Biological Forum – An International Journal **8**(2): 112-119(2016)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Salt Stress on NADP-Malic Enzyme Activity, Proline and Ionic Contents of Durum Wheat Genotypes

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(Received 08 June, 2016, Accepted 27 July, 2016)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Salt stress is a major limiting factor for cereal production in semi-arid regions such as Middle East. Development of salt tolerant durum wheat cultivars is of great importance in salt affected regions. This study was carried out to test the effect of salt stress on NADP-Malic enzyme (NADP-ME) activity, proline, sodium and potassium contents as well as grain yield of durum wheat genotypes and to identify biochemical indicators for salt tolerance in durum wheat. The eleven wheat genotypes including three commercial durum wheat cultivars, seven advanced lines of durum wheat and one bread wheat cultivar were exposed to normal and NaCl salinity stress conditions. Significant differences were observed among the genotypes for all the biochemical and agronomic traits except NADP-ME. In addition, salt stress had a significant effect on the traits except spike length and protein content. The results led to identification of some durum wheat genotypes which showed more salt tolerance than the commercial cultivars examined in this study. Therefore, they can be utilized for salt tolerance improvement programs in durum wheat. The increased activity of NADP-ME and proline accumulation occurred under salt stress, although no relationships were found between these biochemical characteristics and salt tolerance in wheat genotypes. The results indicated that the ionic content was associated with salt tolerance and also K/Na ratio had a significant correlation with grain yield. Thus K/Na ratio can be considered as a reliable indicator for screening salt tolerant durum wheat genotypes.

Keywords: Salt tolerance; NADP-ME; Sodium; Potassium; K/Na ratio; Wheat

INTRODUCTION

Salt stress is a major constraint for cereal production mainly in arid and semi-arid regions (Capriotti *et al*., 2014). Methods of cultivation, excessive use of fertilizers, irrigation with saline water and deforestation have enhanced concentration of salts in the root zone and made salinity a more widespread problem (Munns & Tester, 2008). FAO estimated that about 25.5 and 5.8 million hectares worldwide are saline and extremely saline, respectively (FAOSTAT: http://faostat3.fao.org/download/Q/*/E). The growth and production of crops are adversely affected by salinity due to low water absorption, ion toxicity and photosynthetic inefficiency (Gaber, 2010).

Durum wheat (*Triticum turgidum* ssp. durum) is an economically important cereal because of its quality factors for pasta industry. It is commonly grown in semi-arid regions such as Middle East where soil salinization is becoming a more challenging problem. Salinity threshold level of durum wheat is 5.7 dSm^{-1} (Ayers & Westcot, 1985) and this tetraploid species shows lower salt tolerance compared with bread wheat partly because of its poor ability to exclude sodium from the cells (Gorham, 1990; Munns & James, 2003). Therefore, development of salt tolerant durum wheat cultivars is of primary importance in salt affected regions. Osmotic effects of salinity cause rapid growth inhibition and reduction of grain yield in durum wheat (Davenport *et al*., 2005), however considerable variation exists among wheat lines and cultivars for salt tolerance.

Since selection of salt-tolerant genotypes merely based on grain yield is not effective, better understanding of physiological and biochemical mechanisms in response to salt stress may eventually provide more reliable indicators for improvement of salt tolerance in crops. Under salt stress conditions, monitoring ionic status in plant shoots is an efficient tool to identify salt tolerant genotypes. Salinity causes high concentration of Na+ which imbalances the uptake of other nutrients particularly K+ in plants (Munns & Tester, 2008).

K/Na ratio is presumed to be a salt tolerance indicator in some glycophyte plants (Chhipa & Lal, 1995; Munns *et al*., 2006). The optimal K/Na ratio can be maintained in the plant cells by sodium efflux from the cells or by the ability of plants to retain potassium in the leaf mesophyll (Wu *et al*., 2014). Salt tolerance in wheat species is associated with sodium exclusion (Gorham, 1990; Shah *et al*., 1987; Husain *et al*., 2003). Salt tolerant cultivars of bread wheat translocate low rates of Na+ from roots to shoots and maintain a high K/Na ratio in leaves (Gorham, 1990; Shah *et al*., 1987). A salt tolerant line of durum wheat was also identified to have low levels of Na+ concentration in the leaves, as low as bread wheat (Munns *et al*., 2000). This genotype maintained a high rate of K+ accumulation and as a result high K/Na ratio (Munns *et al*., 2000).

In addition to ionic changes, salt stress causes accumulation of proline as an osmoregulatory agent (Huang *et al*., 2009; Maggio *et al*., 2000). Proline can scavenge free radical molecules in order to prevent oxidative damage caused by reactive oxigen species (Rejeb *et al*., 2014). Several studies evaluated whether proline accumulation is simply a common adaptive response to stress or can be taken into account as an indicator associated with the level of salt tolerance in a given genotype. While some reports indicated that salt tolerant wheat genotypes showed higher proline accumulation (Gupta & Srivastava, 1990; Bajji *et al*., 2001; Goudarzi & Pakniyat, 2009), a number of studies failed to find a correlation between proline content and salt tolerance (Lacerda *et al*., 2005; Poustini *et al*., 2007; Shahbaz *et al*., 2013).

CO_2 fixation mechanisms also are essential in plant survival and tolerance to environmental stresses. Some of the enzymes such as NADP malic enzyme (NADP-ME) involved in photosynthesis are effective in oxidative stress tolerance (Shao *et al*., 2011). NADP-ME catalyzes the oxidative decarboxylation of L-malate and NADP+ to produce pyruvate, CO_2 and NADPH (Maurino *et al*., 2001).

