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Evaluation of morpho-physiological indices in autumn sugar beet (*Beta vulgaris* L.) cultivars under freezing stress at seedling stage

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ABSTRACT

The physiological and morphological responses of seven sugar beet cultivars including three domestic cultivars (Jolge, PPB and SBSI1) and four exotic cultivars (Giada, Monotunno, Palma and Suprema) to freezing stress were studied in controlled environment in Agricultural College of Ferdowsi University of Mashhad in Iran. The experiment was performed as factorial with randomized complete block design arrangement in three replications. Plants were exposed to different freezing temperatures (0, - 2, -4, -6, -8, -10, -12, -14, -16 and -18 ^{o}C) at seedling stage. Percentage of electrolyte leakage, minimal fluorescence level in light-adapted leaves (*Fs*), maximal fluorescence level in light-adapted leaves (*Fms*), variable fluorescence (ΔF), efficiency of PSII photochemistry ($\Delta F/F_{ms}$), net photosynthesis rate, leaf number and area, radicle length and diameter, and survival percentage were measured. Monotunno cultivar showed higher survival (88%) and electrolyte leakage (26%) compared with other cultivars. The minimum (-16.9 ^{o}C) and the maximum (-15.2 ^{o}C) of lethal temperature 50% level according to survival percentage, and also the maximum (0.7%) and the minimum (0.59%) average of efficiency of PSII photochemistry, were observed in cultivars Monotunno and SBSI1, respectively. A negative and significant correlation (r=-0.65, P<0.001) was found between electrolyte leakage and survival percentage, and also among recovered plants traits. Survival percentage showed significant and positive correlation with leaf number (r=0.88, p<0.001) and radicle length (r=0.87, p<0.001). A positive and significant correlation between survival percentage and $\Delta F/F_{ms}$ ratio and also, a negative and significant correlation between $\Delta F/F_{ms}$ with LT_{50su} (r=-0.85, p<0.001) and lethal temperature 50% according to electrolyte leakage (LT_{50e1}, r=-0.84, p<0.001), indicated that the cultivars which had a lower electrolyte leakage percentage and higher survival after recovering period, showed higher $\Delta F/F_{ms}$ ratio. In tolerant cultivars, with decrease of electrolyte leakage, the LT_{50su} and LT_{50e1} indices were decreased significantly and a significant and positive correlation was observed between these indices (r=0.75, p<0.05).

Keywords: autumn sugar beet, freezing stress, morpho-physiological traits, seedling

INTRODUCTION

Freezing tolerance is an important characteristic for overwintering plants to survive a harsh winter (Sasaki et al. 1998). Sugar beet planting in Iran has the minimum limitation of receiving sun radiation and is dependent on irrigation in arid and semi-arid regions. Due to excess of water usage in spring planting and water resource boundaries, the spring planting is risky. For this reason, autumn planting has been considered in some regions of Khorasan province. Proper usage of winter raining and avoidance of summer droughts, led to autumn planting of sugar beet in southern area of Spain, Italy and Greece (Rinaldi and Vonella 2006, Caliandro et al. 1996). Contrary to these regions, which have Mediterranean climate and semi-mild winter (Scott et al. 1973), Khorasan province mostly has a cold winter and therefore, plants may be exposed to cold stress.

Too many studies have been done to find rapid and effective methods for evaluation of the plant's cold hardiness (Fowler et al. 1981). Electrolyte leakage is one of these methods which is measured according to the damage of cell mem-

