

Evaluation of Freezing Tolerance of Hexaploid Triticale Genotypes under Controlled Conditions

Ahmad NEZAMI¹⁾, Mohammad Reza SOLEIMANI¹⁾, Masoud ZIAEE²⁾

Masoud GHODSI³⁾, Mohammad BANNAYAN AVAL¹⁾

¹⁾Ferdowsi University of Mashhad, Faculty of Agriculture, Mashhad, Iran; bannayan@um.ac.ir (corresponding author)

²⁾University of Sistan and Baluchestan, Faculty of Agriculture and Natural Resource of Saravan, Department of Medicinal Plant, Saravan, Iran

³⁾Khorasan Agriculture Center of Cereals Division, Mashhad, Iran

Abstract

In order to evaluate freezing tolerance of different triticale (*X Triticosecale* Wittmack) genotypes, an experiment was carried out under controlled conditions in 2007 and 2008 at college of agriculture, Ferdowsi University of Mashhad. In this study seven triticale genotypes ('Juanilo-92', 'ET-82-15', 'ET-82-8', 'ET-83-20', 'ET-83-19', 'ET-83-18' and 'ET-79-17'), across six temperatures (0°C, -4°C, -8°C, -12°C, -16°C and -20°C) were evaluated within a factorial-completely randomized design with three replications. Plants were kept until 2 leaf stage in chamber with temperature of 20/15°C (day/night) and 12.5 h photoperiod. At the end of this stage, plants were under acclimation for three weeks. After exposing to acclimation freezing the cell membrane integrity was measured through electrolyte leakage (EL) and the lethal temperature (LT50) of samples was measured. After the exposure to freezing temperatures the samples were transferred to the greenhouse. Survival percentage, plant height, leaf area and number, chlorophyll content, and plant dry weight were determined after 3 weeks. Results showed that the effect of different freezing temperature and genotypes were significant on all plant characteristics. As temperature decreased, %EL of all genotypes was increased. Minimum and Maximum EL % in leaf and crown were observed at 0°C (21%) and -20°C (88.5%). 'ET-79-17' and 'Juanilo-92' genotypes showed the highest EL% (55.5% and 44.8%) and 'ET-83-20' the lowest EL% (47.3% and 41.2%) in leaf and crown. Dry weight and leaf area decreased by 48% and 42% respectively compared to non frozen control plants. 'ET-79-17' and 'ET-82-15' genotypes showed the highest dry weight (83.8 mg) and highest leaf area (14.3 cm²) respectively and 'ET-83-20' cultivar showed the lowest dry weight and leaf area (58.2 mg and 8.7 cm²).

Keywords: plant cold acclimation, electrolyte leakage, plant freezing recovery, LT50

Introduction

Triticale (*X Triticosecale* Wittmack) has been deliberately produced by crossing wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) plants. Triticale contains the privileges of both parents, high quality for making various food products along with robustness of adaptability to hard environment conditions and disease resistance (Ammar, 2004). The grains of triticale contain more protein, lysine and minerals compared to cereals such as wheat, rice and corn with equal amount of vitamins compared to wheat (Barnett *et al.*, 1971). Triticale is grown for grain and also forages for livestock in the temperate winter zone (Brown and Almodares, 1976). According to FAO statistics (FAO, 2008) 3.5 million ha triticale are grown in the world with 14 million tones production of more than 200 cultivars in almost 35 countries.

Average annual precipitation in Iran is about 250 mm, only about one third of the world rainfall. On the other hand limited precipitation is mainly confined to cold and winter month (Ghamarnia and Gowing, 2005). Therefore farmers in Iran desire more winter cropping for from

the benefit of winter rainfall, which increase with 15-25 percent the yield and avoid drought stress (Dashti *et al.*, 2001).

This crop is relative tolerant to the environmental stresses (Brown and Almodares, 1976). One of the most important environment stresses is the frost which limits the growth and the yield of winter cereals including triticale. Freezing causes serious damages to plants by injuring the plant cells and tissues. Hence, the cold tolerant cultivars play an important role in the success of the production of the winter cereals (Moshiri *et al.*, 2006).

Many studies have been conducted to find an effective and rapid method to evaluate the plants tolerance to freezing temperatures. One of these methods is measuring the cytoplasm membrane electrolyte leakage or the conductivity of acclimated organs which can be damaged due to the freezing stress (Mirzai-Asl *et al.*, 2002). Since the cytoplasm membrane is the first place that can be damaged, it is possible to determine the amount of injury through the electrolyte leakage of damaged tissues. It is expected that susceptible cells can be damaged more than tolerant ones and show higher electrolyte leakage (Beyrami Zade, 2006).

