season after winter with grain yield and biomass production under post anthesis water stress in different wheat cultivars.

Trait		Control		Water stress		Soluble sugar	Insoluble sugar	Survival at -3°C	Survival at -5°C
		Biological yield	Grain yield	Biological yield	Grain yield				
Control	Biological yield	1							
	Grain yield	0.83*Í	1						
Water stress	Biological yield	0.81*	0.68	1					
	Grain yield	0.51	0.52	0.54	1				
Soluble sugars		-0.62	-0.31	-0.19	-0.40	1			
Insoluble sugar		0.16	0.46	0.24	0.38	-0.05	1		
Survival at -3°C		-0.33	-0.37	-0.25	-0.87**	0.35	-0.32	1	
Survival at -5°C		-0.64	-0.56	-0.71	-0.64	0.40	-0.18	0.46	1

Í * and ** Indicating significant correlation at Ŭ = 0.05 and 0.01, respectively.

treatment also showed that this cultivar probably has the highest resistance to postanthesis water stress. Reduction in harvest index under post anthesis water stress also was reported by Araus et al. (2002) and Liu and Li (2005). These experiments are conducted considering the main role of crowns in survival of winter wheat cultivars in prevailing of freezing stress at the beginning of growing season after over wintering of winter wheat cultivars. Thus, Marvdasht and Pishtaz cultivars were most resistant and sensitive cultivars to freezing stress, respectively. Considering the main role of sugars in resistance to freezing stress (Ristic and Ashworth, 1993; Wanner and Junttila, 1999), soluble and insoluble sugars

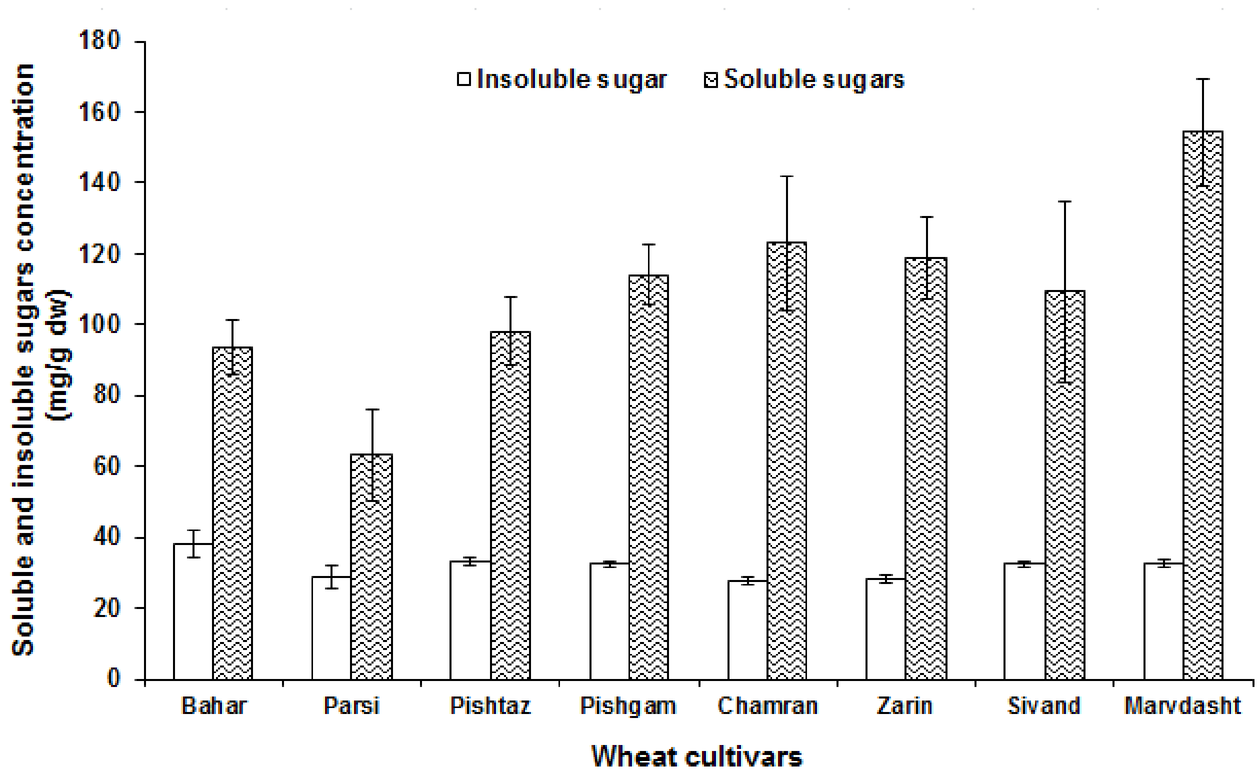

Figure 2. Mean comparisons of soluble and insoluble sugars in crowns of different wheat cultivars at early growing season after winter before freezing stress. ɯIndicating standard error (n=4).