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brane, caused by freezing stress. The basis of this method is on the measurement of cellular solutions such as potassium, amino acids, carbohydrates, and in general increase of electrolyte leakage to out of the cell (Mirzaee et al. 1989). This method is fast, inexpensive, and gives a proper estimation of the degree of freezing tolerance and the level of cell membrane damage in a large volume of plant samples (Colombo and Raitanen 1993; Odlum and Blake 1996; Mirzaee et al. 2002; Nezami 2002; Cardona et al. 1997). Nezami et al. (2006) studied the electrolyte leakage as an index of freezing damage in rapeseed and reported the increase of electrolyte leakage degree as a result of temperature decrease in all cultivars. Temperature which induces electrolyte leakage 50% is suggested as electrolyte leakage lethal temperature (LT_{50e1}) (Shashikumar and Nus 1993; Gusta et al. 1982). Abiotic stresses influence directly or indirectly the photosynthetic characters of the leaves and also change their fluorescence characters (Gray et al. 2003). Freezing stress may strongly hamper the leaves metabolism and causes damages due to exacerbation of light irradiance on photosystem II, and the reduction in electron transport rate in photosynthesis system (Baker and Rosenquist 2004). Measurement of chlorophyll fluorescence parameters is an appropriate and non destructive method for determination of the differences existing among plant species (Neuner and Larcher 1990) in relation to environmental stress tolerance and as an important criteria for quantification of the cold hardiness in maize and rice cultivars and lines, and in tolerant sunflower lines to heat stress (Dobrowski et al. 2005). Efficiency of PSII photochemistry is the main parameter in stress evaluation and the rate range between 0.75 to 0.85%. However, the stress will change this range (Petite et al. 2005). Levitt (1980) suggested lethal temperature 50% of plants in controlled conditions as a proper method for cold hardiness measurement. In cereals also, the crown LT_{50} determination is used as a method for estimation of the plant survival after cold stress (Gusta and Chen 1987; Fowleret al. 1981). Huner et al. (1993) reported that the cold hardiness index in cereal has a linear and negative relationship with the increase of photosynthesis capacity. Hence, any factor which has a direct or indirect influence on plant photosynthesis, affects cold hardiness of plants.

The objectives of this study were (1) to evaluate the correlation between morphological and physiological traits of autumn sugar beet cultivars under freezing stress situation in seedling stage, (2) to determine the indices with higher role in cultivar variation and (3) to determine tolerant cultivars to freezing stress.

MATERIALS AND METHODS

The experiment was conducted at Agriculture College of Ferdowsi University in mid-October, 2009. The experiment was conducted in factorial design with randomized complete block design arrangement in three replications. Seven cultivars (Suprema, Jolge, Monotunno, Giada, PP8, SBSI1and Palma) used in the present study were exposed to ten freezing temperatures (0, -2, -4, -6, -8, -10, -12, -14, -16 and -18 ^{o}C). In mid November, the seeds were sown ar the depth of 1-2 cm of each pot (20 cm height and 12 cm diameter). Pots were filled with a same composition of sand, field soil, and peat moss. Seedlings were thinned to five plants after establishment. To apply cold acclimation, seedlings were kept at normal temperature until 6-8 leaf stage. Twenty four hours before freezing temperature application, the pots were watered and then transferred to a thermogradient freezer to apply freezing stress. The primary temperature of the freezer was set at 5 ^{o}C, and after transferring the samples, it was decreased 2 ^{o}C in every one hour. To induce ice nucleation activity, leaf surface of the seedlings were sprayed with a thin layer of ice nucleation active bacteria (INAB) solution at -3 ^{o}C. To balance the temperature of the experiment area, plants were kept in freezing treatment for one hour, then the samples were transferred to growth chamber with 4±2 ^{o}C temperature and kept for 24 hours to decrease melting rate. Then, plants were transferred to cold frame.

Electrolyte leakage percentage was evaluated by detaching young and completely developed leaves (5 leaves from each pot), placing in Mcartini glasses containing 75 mL of double distilled water and shaken at room temperature for 24 hours. Electrical conductivity (E_1) of each sample was measured using EC meter (Senway model). The total rate of electrolyte leakage after cell death was measured by placing the samples in an autoclave with 15 pound pressure per inches (PSI) equal to 1.03 bar, and approximate temperature of 121 ^{o}C for 20 minutes. Samples were placed on a shaker again for 24 hours and the electrical conductivity was recorded (E_2). Electrolyte leakage percentage was estimated using equation 1. After 21 days of transferring the pots to cold frame,