Electrolyte leakage (EL) method is easy, fast, and reliable and demand less expenses compared to the other methods, thus it is a suitable method to determine the plant tolerance to coldness (Lyons *et al.*, 1990). Hommo (1994), working on 13, 10, 3 and 6 cultivars of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), triticale (X *Triticosecale* Wittmack) and winter barley (*Hordeum vulgare* L.) respectively, found a significant correlation between the EL% of plant leaves and crop survival from freezing.

Similarly, Pulli (1994) studied 9, 10 and 12 cultivars of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.) red clover (*Trifolium pretense*) and found a significant correlation between crop survival and EL% of leaves though this relation was not significant for rye crops.

Another evaluation method for freezing tolerance is to measure the growth characteristics and the plant regrowth after the recovery period which is followed by the freezing test in controlled conditions. Fowler and Carlers (1979), on wheat (*Triticum aestivum* L.) study, found a significant correlation between the plants shoot dry weight during the recovery period and Lethal temperature 50 (LT50) in field condition. In addition, a positive significant correlation was found between the chlorophyll amount (SPAD), plant height, leaf area and dry weight with LT50 under the recovery period (Azizi *et al.*, 2007). Nezami *et al.*, (2007) observed that at -12°C the plant dry weight of tolerant genotypes was 40%-45% less than the control plant (0°C) dry weight. However the dry weight of sensitive genotypes was only 10% the control plant. The objective of this study was to evaluate various triticale genotypes exposed to the freezing stress under controlled conditions.

Materials and methods

Plant establishment

Seven triticale genotypes ('Juanilo-92', 'ET-82-15', 'ET-82-8', 'ET-83-20', 'ET-83-19', 'ET-83-18' and 'ET-79-17') across six different temperatures (0°C, -4°C, -8°C, -12°C, -16°C and -20°C) were employed in this research. The seeds were put to germinate in the Petri-dishes using the moist filter papers. After 3 days, germinated seeds were sown in plastic pots (9.5 cm diameter) that were primarily filled with an equivalent ratio of sand, compost and farm soil with 2-3 cm depth and 10 seeds of each genotype were planted. After that, pots were immediately transferred to the growth chambers. The growth chamber temperature and photoperiod for seedlings were set as 20/15°C (day/night) and 12.5 h light duration.

Acclimation conditions

At the plants' second leaf stage (when 50% of plants second true leaf appeared) freezing acclimation conditions were set for three weeks as described below.

The first week, 10/8°C (day/night) and 11.5 h light; the second week, 7/5°C (day/night) and 10.5 h light and the third week 5/2°C (day/night) and 10.5 h light. Af-

ter three weeks, the pots were transferred to the Thermo Gradient Freezer. The initial temperature of the freezing chamber was 5°C, however the temperature was decreased linearly at the rate of 2°C per hour. When the temperature cooled down to -3°C, the seedlings were sprayed with the Ice Nucleation Active Bacteria (INAB) to help the formation of the ice nucleic in the seedlings tissues (Lindow and *et al.*, 1982). Followed by the freezing treatment, the samples were transferred to the growth chamber at 5±1°C for 24 h to decrease the rate of thawing.

Electrolyte leakage evaluation

To employ the EL method, three plants from each pot were removed and their leaves and crowns were separated and placed in the vials containing the double distilled water. The samples were then placed on a shaker for 6h before measuring the primary EL. The EL measurement was made using an EC meter (Jenway-Model). To evaluate total electrolyte leakage of the dead cells, the samples were frozen at -70°C for 24 h in a cold chamber. Subsequently the samples were transferred to the laboratory and exposed to the natural temperature to defreeze. The second EL measurement was then made by placing the samples on the shaker for 6 h. The EL % was calculated as:

$$EL = (EL_1 / EL_2) \times 100 \quad (1)$$

EL₁ = Primary reading electrolyte leakage after treatment

EL₂ = Total reading electrolyte leakage after freezing to -70°C

Recovery evaluation

The pots containing the other remained seven plants were transferred to the greenhouse for 21 days and their re-growth was evaluated. The percentage of the survived seedlings was determined by counting the alive plants from each pot as:

$$PSI = (A/B) \times 100 \quad (2)$$

PSI = Survival Percentage Index

A = Number of alive plants in recovery period after 3 weeks

B = Number of plants before freezing treatment

After the recovery period, five plants from each pot were harvested to determine the plants height (from the soil surface to the last node), the number of leaves, leaf area, leaf chlorophyll content (using three young expanded leaves) and plants dry weight. Plant height was also measured again at heading stage.