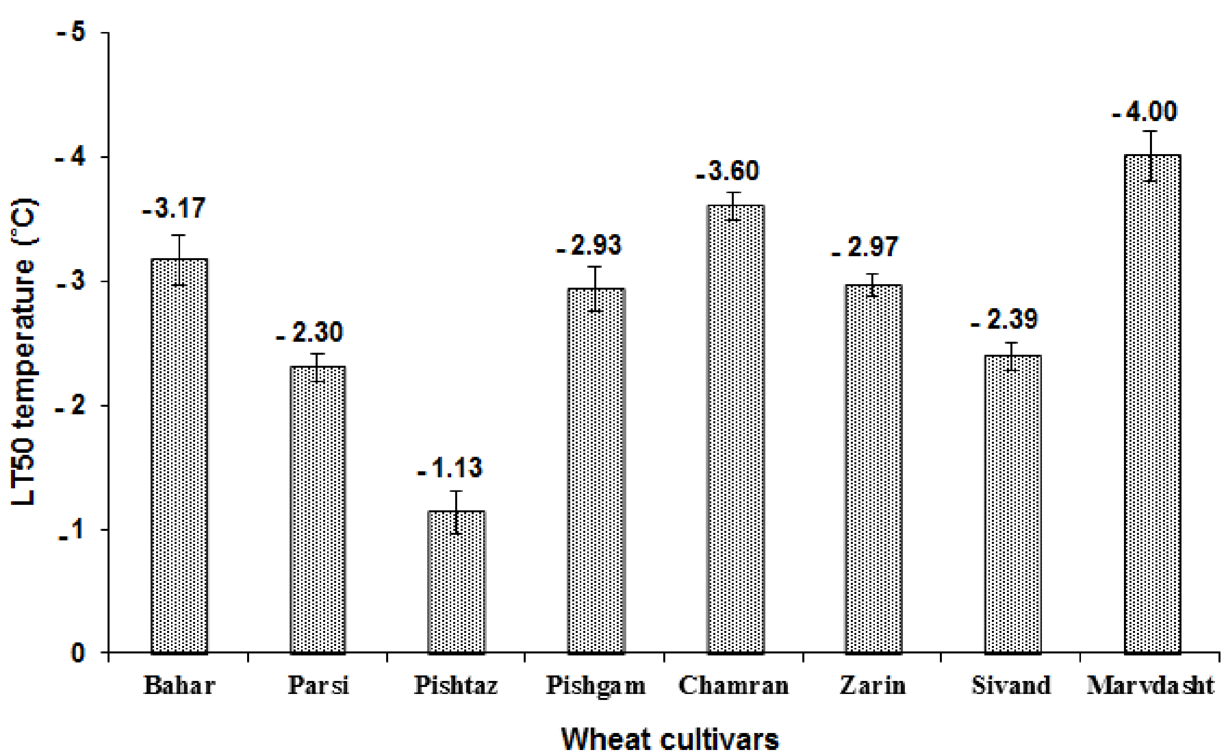

Figure 3. Mean comparisons of LT50ÿs temperatures of crowns of different winter wheat cultivars at early growing season after winter before freezing stress. ɯIndicating standard error (n=4).

concentration in the crowns at the beginning of freezing stress, also were studied. Correlation coefficients between freezing resis-tance with soluble sugar (at -3 and -5°C, r = 0.40 and r=0.34, respectively) and with insoluble sugars (at -3 and -5°C, r=-0.18 and r = -0.32, respectively) showed that soluble sugars have more important role than insoluble sugars in resistance to freezing stress.

In freezing stress, soluble sugars with protection of plasma membrane (Shao et al., 2006; Shalaev and Steponkus, 2001) may maintain hydrophobic interaction (Cacela and Hincha, 2006; Tsvetkova et al., 1989), and scavenging reactive oxygen species (Bolouri-Moghaddam et al., 2010; Couee et al., 2006; Ramel et al., 2009) help the resistance of plant. The correlation coefficient between the concentration of soluble sugars and freezing stress resistance (at -3 and -5°C, r = 0.40 and r=0.34, respectively) showed that in addition to soluble sugars, other factors such as sugar alcohols, low molecular weight of nitrogenous compound (proline, glycine, betaein) and also starch may involve in resistance to freezing stress through degradation into other compounds. This finding is in agreement with Ritte et al. (2004) and Yano et al. (2005) findings which showed starch degradation is involved in the freezing tolerance enhancement. Kaplan and Guy (2004) presented more direct evidence for cold-induced starch degradation in arabidopsis.