Table 1. Physiological traits of autumn sugar beet cultivars in different temperatures averaged over replication

Cultivar	Survival (%)	LT_{50el}	LT_{50su}	$RDMT_{50}$	Leakage (%)	Maximum leakage (%)	Leakage percentage in LT_{50el}	Leakage percentage in LT_{50su}	$\Delta F/F_{ms}$	Photosynthesis, 3 weeks after stress
Suprema	88.3 a	-11.8 c	-16.9 c	-16.8 ac	37.0 a	81.4 a	48.2 a	76.7 a	0.658 b	8.7 b
Jolge	85.3 ab	-11.9 c	-16.3 bc	-14.6 a	38.7 a	79.9 a	49.3 a	77 a	0.655 b	7.9 bc
PP8	80 b	-11.4 abc	-15.2 a	-14.8 a	38.5 a	76.2 a	46.2 a	72.7 a	0.618 c	7.1 c
SBSI1	80 b	-11.1 ab	-15.2 a	-15.4 ab	31.1 b	63.3 bc	39.9 b	58.5 bc	0.599 e	8.2 bc
Monotunno	88.7 a	-12.0 c	-16.9 c	-15.9 abc	26.0 c	54.2 d	32.7 b	53.8 c	0.700 a	10.8 a
Giada	85.3 ab	-10.9 ab	-16.0 abc	-16.3 bc	29.5 b	60.5 c	34.9 b	57.5 bc	0.606 d	10.3 a
Palma	81.1 b	-10.6 a	-15.4 ab	-14.6 a	31.6 b	66.8 b	36.5 b	62.5 b	0.616 c	7.6 c

Means with the same letter in each column are not significantly different.

survival percentage and seedlings recovery were estimated by counting alive seedlings through equation 2. In addition, growth characteristics such as leaf area, number and dry weight, and also root length and diameter were measured.

Lethal temperature 50% according to electrolyte leakage (LT_{50e1}) was measured on the basis of average leaf leakage percentage diagrams vs freezing temperature (Anderson et al. 1988; Ingram 1985) using equation 3:

(1) $(E_1/E_2) \times 100$ = electrolyte leakage percentage

(2) [(number of survived plants after freezing treatment)/(number of plants before freezing treatment) × 100]

(3) $$E1_p = E1_1+[(E1_m-E1_1)/(1+e^{-B(T-Tm)})]$$

where $E1_p$ is the estimated electrolyte leakage, $E1_1$ is the minimum limit of electrolyte leakage, $E1_m$ is the maximum electrolyte leakage, e is equal to 2.714, B is the rate of curve slope increase, T is absolute value of the thermal treatment, and T_m is the value of LT_{50e1} – turning point of the curve (Zhu and Liu 1987).

Lethal temperature 50 according to the plant survival percentage (LT_{50su}) and reduced dry matter temperature 50 percentage (RDMT50) were estimated according to survival percentage and aerial dry matter of each cultivar against freezing temperature, respectively.

A fluorescence meter (OS1-F1 chlorophyll Fluorometer) was used to measure the value of chlorophyll fluorescence by measuring the side edge of fully developed young leaves (with a distance from the midrib) in a period of 2 to 72 hours after freezing. Measured parameters were: minimal fluorescence level in light-adapted leaves (*Fs*), maximal fluorescence level in light-adapted leaves (*Fms*), variable fluorescence (ΔF), and efficiency of PSII photochemistry ($\Delta F/F_{ms}$). Plant net photosynthesis rate was measured with an IRGA (LCA4, ADC Company, Hoddeson, England). The rate of net photosynthesis was measured at PAR of 900 µmol m^{-2} s^{-1} for a completely developed young leaf.

Data were analysed using SAS, SPSS 15 and Sigma Plot 17 software. For drawing the diagrams, Excel 2010 and Curve Expert 1.3 were used.