Statistical analysis

The experiment was arranged in a factorial completely randomized design with three replications. LT50 was calculated from a graph of EL% verses temperature and data were analyzed by using the statistical software MSTAT-C followed by the calculation of LSD test at 5% probability level. The percentage data were transformed to arcsine prior to analysis.

Results and discussion

Electrolyte leakage method

Electrolyte leakage percentage

Significant effect of different freezing temperatures over EL% of both the leaves and crowns were found ($P \leq 0.01$) (Tab. 1). As temperature decreased, the EL% increased. The leaves EL% were 1.3, 1.9, 2.6, 3.5 and 4.2 times higher at -4°C , -8°C , -12°C , -16°C and -20°C compared to the control condition (Tab. 2). For leaves the minimum and the maximum EL% were obtained at 0°C (21%) and -20°C (88.5%), respectively (Tab. 2). Similar results were found for the EL% of the crowns. By decreasing the temperature EL% of the crowns increased. The EL% of crowns was 1.1, 1.3, 1.6, 1.9 and 2.2 times higher at -4°C , -8°C , -12°C , -16°C and -20°C compared to the control. The minimum and the maximum EL% level were observed at 0°C (29.2%) and -20°C (64.6%), respectively (Tab. 2). When the leaves of rose clover (*Trifolium hirtum* All.) genotypes were exposed to the different freezing temperatures, there was a significant difference between

the 'ET-83-20' about 47.3%. The same results were found by Eugenia *et al.*, (2003). They found a significant difference between the EL% of leaves within the rose clover genotypes. Since, the EL% ranged from 51.1% to 61.5%.

Lethal temperature (LT50)

The temperature in which the electrolyte leakage is 50% is known as lethal LT50 (Gusta *et al.*, 1982). In this experiment, there was a significant difference ($P \leq 0.01$) between triticale genotypes for the leaves and crowns LT50 (Tab. 4). Since, 'ET-83-18' and 'ET-83-19' having the leaf LT50 of -13.6°C and -11°C were noted as the most tolerant and the most sensitive genotypes, respectively (Tab. 3). However, when comparing the crowns LT50 within the genotypes, Juanilo-92 (-13.9°C) and 'ET-82-8' (-9.2°C) were the most tolerant and the most sensitive genotypes, respectively (Tab. 3). Anderson *et al.*, (1993) studying on Bermudagrass [*Cynodon dactylon* (L.) Pers. \times *C. transvaalensis* Burt-Davy] reported that there is a significant difference between the genotypes for LT50. The LT50 ranged from -7°C to -11°C between the genotypes. Similarly, Nezami *et al.* (2007) found a clear difference on

Tab. 1. Source of variation (SV), degree of freedom (df) and mean square (Ms) of measured plant parameters exposed to different freezing temperature and recovery in greenhouse of different triticale genotypes

SV	df	Ms							
		El leaf (%)	El crown (%)	Height (cm)	LN	LA (cm)	SPAD	DW (mg)	HH (cm)
Temperature	5	14625.11 ^{**}	3926.39 ^{**}	437.79 ^{**}	107.60 ^{**}	2039.48 ^{**}	7651.90 ^{**}	78240.36 ^{**}	8774.25 ^{**}
Genotype	6	157/11 [*]	43.52 ^{ns}	14.58 ^{**}	1.43 ^{**}	55.66 ^{**}	21.22 ^{**}	1528.85 ^{**}	2314.84 ^{**}
T \times G	30	41/44 ^{ns}	43.36 ^{ns}	1.67 ^{**}	0.20 ^{**}	9.42 ^{**}	3.86 ^{ns}	239.13 ^{ns}	268.82 ^{**}
Error	84	69.23	53.91	0.19	0.05	5.17	2.85	271.43	22.14

(EL%) Electrolyte leakage percentage; (LN) Leaf number; (LA) Leaf area; (SPAD) Chlorophyll content; (DW) Total dry weight; (HH) Height at heading stage

the EL% at different freezing temperatures (Eugenia *et al.*, 2003). Thus decreasing the temperature down to -18°C caused an increase in the EL% of leaves up to 5.7 times more than at -6°C . Similar result was reported by Murray *et al.* (1994). They found a significant correlation between the EL% leaves of winter oat (*Avena sativa* L. cv. 'Kanota') at different freezing temperature, since the EL% at -28°C was 80% higher than control condition (0°C).