It seems possible that these results are due to chloroplast-targeted ɓ-amylase isogene encoded by BMY8 positively responds to low temperature and the cold-induced enhancement of BMY8 transcripts is correlated with maltose accumulation (Kaplan and Guy, 2004). Marvdasht cultivar with the most resistance to freezing stress at the beginning of growing season had the highest concentration of soluble sugars in its crown. Many reports emphasize critical roles of soluble sugars as compatible solute in freezing tolerance (Klotke et al., 2004; Iftime et al., 2010; Livingston et al., 2009). After the passage of winter and favorable conditions in early spring under freezing stress, the average LT50 of crown was equal to about -2.8°C. In this condition, Marvdasht and Pishtaz with LT50 of -4 and 1.14°C had the highest and lowest resistance to freezing stress, respectively. According to these results and conventional and sudden dropping of air temperature in early growing season after winter, planting of cultivars with high LT50 led to high risk for the highest yield potential. Among different wheat cultivars, Marvdasht had the highest resistant to freezing stress in the early growing season and its planting in these conditions will have less at risk. However, this cultivar has low yield potential in these conditions.

The correlation coefficients between grain yield in the control and postanthesis water stress conditions with crown viability under freezing stress (temperatures below zero) was negative. There were high correlation coefficients between grain yield in postanthesis water stress conditions with crown viability under freezing stress at -3 and -5°C, r = 0.86 and r = 0.64, respectively. Lower yielding cultivars, particularly at postanthesis water stress were more resistant to prevailing of freezing stress at early growing season after winter. Then, according to the most likely occurrence of below zero temperatures at the beginning of the growing season after winter in temperate zones, These findings suggest that wheat breeding programs should be in the direction that higher yielding cultivars in terms of postanthesis water stress also had more resistant to freezing stress at early growing season. Such wheat cultivars will be reduced risk to achieve higher grain yields in temperate zones that prevailing of postanthesis water stress and freezing stress at early growing season after winter is almost always.

REFERENCES

Aniol A (2002). Environmental stress in cereals: an overview. Proceeding of the 5th International Triticale Symposium, Jun 30-July 5, 2000, Radzikow, Poland. pp. 112-121.

AOAC (1995). Official method of analysis (16th ed.), Arlington, VA., USA: AOAC.

Araus JL, Slafer GA, Reynolds MP, Royo C (2002). Plant breeding and drought in C_3 cereals: what should we breed for? Ann. Bot. 89: 925-940.

Ashraf M, Taylor GA (1974). Morpho-development factors related to winter survival of wheat. Association of characteristics of dark green seedling and winter survival. Crop Sci. 14: 499-502

Bolouri-Moghaddam MR, Le Roy K, Xiang L, Rolland F, Van den Ende W (2010). Sugar signalling and antioxidant network connections in plant cells. FEBS J. 277: 2022-37.

Brule-Bable AL, Fowler DB (1989). Use of controlled environment for winter cereal cold hardiness evaluation. Can. J. Plant Sci. 69: 355-366.

Cacela C, Hincha DK (2006). Monosaccharide composition, chain length and linkage type influence the interactions of oligosaccharides with dry phosphatidylcholine membranes. Biochimica Et Biophysica Acta-Biomembranes, 1758: 680-691

Chen TH, Murata N (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr. Opin. Biotechnol. 5: 250-257.

Chen TH, Murata N (2008). Glycine betaine: an effective protectant against abiotic stress in plants. Trends Plant Sci. 13: 499-505.

Chu J, Yao X, Zhang Z (2010). Responses of wheat seedlings to exogenous selenium supply under cold stress. Biological Trace Element Res. 136: 355-363.

Clarke JM, De Pauw RM, Townley-Smith TM (1992). Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 32: 728-732.

Couee I, Sulmon C, Gouesbet G, El- Amrani A (2006). An involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. Exp. Bot. 57: 449-459.

Harvey RB (1998). Hardening process in plant and development from frost injury. J. Agric. Res. 15: 83-112.

Hossein ABS, Sears A, Cox TS, Paulsen GM (1990). Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. Crop Sci. 30: 622-627.

Iftime D, Hannah MA, Peterbauer T, Heyer AG (2010). Stachyose in the cytosol does not influence freezing tolerance of transgenic Arabidopsis expressing stachyose synthase from adzuki bean. Plant Sci. 180: 24-30.