Results and Discussion

Survival percentage was not affected until -14°C, and also after recovery period, however, as the temperature decreased further, survival percentage declined with a sharper gradient. Cultivars Monotunno and Suprema with average survival percentage of more than 88% had higher tolerance (Table 1). No cultivar could tolerate -18 °C. Cultivars Monotunno and Giada had higher number of leaf area and dry weight, and also higher radicle diameter after recovery compared with sensitive cultivars (Table 2). There was a significant and positive correlation between survival percentage and all morphological traits (p<0.001). Survival percentage showed high correlation with leaf number (r=0.88, p<0.0001), and radicle length (r=0.87, p<0.0001). Electrolyte leakage percentage showed significant difference among cultivars (P<0.01) (Table 1). The degree of electrolyte leakage was negligible until -5 °C and as the temperature decreased further, the cultivars which achieved the maximum degree of leakage through a mild gradient with lower total leakage, showed higher tolerance to freezing stress compared with other cultivars.

Cultivar Monotunno with average leakage of 26% and maximum leakage of 54%, and cultivar Jolge with average leakage of 39% and maximum leakage of 80% had the lowest and highest electrolyte leakage degree, respectively (Table 1). Through maintaining the plasma membrane integrity and decreasing electrolyte leakage percentage after freezing treatment, survival percentage in-

Table 2. Morphological traits of autumn sugar beet cultivars in different temperatures averaged over replication

Cultivar	Root length (mm)	Root diameter (mm)	Leaf dry weight (mg/plant)	Leaf area (cm^{-2})	Leaf number
Suprema	62.0 b	2.7 b	124.0 d	13.8 cd	5.3 d
Jolge	72.2 a	2.8 b	150.9 c	15.5 c	5.9 bc
PP8	50.2 c	2.4 c	111.7 d	12.2 d	4.7 e
SBSI1	51.7 c	2.0 d	87.4 e	9.5 e	4.0 f
Monotunno	47.3 c	3.0 b	284.2 a	32.6 a	6.1 b
Giada	58.4 b	4.1 a	201.1 b	20.4 b	7.7 a
Palma	59.4 b	2.7 b	108 de	12.2 d	5.8 a

Means with the same letter in each column are not significantly different.

creased in tolerant cultivars. The influence of cold stress on electrolyte leakage differs based on cultivar's freezing tolerance (Cadona et al. 1997). In study by Nezami et al. (2006) and Cadona et al. (1997), the gradient of the electrolyte leakage percentage curve versus freezing temperature was lower in tolerant cultivars rather than sensitive cultivars, and may be considered as a proper index for evaluation of cold tolerance.

There was a negative and significant correlation between electrolyte leakage percentage and survival percentage (r=-0.65, p<0.0001) (Table 3). A negative and significant correlation was also observed between leakage percentage and all morphological traits such as leaf area and dry weight, and also the length and diameter of root in recovery stage (Table 3). Similar correlation coefficient was obtained in Triticale (Nezami et al. 2010).

Minimum and maximum LT_{50su} with -16.9 and -15.2 ^oC were found in Monotunno and SBSI1 cultivars, respectively. A negative and strong correlation between LT_{50su} and survival percentage (r=-0.99, p<0.0001) illustrated that the cultivars with negative LT_{50su} had higher survival percentage after recovery period (Table 4). Although, Monotunno cultivar had a lower LT_{50su} among other culcultivars but showed more positive $RDMT_{50}$ ratio (-15.9^oC) than Suprema (-16.8^oC) and Giada (-16.3^oC) cultivars (Table 1). No significant correlation was found between $RDMT_{50}$ and survival percentage (r=-0.68) and also LT_{50su} (r=0.65). These results are in contrary to the findings previously reported in a study by Nezami (2002) on bean, and Nezami et al. (2010) on spring sugar beet which showed positive and significant correlation of $RDMT_{50}$ with survival percentage and LT_{50su}. It does not seem that this index could give an accurate estimation of cold damage in sugar beet cultivars.