Different genotypes showed significant different leaves EL% ($P \leq 0.05$) however it was not significant for the EL% of the crowns (Tab. 1). The 'ET-79-17' had the highest leaf EL% (55.5%) and the lowest EL% was obtained for

the leaf LT50 among different genotypes of colza (*Brassica napus* L.) under the freezing condition.

Plant re-growth

Survival percentage

There were no differences between the genotypes for percentage of survived seedlings. The survived seedlings percentage for all genotypes was 100% until the temperature decreased to -12°C . All seedlings were lost at -16°C and -20°C . Therefore we couldn't measure LT50 by survival percentage.

Plant height

As temperature decreased, the plants height also decreased (Tab. 2). The effect of different freezing temperatures on plant height was significant ($P \leq 0.01$) (Tab. 1). In living plants, the maximum and the minimum height were obtained at 0°C (9.8 cm) and -12°C (7.7 cm), respectively. The same result for wheat was observed by Azizi *et al.* (2007). They reported that the plant height decreased as temperature decreased and shoot height was 16% lower

Tab. 2. Source of variation (SV), degree of freedom (df) and mean square (Ms) of lethal temperature 50 (LT50) in leaf and crown of different triticale genotypes

SV	df	MS	
		LT50 leaf	LT50 crown
Genotype	6	1.712 ^{**}	31.829 ^{**}
Error	14	0.448	1.815

Tab. 3. Freezing temperature effects on various measured plant parameters

Treatment	EL leaf (%)	EL crown (%)	SPI	Height (cm)	LN	LA (cm ²)	SPAD	DW (mg)	HH (cm)
Freezing temperature (°C)									
0	21.0	29.2	100.0	9.8	4.8	22.9	39.8	145.0	46.9
-4	28.0	32.7	100.0	9.1	4.5	19.7	38.0	119.5	40.4
-8	40.1	38.5	100.0	8.4	4.3	16.3	35.5	97.7	35.5
-12	53.9	45.3	100.0	7.7	3.7	13.2	33.6	75.1	28.5
-16	73.9	55.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-20	88.5	64.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD (0.05)	5.1	4.5	- ^s	0.3	0.1	1.4	1.0	10.1	2.9

§ Data analyzed is not possible; (EL%) Electrolyte leakage percentage; (LN) Leaf number; (LA) Leaf area; (SPAD) Chlorophyll content; (DW) Total dry weight; (HH) Height at heading stage

at -12°C compared to the control condition. Similarly, the height of chickpea plants (*Cicer arietinum* L.) at -12°C was 45% lower than those under the control condition (Nezami, 2002). The role of water in the frost hardness of plant cells has been recognized primarily in terms of tissue water content and frost survival, so there are similar indications injury of freezing stress and drought stress (Cloutier and Siminovitch, 1982). Probably freezing stress decreased the plant height by reducing the cell division and size cells. These differences in plants height were also significant ($P \leq 0.01$) between the plant genotypes (Tab. 1). The tallest plants were obtained from 'ET-82-8' whereas the 'ET-83-20' produced the shortest plants (Tab. 3). Previously, Mirzai-Asl *et al.* (2002) by working on wheat observed a significant difference between the height of genotypes while the plant height ranged between 6.8 cm to 19.3 cm. In addition, the interaction between the temperature and the cultivar on plant height was also significant ($P \leq 0.01$) (Tab. 1). 'ET-82-8' was the highest at 0°C (11.9 cm) while 'ET-83-20' produced the shortest plants (5.3 cm) at -12°C (Tab. 5). At -12 °C the height of 'ET-79-17' and 'ET-82-15' were 33% and 17% lower than control treatments, respectively.

Number of leaves

In several crop species severe wilting of the leaves occurs within the first few hours of exposure to freezing tem-

perature. This quickly leads to the development of necrotic patches over the leaf surface and induces drying of the leaf edges (Ristic and Ashworth, 1997). The effect of different freezing temperatures on the number of leaves was significant ($P \leq 0.01$) (Tab. 1). The maximum and the minimum levels were obtained at 0°C (4.8 leaves) and -12°C (3.7 leaves), respectively. The number of leaves at -4°C, -8°C and -12°C was 6.2%, 10.4% and 22.9% lower in comparison with the control condition (Tab. 2). These differences were also significant even between the genotypes (Tab. 1). 'ET-82-8' showed the highest number of leaves (3.3) whereas the 'Juanilo- 92' produced the lowest number of leaves (2.5) at the end of the recovery period (Tab. 3). The interaction between the genotype and the temperature on the number of leaves was also significant ($P \leq 0.01$). The maximum level was observed for the 'ET-82-8' at 0°C (5.4 leaves) and the minimum level was obtained for the Juanilo-92 at -12°C (3.3 leaves) (Tab. 6). Reducing the temperature from 0°C to -12°C caused a 25% decrease of the number of leaves in ET-82-17, however, this reduction was 16% for the 'ET-82-15'.