Kaplan F, Guy CL (2004). b-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol. 135: 1674-1684.

Klotke J, Kopka J, Gatzke N, Heyer AG (2004). Impact of soluble sugar concentrations on the acquisition of freezing tolerance in accessions of *Arabidopsis thaliana* with contrasting cold adaptation ï evidence for a role of raffinose in cold acclimation. Plant Cell Environ. 27: 1395-1404.

Lang P, Zhang CK, Ebel RC, Dane F, Dozier WA (2005). Identification of cold acclimated genes in leaves of *Citrus unshiu* by mRNA differential display. Genetic, 359: 111-118.

Levitt J (1956). The hardiness of plants, part I. low temperature hardiness. Academic Press, Inc, New York.

Liu HS, Li FM (2005). Root respiration, photosynthesis and grain yield of two spring wheat in response to soil drying. Plant Growth Regul. 46: 233-240.

Livingston DP, Hincha DK, Heyer AG (2009). Fructan and its relationship to abiotic stress tolerance in plants. Cell. Mol. Life Sci. 66: 2007-2023

Ramel F, Sulmon C, Bogard M, Couee I, Gouesbet G (2009). Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in *Arabidopsis thaliana* plantlets. BMC Plant Biol. 9: 28-45.

Ristic LA, Ashworth EN (1993). Changes in leaf ultrastructure and carbohydrates in *Arabidopsis thaliana* L. (Heyn) cv. Columbia during rapid cold acclimation. Protoplasma, 172: 111-123.

Ritte G, Scharf A, Eckermann N, Haebel S, Steup M (2004). Phosphorylation of transitory starch is increased during degradation. Plant Physiol. 135: 2068-2077.

Roustaei M (2008). Study of cold tolerance and some agronomic traits in bread and durum wheat genotypes in dryland areas. Seed and Plant Improvement J. 25: 275-295.

Shalaev EY, Steponkus PL (2001). Phase behavior and glass transition of 1,2-dioleoylphosphaditylethanolamine (DOPE) dehydrated in the presence of sucrose. Biochem. Biophy. Acta-Biomembr 1514: 100-116.

Shao HB, Liang ZS, Shao MA (2006). Osmotic regulation of 10 wheat (*Triticum aestivum* L.) genotypes at soil water deficits. Colloids Surfaces B: Biointerfaces, 47: 132-139.

Sidlauskas G, Bernotas S (2003). Some factors affecting seed yield of spring oilseed rape (*Brassica napus* L.). Agron. Res. 1: 229-243.

Tsvetkov TD, Tsonev LI, Tsvetkova NM, Koynova RD, Tenchov BG (1989). Effect of trehalose on the phase properties of hydrated and lyophilized dipalmitoylphosphatidylcholine multilayers. Cryobiology, 26: 162-169.

Vitamvas P, Ilja GS, Prasil LT, Capkovac V, Opatrnac J, Ahme J (2007). WCS120 protein family and proteins soluble upon boiling in coldacclimated winter wheat. J. Plant Physiol. 164: 1197-1207.

Wanner LA, Junttila O (1999). Cold-induced freezing tolerance in Arabidopsis. Plant Physiol. 120: 391-400.

Yang J, Peng S, Zhang Z, Wang Z, Visperas RM, Zhu Q (2002). Grain and dry matter yields and partitioning of assimilate in japonica/indica hybrid rice. Crop Sci. 42: 766-772.

Yang J, Zhang J (2005). Grain filling of cereals under soil drying. New Phytol. 169: 223-236.

Yang J, Zhang J, Huang Z, Zhu Q, Wang L (2000). Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. Crop Sci. 40: 1645-1655.

Yano R, Nakamura M, Yoneyama T, Nishida I (2005). Starch-related a-glucan/water dikinase is involved in the cold-induced development of freezing tolerance in arabidopsis. Plant Physiol. 138: 837-846.

Yuanyuan M, Zhang Y, Lu J, Shao H (2010). Roles of plant soluble sugars and their responses to plant cold stress. Afr. J. Biotechnol. 8: 2004-2010.

Zhang J, Sui X, Li B, Su B, Li J, Zhou D (1998). An improved water-use efficiency for winter wheat grown under reduced irrigation. Field Crop Res. 59: 91-98.

Zhu L, Liang ZS, Xu X, Li SH, Monneveux P (2009). Evidences for the association between carbon isotope discrimination and grain yield-ash content and stem carbohydrate in spring wheat grown in Ningxia (northwest China). Plant Sci. 176: 758-767.