Monotunno indicated a priority to other cultivars with lower LT_{50e1} (-12^oC) and the minimum electrolyte leakage percentage compared with other cultivars. In tolerant cultivars Monotunno and Suprema, LT_{50e1} and $RDMT_{50}$ indices were 1.4 and 2.2^oC lower than sensitive cultivars, respectively (Table 1). In study by Nezami (2002), LT_{50su} index in cold tolerant genotypes of pea was 2^oC lower than sensitive genotypes. Owing to lower value of LT_{50su} compared with LT_{50e1} in all cultivars, electrolyte leakage in LT_{50e1} is less than LT_{50su}. Based on this result, cultivars Monotunno and Giada with the minimum leakage percentage in both lethal temperature 50% can be selected as tolerant cultivars to freezing.

Table 3. Correlation coefficients among leaf number, area, and dry matter, root length and diameter, survival percentage and electrolyte leakage, chlorophyll fluorescence and photosynthesis in autumn sugar beet cultivars

	1	2	3	4	5	6	7	8	9	10	11	12
Leaf number	1											
Leaf area	0.7***	1										
Leaf weight	0.73***	0.98***	1									
Root diameter	0.93***	0.67***	0.74***	1								
Root length	0.8***	0.49***	0.51***	0.75***	1							
Survival percentage	0.88***	0.63***	0.64***	0.83***	0.87***	1						
Leakage percentage	-0.62***	-0.54***	-0.54***	-0.54***	-0.6***	-0.65***	1					
Fs -8	0.68***	0.4***	0.43***	0.65***	0.68***	0.74***	-0.61***	1				
FMS -9	0.73***	0.67***	0.68***	0.65***	0.73***	0.76***	-0.78***	0.82***	1			
ΔF -10	0.72***	0.69***	0.69***	0.64***	0.71***	0.83***	-0.78***	0.78***	0.99***	1		
ΔF/Fms -11	0.84***	0.69***	0.69***	0.78***	0.85***	0.97***	-0.70***	0.76***	0.92***	0.9***	1	
Photosynthesis, 1 week after stress	0.61***	0.69***	0.69***	0.54***	0.55***	0.58***	-0.87***	0.54***	0.79***	0.8***	0.67***	1
Photosynthesis, 3 weeks after stress	0.65***	0.65***	0.66***	0.59***	0.62***	0.67***	-0.94***	0.66***	0.83***	0.84***	0.73***	0.95***

*** Significant at p < 0.0001

Table 4. Correlation coefficients between LT_{50e1}, LT_{50su}, $RDMT_{50}$, leakage percentage in LT_{50e1} and LT_{50su} in autumn sugar beet cultivars

Traits	LT_{50el}	LT_{50su}	$RDMT_{50}$	Leakage percentage in LT_{50el}	Leakage percentage in LT_{50su}
LT_{50su}	0.748	0.648 ^{ns}			
$RDMT_{50}$	0.257 ^{ns}	0.114 ^{ns}			
Leakage percentage average	-0.253 ^{ns}	0.003 ^{ns}	0.255 ^{ns}		
Maximum leakage percentage	-0.273 ^{ns}	-0.075 ^{ns}	0.173 ^{ns}		
Leakage percentage in LT_{50el}	-0.438 ^{ns}	-0.077 ^{ns}	0.156 ^{ns}		
Leakage percentage in LT_{50el}	-0.374 ^{ns}	-0.849 **	0.172 ^{ns}	0.989 ***	
$\Delta F/F_{ms}$	-0.842 **	-0.99 ***	-0.267 ^{ns}	0.074 ^{ns}	0.068 ^{ns}
Survival percentage	-0.699 ^{ns}	-0.706 *	-0.679 ^{ns}	0.025 ^{ns}	0.0298 ^{ns}
Photosynthesis, one week after stress	-0.321 ^{ns}	-0.677 ^{ns}	-0.570 ^{ns}	-0.608 ^{ns}	-0.611 ^{ns}
Photosynthesis, three weeks after stress	-0.291 ^{ns}	-0.687 ^{ns}	-0.701 ^{ns}	-0.589 ^{ns}	-0.613 ^{ns}
Leaf area	-0.470 ^{ns}	-0.681 ^{ns}	-0.381 ^{ns}	-0.487 ^{ns}	-0.501 ^{ns}
Leaf dry weight	-0.446 ^{ns}	-0.283 ^{ns}	-0.391 ^{ns}	-0.473 ^{ns}	-0.489 ^{ns}
Root diameter	0.146 ^{ns}	-0.208 ^{ns}	-0.364 ^{ns}	-0.206 ^{ns}	-0.163 ^{ns}
Root length	-0.155 ^{ns}	0.648 ^{ns}	0.158 ^{ns}	0.586 ^{ns}	0.628 ^{ns}