Leaf area

One of the first symptoms of drought and cold is the reduction of cell expansion and therefore leaf growth. Temperature showed a significant effect ($P \leq 0.01$) on leaf area (Tab. 1). As the temperature decreased, the leaf area

Tab. 4. Plant parameters of different genotype exposed to freezing temperature

Genotype	EL leaf (%)	LT50 leaf	LT50 crown	Height (cm)	LN	LA (cm ²)	SPAD	DW (mg)	HH (cm)
'ET-83-19'	52.3	-11.0	-13.5	5.6	3.1	11.6	23.1	75.1	29.9
'ET-83-18'	47.9	-13.6	-12.2	6.0	3.1	12.1	24.6	75.9	24.9
'ET-82-8'	51.0	-12.5	-9.2	7.1	3.3	13.1	23.0	70.6	31.2
'ET-82-15'	53.0	-11.3	-12.4	5.9	2.7	14.3	25.2	82.2	31.6
Juanilo-92	49.3	-12.6	-13.9	6.1	2.5	11.4	24.8	64.6	28.9
'ET-79-17'	55.5	-12.0	-10.9	6.1	2.7	12.9	24.5	83.8	30.1
'ET-83-20'	47.3	-12.2	-13.6	4.1	2.8	8.7	26.1	58.2	0.0
LSD(0.05)	5.5	1.2	2.7	0.3	0.1	1.5	1.1	10.9	3.1

(EL%) Electrolyte leakage percentage; (LN) Leaf number; (LA) Leaf area; (SPAD) Chlorophyll content; (DW) Total dry weight; (HH) Height at heading stage

Tab. 5. The interactive effects of temperature and genotypes on various measured plant parameters exposed to freezing temperature

Genotype	Temperature	Height (cm)	LN	LA (cm ²)	HH	Genotype	Temperature	Height (cm)	LN	LA (cm ²)	HH
'ET-83-19'	0	9.5	5.1	21.0	53.	'ET-83-18'	0	9.9	5.3	22.7	50.7
	-4	8.8	4.9	19.3	49.3		-4	9.2	5.0	20.7	40.6
	-8	7.9	4.7	16.0	41.3		-8	8.6	4.9	17.0	35.3
	-12	7.4	3.9	13.1	35.2		-12	8.0	3.7	12.0	22.4
	-16	0.0	0.0	0.0	0.0		-16	0.0	0.0	0.0	0.0
	-20	0.0	0.0	0.0	0.0		-20	0.0	0.0	0.0	0.0
'ET-82-8'	0	11.9	5.4	24.7	52.8	'ET-82-15'	0	9.7	4.4	26.7	64.0
	-4	11.3	5.2	20.0	49.8		-4	8.8	4.3	24.7	47.3
	-8	9.9	4.9	18.0	45.7		-8	8.6	3.9	18.0	42.2
	-12	9.4	4.0	15.7	38.7		-12	8.0	3.7	16.7	35.8
	-16	0.0	0.0	0.0	0.0		-16	0.0	0.0	0.0	0.0
	-20	0.0	0.0	0.0	0.0		-20	0.0	0.0	0.0	0.0
'Juanilo-92'	0	10.0	4.2	23.3	52.5	'ET-79-17'	0	11.0	4.4	26.3	54.7
	-4	9.3	3.8	17.0	50.3		-4	9.6	4.3	21.7	45.3
	-8	9.1	3.7	16.3	41.2		-8	8.8	4.2	16.7	43.1
	-12	8.1	3.3	12.0	29.7		-12	7.4	3.3	12.7	37.7
	-16	0.0	0.0	0.0	0.0		-16	0.0	0.0	0.0	0.0
	-20	0.0	0.0	0.0	0.0		-20	0.0	0.0	0.0	0.0
'ET-83-20'	0	6.9	4.7	15.7	- ^s		0				
	-4	6.3	4.3	14.3			-4				
	-8	5.9	4.1	12.0			-8				
	-12	5.3	3.8	10.3			-12				
	-16	0.0	0.0	0.0			-16				
	-20	0.0	0.0	0.0			-20				
LSD (0.05)		0.7	0.4	3.7							

(LN) Leaf number; (LA) Leaf area; (HH) Height at heading stage

also decreased. The maximum and the minimum leaf area were found at 0°C (22.9 cm²) and -12°C (13.2 cm²), respectively (Tab. 2). The leaf area was 14%, 29% and 42% lower at -4°C, -8°C, and -12°C compared to the control condition (Tab. 2). Azizi *et al.* (2007) reported that leaf area of different wheat genotype was 28% lower at -12°C compared to the control treatment. 'ET-82-15' showed the highest leaf area at the end of the recovery period and the minimum level was found for the 'ET-83-20' (Tab. 3). Azizi (2006) also found similar effect for wheat genotypes. He found the maximum and the minimum levels for 'Bezostaya' (8.6 cm) and 'Maron' (3.2 cm) genotype, respectively. The interaction between the genotype and the temperature on the leaf area was also significant ($P \leq 0.01$) (Tab. 1). The 'ET-82-15' showed the maximum leaf area at 0°C (26.7 cm) and the minimum was obtained for 'ET-83-20' at -12°C (10.3 cm) (Tab. 5). When the temperature decreased from 0°C to -12°C the reduction in the leaf area of 'ET-83-20' was 34%. In this experiment the effect of freezing stress on the leaf area was more significant in relation to the number of leaves. The number of leaves decreased up to 23% by reducing the temperature

from 0°C to -12°C, whereas the plant leaf area was more affected and decreased up to 42%.

Chlorophyll content (SPAD)

Chlorophyll synthesis is one of the most temperature sensitive processes (Ilker *et al.*, 1979). Chlorophyll content can be used as an indicator to determine the plants tolerance to coldness. Freezing temperature showed significant effect on leaf chlorophyll content ($P \leq 0.01$) of all genotypes (Tab. 1). As the temperature decreased, the plant chlorophyll content was also decreased. The maximum and the minimum chlorophyll content were found at 0°C (39.8) and -12°C (33.6), respectively (Tab. 2). The chlorophyll content was 4.5%, 10.8% and 15.6% lower at -4°C, -8°C, and -12°C compared to the control condition (Tab. 2). 'ET-83-20' and 'ET-82-8' showed the highest and the lowest chlorophyll content, respectively. It seems that low temperature not only affected the plant leaf area but also caused a clear reduction in the leaf chlorophyll content. Low temperature influenced the chlorophyll synthesis by changing the chloroplast structures and disorder the thylakoid membranes (McWilliams *et al.*, 1979). Previously,

Tab. 6. Correlation coefficients between plant parameters exposed to different freezing temperature and recovery in greenhouse

Trait	1	2	3	4	5	6	7	8
1- EL leaf	1							
2-EL crown	0.85**	1						
3-Height	-0.84**	-0.78**	1					
4-LN	-0.89**	-0.80**	0.96**	1				
5-LA	-0.87**	-0.81**	0.95**	0.93**	1			
6-SPAD	-0.88**	-0.81**	0.95**	0.97**	0.92**	1		
7-DW	-0.88**	-0.80**	0.92**	0.92**	0.95**	0.91**	1	
8-HH	-0.70**	-0.65**	0.88**	0.79**	0.86**	0.76**	0.84**	1

(EL%) Electrolyte leakage percentage; (LN) Leaf number; (LA) Leaf area; (SPAD) Chlorophyll content; (DW) Total dry weight; (HH) Height at heading stage.

** significant effects at 99%

Azizi *et al.* (2006) working on wheat genotypes found that at -12°C leaf chlorophyll content was 79% lower compared to the control condition.

Dry weight

The maximum and the minimum dry weight were found at 0°C (145 mg) and -12°C (75.1 mg), respectively (Tab. 2). The plant dry weight was 17.6%, 32.6 % and 48.2% lower at -4°C, -8°C, and -12°C compared to the control condition. This could be explained as a negative effect of the freezing stress on plant regrowth in the recovery period. Similarly, Chen *et al.* (1983) found the freezing temperatures of -18°C and -20°C decreased the wheat (*Triticum aestivum* L. cv 'Norstar' and 'Cappelle') plants regrowth 80 % and 90%, respectively. Azizi *et al.* (2006) also found that wheat dry weight was 81% lower at -12°C compared to the control condition. In addition, the effect of different freezing temperatures on chickpea dry weight was significant (Nezami *et al.*, 2007) and decreasing temperature from -4°C to -8°C caused 42% reduction in plants dry weight.