ns=not significant, *p<0.05, **P<0.001, ***p<0.0001

Although electrolyte leakage percentage showed a non significant correlation with LT_{50e1} (r=-0.25) and LT_{50su} (r=0.12) in all cultivars (Table 1), Table 5 and Figure 2 show that this trend was different in both sensitive and tolerant cultivars. Cultivars were placed into two separate groups based on correlation between lethal temperature 50%according to leakage and survival with electrolyte leakage and survival percentage indices. In Monotunno, Giada and Palma cultivars (Group A) correlations were positive and in some cases significant but in group B, it was negative and non significant, and the resultant of these two groups dsiplayed an unstable and non significant trend (Table 4). Gusta et al. (2001) reported negative and significant correlation between survival LT_{50} and field survival index (FSI) (r=-0.509, p<0.05), and also between FSI and crown water contain (r=-0.649, p<0.05) indices of different tolerant genotypes of wheat, classified in semi-tolerant (A) and tolerant (B) groups. However, these correlations were not significant in semi-tolerant genotypes.

In tolerant cultivars, with electrolyte leakage reduction, LT_{50e1} and LT_{50su} were decreased significantly and a positive and significant correlation was observed between LT_{50e1} with LT_{50su} (Table 5, Figure 2). Positive and significant correlation between discussed indices were also observed in rapeseed (Nezami et al. 2002), wheat (Mirzaee et al. 2002), sugar beet (Nezami et al. 2010) and safflower (Nezami and Naghedinia 2010). Despite this, Cardona et al. (1997) reported that the paspalum cultivars which had more negative LT_{50su}, had also higher leakage percentage in both cold acclimation and de-acclimation. Table 5 shows similar correlations between morphological traits with LT_{50e1} and LT_{50su} indices in both groups of A and B. The efficiency of PSII photochemistry was found to be higher in Monotunno with average of 0.7 and the lower value was found in SBSI1 with average of 0.59 among sugar beet cultivars affected by different freezing temperatures (Table 1). The index changes in various freezing temperatures showed that similar to survival percentage index, no significant differences were observed among sugar beet cultivars until -14^{o}C and with gradual decrease of temperature to below -16^{o}C, the difference was significant and at -18^{o}C reached to ziro (data not reported).

Table 5. Correlations among autumn sugar beet cultivars based on LT_{50e1} and LT_{50su}, Group A: Monotunno, Giada and Palma, group B: remained cultivars

	LT_{50el}		LT_{50su}	
	A	B	A	B
Leakage percentage average	+ ^{ns}	– ^{ns}	+ *	– ^{ns}
Maximum leakage percentage	+ ^{ns}	– ^{ns}	+ *	– ^{ns}
Leakage percentage in LT_{50el}	+ ^{ns}	– ^{ns}	+ ***	– ^{ns}
Leaf area	– ^{ns}	– **	– *	– ^{ns}
Leaf dry weight	– ^{ns}	– *	– ^{ns}	– ^{ns}
Root diameter	+ ^{ns}	– *	+ ^{ns}	– ^{ns}
Root length	+ ^{ns}	– ^{ns}	+ ^{ns}	– ^{ns}

ns=not significant, *: significant at p<0.05, **: significant at P<0.001, ***: significant at p<0.0001