These differences ($P \leq 0.01$) were significant between the genotypes in their dry weight (Tab. 1). The 'ET-83-20' showed the minimum level of dry weight (58 mg) while the maximum level was obtained for 'ET-79-17' (83 mg) (Tab. 3). In one experiment, Qian *et al.* (2001) observed that the shoot regrowth of buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) was extremely influenced by the freezing temperatures. They showed that for the sensitive genotypes, the decrease of temperature from -8°C to -12°C caused 60% reduction in plants dry weight.

Plant height at heading stage

The effect of different freezing temperatures on plant height at heading stage was significant (Tab. 1). The maximum and the minimum height were found at 0°C (46.9 cm) and -12°C (28.5 cm), respectively (Tab. 2). The plant height was 13.9%, 24.3 % and 39.2% lower at -4°C, -8°C, and -12°C compared to the control condition. These differences were also significant ($P \leq 0.01$) even between genotypes (Tab. 1). Since, 'ET-82-15' had the maximum height and the shortest plants were produced from 'ET-83-20' (Tab. 3). The interaction between the genotype

and temperature on height was also significant ($P \leq 0.01$) (Tab. 1). The 'ET-82-15' had a maximum height at 0°C (64 cm) and the minimum level was obtained from the 'ET-83-20' at -12°C (Tab. 5).

Conclusions

In this experiment, the leaves and crowns EL% were extremely affected by the different freezing temperatures. Decreasing temperature down to -4°C and -8°C caused a significant increase in the EL% of leaves and crowns, respectively. The EL% of leaves and crowns at -20°C was 3.2 and 1.2 times higher than control condition (0°C). However, there were significant differences between the genotypes in their dry weight, height, leaf area, chlorophyll content and the height at the heading stage. The maximum leaf area and height at the heading stage was obtained for 'ET-82-15', while 'ET-79-18' produced the tallest seedlings with the most number of leaves and 'ET-79-17' had a maximum dry weight. The minimum dry weight, height and leaf area was obtained for 'ET-83-20' and did not produce any spike at all.

In this trial there was a positive correlation ($r = 0.85^{**}$) between the EL% of leaves with the EL% of crowns. In addition, a correlation between the EL% with the other different measured parameters after the recovery period was negatively significant. The number of leaves showed highest negative correlation ($r = -0.89^{**}$) between the EL% of leaves and the chlorophyll content. However, the leaf area had a good correlation ($r = 0.81^{**}$) with the EL% of crowns. The leaves chlorophyll content and the plant height had highest correlation ($r = 0.97^{**}$), ($r = 0.96^{**}$) with the number of leaves. While, the dry weight and height at heading stage showed a good relation ($r = 0.88^{**}$) with the leaf area and the plant height, respectively (Tab. 6).

References

- Ammar, K., M. Mergoum and S. Rajaram (2004). The history and evolution of triticale, p.1-11. In: M. Mergoum, Gomez-H. Macpherson, (Eds.). *Triticale Improvement and Production*. FAO Plant Production and Protection Paper.