The average value of the efficiency of PSII photochemistry in recovery period showed that applying freezing stress had a different influence on this trait. In the first 24 hours after applying temperature treatments, the trend was decreasing, but 72 hours after freezing stress and together with plant recovery, the values increased and reached approximately the level before stress (Figure 1). The effect of freezing stress on barley showed that this plant has an ability to recover the efficiency of PSII photochemistry after 72 hours of experiment but

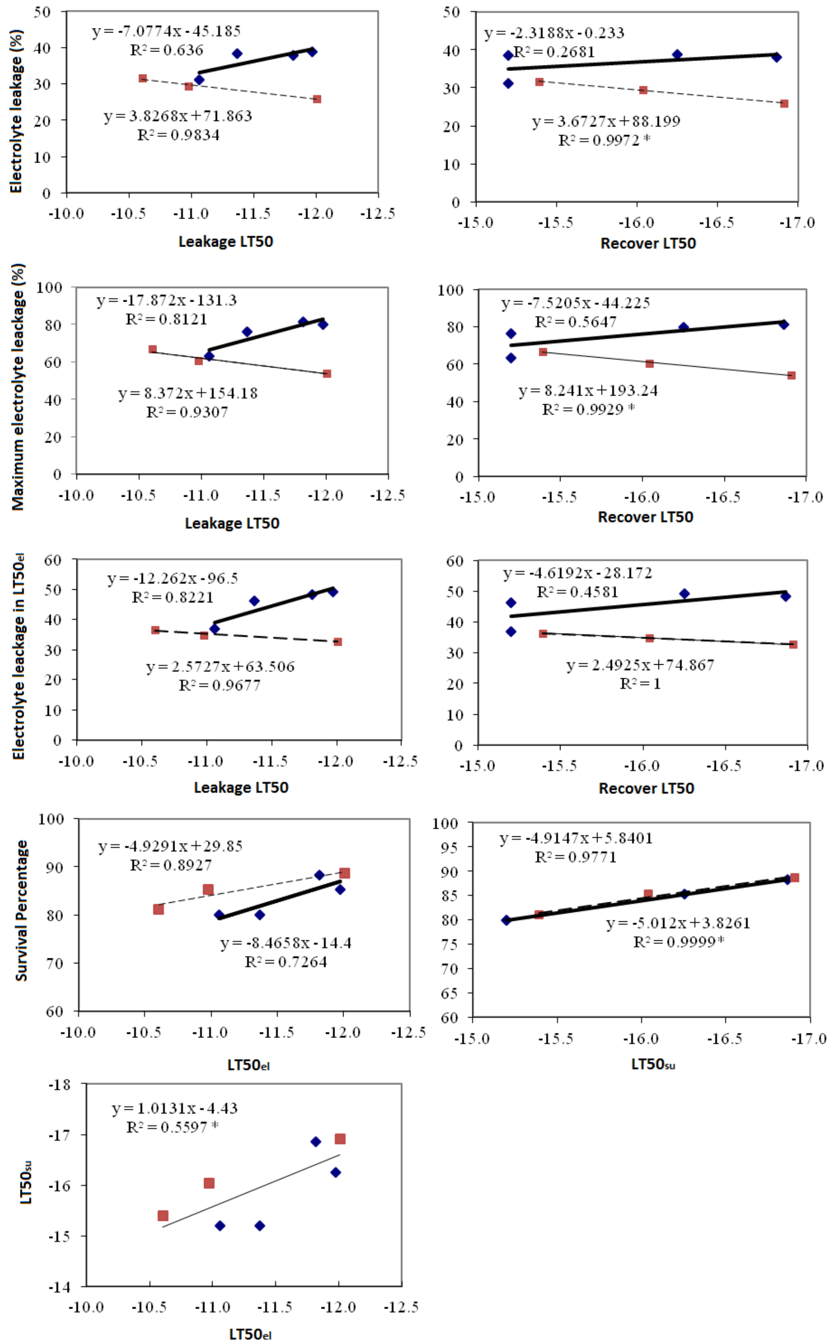

Fig. 2. Coefficient of determination between LT_{50e1} and LT_{50su} with survival and leakage indices in tolerant and sensitive cultivars o autumn sugar beet

the data were not same as reported before stress, and the reason of this inability was the reversible inducted electron capacity and damages to photosynthetic reaction centres (Dai et al. 2007).