- Anderson, J. A., C. M. Taliaferro and D. L. Martin (1993). Evaluating freeze tolerance of bermudagrass in a controlled environment. *Hort Sci.* 28:955.
- Azizi, H., A. Nezami, M. Nassiri Mahallati and H. R. Khazaie (2007). Evaluation of cold tolerance in wheat (*Triticum aestivum*) cultivars under controlled conditions. *Iranian J. Field Crops Resour.* 5:109-121.
- Barnett, R. D., R. L. Stanley, W. H. Chapman and R. L. Stith (1971). *Triticale*. New feed and forage crop for florida. *Sunshine state Agric. Res.* 12-14.
- Brown, A. R. and A. Almodares (1976). Quantity and quality of triticale forage compared to other small grains. *Agron. J.* 68:264-266.
- Chen, T. H., L. V. Gusta and D. B. Fowler (1983). Freezing injury and root development in winter cereals. *Plant Physiol.* 73:773-777.
- Cloutier, Y. and D. Siminovitch (1982). Correlation between cold- and drought-induced frost hardiness in winter wheat and rye varieties. *Plant Physiol.* 69:256-258.
- Dashti, H., B. Yazdi-Samadi, M. Ghannadha, S. Abd-Mishani and A. Sarafi (2001). Identification of chromosomes contributing to cold resistance in winter wheat by using substitution lines. *Iranian, J. Agric. Sci.* 32, 4. 825-833.
- Eugenia, M., S. Nunes and G. Ray Smith (2003). Electrolyte leakage assay capable of quantifying freezing resistance in rose clover. *Crop Sci.* 43:1349-1357.
- Fowler, D. B. and R. J. Carlers (1979). Growth, development, and cold tolerance of fall-acclimated cereal grains. *Crop Sci.* 19:915-922.
- Ghamarnia, H. and J. W. Gowing (2005). Effect of Water Stress on Three Wheat Cultivars. *ICID 21st European Regional Conference 2005-15-19 May. Frankfurt (Oder) and Slubice, Germany and Poland.*
- Gusta, L. V., D. B. Fowler and N. J. Tyler (1982). In: Factors influencing hardening and survival in winter wheat. P. H., Li, and Sakai, A. (Eds.). *Plant Cold Hardiness and Freezing Stress. Mechanisms and Crop Implications.* Academic Press, London.
- Hommo, L. M. (1994). Hardening of some winter wheat (*Triticum aestivum* L.) rye (*Secale cereale* L.) and triticale (*X Triticosecale wittmack*) and winter barley (*Hordeum vulgare* L.) genotypes during autumn and the final winter survival in Finland. *Plant Breed.* 112:285-293.
- Ilker, R., R. W. Breidenbach and J. M. Lyons (1979). Sequence of ultra structural changes in tomato cotyledons during short periods of chilling, p. 97-114. In: Lyons, J. M., D. Graham and J. K. Raison (Eds.). *Low Temperature Stress in Crop Plants.* New York Academic Press.
- Lindow, S. E., D. C. Arny and C. D. Upper (1982). Bacterial ice nucleation: A factor in frost injury to plants. *Plant Physiol.* 70:1084-1089.
- Lyons, J. M. and J. K. Raison (1990). Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Plant Physiol.* 45:386-389.
- McWilliams, W., W. Manokaran and T. Kipnis (1979). In: *Adaptation to chilling stress in sorghum.* J. M. Lyons, D. Graham, and J. K. Raison (Eds.). *Low Temperature Stress in Crop Plants: The Role of Membrane.* New York: Academic Press.
- Mirzai-Asl, A., B. Yazdi-Samadi, A. Zali and Y. Sadeghian-Motahhar (2002). Measuring cold resistance in wheat by laboratory tests. *Iranian J. Sci and Technol. Agric. and Natur. Resour.* 6:177-186.
- Moshiri, F., A. Bagheri and A. Safarnejad (2006). The effect of cold acclimation on freezing tolerance of three chickpea (*Cicer arietinum* L.) cultivars. *Iranian J. Agri. Sci. Natur. Reasour.* 12:153-160.
- Murray, S., M. U. Webb and P. Steponkus (1994). A comparison of freezing injury in oat and rye: Two cereals at the extremes of freezing tolerance. *Plant Physiol.* 104:467-478.
- Murray, G. A., D. Eser, L. V. Gusta and G. Eteve. (1994). Winter hardiness in pea, lentil, faba bean and chickpea p. 831-843. In: R.J. Summerfield (Eds.), *World Crops: Cool Season Food Legumes.* Kluwer Academic Publishers, Dordrecht The Netherlands.
- Nezami, A. (2002). Evaluation of chilling stress in chickpea (*Cicer arietinum* L.). PhD Thesis, Ferdowsi University of Mashhad. (In Farsi).
- Nezami, A., A. Bagheri, H. Rahimian, M. Kafi and M. Nasiri Mahalati (2007). Evaluation of freezing tolerance of chickpea (*Cicer arietinum* L.) genotypes under controlled conditions. *Iranian J. Sci and Technol. Agric. and Natur. Resour.* 10:257-269.
- Nezami, A., A. Borzooei, M. Jahani, M. Azizi and A. Sharif (2007). Electrolyte leakage as an indicator of freezing injury in colza (*Brassica napus* L.). *Iranian J. Field Crops Resour.* 5:167-177.
- Pulli, S. (1994). Conductivity testing for screening the winter hardiness of cereals, grasses and legumes. *Nordisk Jordisk Jordbruksforskning* 76:58-59.
- Qian, Y. L., S. Z. Ball, A. J. Tan, S. J. Koski and S. J. Wilhelm (2001). Freezing tolerance of six genotypes of buffalograss. *Crop Sci.* 41:1174-1178.
- Ristic, Z. and E. N. Ashworth (1997). Mechanisms of freezing resistance of wood tissues, p.123-137: In: *Recent advancements.* A. S. Basra, R. K. Basra (Eds.). *Mechanisms of Environmental Stress Resistance in Plants.* Amsterdam B. V.