The results also indicated a positive and significant correlation between chlorophyll fluorescence and specially the efficiency of PSII photochemistry with survival percentage (r=0.97, p<0.0001) and also all morphological traits (table 3). So, it seems that cultivars with higher survival percentage after recovery period have a higher efficiency of PSII photochemistry compared with the influenced plants.

Jalilian et al. (1999) also reported the reduction of maximum chlorophyll fluorescence (Fm) and efficiency of PSII photochemistry in spring sugar beet cultivars as a reason of freezing damage increase in plants. Dai et al. (2007) suggested electron transport rate of photosystem II as a proper index for identification and evaluation of cold tol-

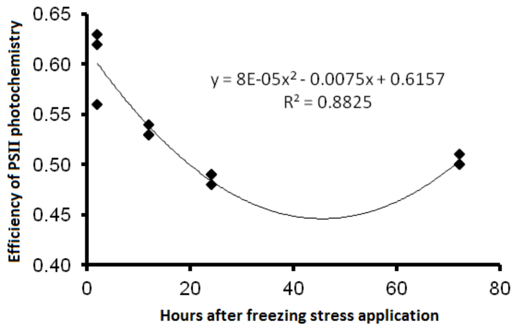

Fig. 1. Variation of the efficiency of PSII photochemistry in autumn sugar beet cultivars during different times of freezing stress application in controlled conditions (each point is the average of 70 measurements)

erance in barley, during recovery from freezing shock. Table 3 also shows a negative and strong correlation between $\Delta F/F_{ms}$ and the average of leakage percentage (r=-0.7, p<0.0001). This index was higher in cultivars having minimum degree of electrolyte leakage at freezing temperatures than sensitive cultivars. In tolerant cultivar Monotunno, this ratio was equal for both recovery period and before stress. Difference in cultivars genetic composition for freezing tolerance potential is also effective to this situation. Since the origin of tolerant cultivars Monotunno and Giada is cold regions of Switzerland and Germany, they will have higher cold tolerance compared with the other cultivars with tropical and warm regions origin. The results of the efficiency of PSII photochemistry reduction owing to freezing damage on soybean (Strauss et al. 2006) and wheat (Majdi et al. 2007) showed that the reduction was significantly lower in tolerant cultivars compared with the sensitive ones.

A negative and strong correlation between $\Delta F/F_{ms}$ with LT_{50e1} (r=-0.84, p<0.0001) and LT_{50su} (r=-0.85, p<0.0001) indicated that cultivars with lower lethal temperature 50% had higher quantum yield compared with the sensitive cultivars. Hasselt (1996) also showed that the efficiency of PSII photochemistry after freezing stress is a fast and suitable method for estimation of wheat leaves tolerance to freezing and have a high correlation with current methods such as leakage LT_{50}, recovery of triphenyltetrazolium chloride and visual assessments. Freezing stress influenced significantly the plant net photosynthesis. Cultivars Monotunno and Giada with average of 10.8 and 10.3 µmol CO_2 m^{-2} S^{-1}, had higher photosynthesis rate after three weeks of recovery, respectively. All studied cultivars could recover their photosynthesis systems after 72 hours in the range of 0 to -14°C but temperatures of -16 and -18°C led to irreversible damages to plant photosynthesis systems.

A positive and strong correlation between photosynthesis rate and morphological traits was observed after freezing. However, a negative and highly significant correlation between electrolyte leakage percentage with photosynthesis rate was seen in first (r=-0.87, p<0.0001) and third (r=-0.94, p<0.0001) weeks after freezing (Table 3). Results also indicated negative correlation between photosynthesis rate and LT_{50e1}, LT_{50su} and $RDMT_{50}$ indices after freezing and the only significant correlation was with LT_{50su} (r=-0.71, p<0.05) (Table 4).

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