Cytosolic and plastidic isoforms of NADP-ME play important roles in many biological processes such as flavonoids production, lignin and lipid biosynthesis, Malate exchanges in guard cells and response to environmental stresses (Shao *et al*., 2011; Casati *et al*., 1999; Drincovich *et al*., 2001). Evaluation of the trend of NADP-ME activity in different abiotic stresses may help to improve plant abiotic tolerance. The increased activity of NADP-ME was reported in wheat under oxidative stress (Yang *et al*., 2006). Although the current information is not enough to discern the relationship between NADP-ME and salinity, but the evidence show the protective role of NADP-ME and other members of NADP enzyme family in salt stress (Leterrier *et al*., 2012; Manai *et al*., 2014). Salt stress can affect NADP-ME gene expression and consequently regulate its activity (Fu *et al*., 2009). Overexpression of NADP-ME gene conferred osmotic and salt tolerance to transgenic Arabidopsis plants (Cheng & Long, 2007; Liu *et al*., 2007). The present study was performed to evaluate the effect of salt stress on the ionic and proline contents as well as NADP-ME activity of durum wheat genotypes, and to eventually identify biochemical indicators for salt tolerance in durum wheat.

MATERIALS AND METHODS

A factorial experiment based on completely randomized design with three replicates was conducted at the greenhouse of Department of Crop Production and Plant Breeding, Shiraz University. Eleven wheat genotypes including three commercial durum wheat cultivars (Seymareh, Aria and Yavarus), seven advanced lines of durum wheat with known pedigree information (Table 1) and one bread wheat cultivar (Chamran) were exposed to NaCl salinity levels with electrical conductivities (EC) of 2.2 dSm^{-1} (Non stress) and 13.5 dSm^{-1}. Salt stress was imposed at the beginning of stem elongation stage.

Table 1: The list of durum wheat lines used in the experiment and their pedigrees.

Name	Pedigree
ADYTW1/4	STOT//ALTAR34/AD/3/GREEN_18/FO
ADYTW1/5	CAMAYO/LLARET2/INIA//CADO/BO
ADYTW1/7	AINTZEN_1/6/CMB82A1062/3/GGOVZ394/
ADYTW1/14	ALTAR84/STINIT//SLVER_45/3/POHO
ADYTW1/18	LLARETA INIA/YEBAS_8/3/MINIMUS
ADYTW1/19	SRN_3/AJAIA_15//DUKEM_1/3/DION_2/4
D-81-18	ZONGZO_2/GREEN_3

The seeds were obtained from Agricultural and Natural Resources Research Center of Fars Province, Iran. The names of the advanced lines beginning with ADYWT were abbreviated as AD4, AD5, AD7, AD14, AD18 and AD19. Five plants were grown in each pot filled with 5 Kg sterilized silty loam soil under greenhouse conditions with 14 hours daylight and 25°C/15°C day/night temperature. In order to meet vernalization requirement, the pots were exposed to low temperature for four weeks after sowing. Soil EC was regularly measured and the levels of soil salinity were maintained at the mentioned ECs during the experiment. The pots were irrigated with tap water (EC: 0.32 dSm^{-1}) to keep soil moisture around field capacity during the experiment.

Leaf samples were collected at spike emergence stage to measure biochemical and ionic parameters. Total protein content was assayed according to the method of Bradford (Bradford, 1976). NADP-ME activity was measured spectrophotometrically as described by Casati *et al*. 1997. The proline content was determined using the method of Bates *et al.,* 1973. Sodium and potassium contents were measured by standard flame photometry (Flame Photometer Jenway, model PFP7) method (Bernstein, 1952).

The plants were harvested at maturity stage, and grain yield per plant, plant height and some yield-related traits including spike length, number of grain per spike, 100-grain weight, and number of spikelets per spike were determined. In order to assess the relative tolerance of the genotypes to salt stress, stress tolerance index (STI) (Fernandez, 1992) was calculated for each of the genotypes according to following formula in which Yp and Ys denote yield of a given genotype under non-stress and salt stress conditions, respectively and $\bar{Y}_p$ is mean yield of all the genotypes under non-stress conditions.

$$STI= (Yp \times Ys)/ \bar{Y}^2_p$$

Analysis of variance (ANOVA) was performed to test the effects of genotype and salt treatments and their interaction. The data were then subjected to means comparisons by Least Significant Difference (LSD) test. Correlation coefficients were calculated to find out the relationships among different characteristics. All the analyses were carried out by SAS9.1.3 software package.

RESULTS AND DISCUSSION

The results of analysis of variance revealed that salt stress had a significant effect on all the traits except spike length and total protein content (Table 2). Significant differences were also observed among the genotypes for all the traits except to NADP-ME activity. The existence of considerable variation provides opportunities for further genetic improvement of durum wheat using the advanced lines. Salt stress caused a significant reduction (over 40%) in grain yield (Table 3). The grain yield reduction of the wheat genotypes under salt stress is consistent with the previous studies (Poustini & Siosemardeh, 2004; Poustini *et al*., 2007; Royo & Abio, 2003; Sharbatkhari *et al*., 2013). Decrease in transpiration and photosynthesis rate limit grain yield under salt stress conditions (Radi *et al*., 2013). The genotypes AD18, Yavarus and AD4 not only showed the maximum grain yield per plant in salt stress conditions but also had the lowest reduction in this trait due to salt stress (Table 4). Stress Tolerance Index (STI) varied from 0.210 to 1.754 implying remarkable variation among the genotypes for salt tolerance (Table 4).

Table 2: Analysis of variance for the traits measured in the eleven wheat genotypes under non-stress and salt stress conditions.

Source	df	100-grain weight	Grain yield per plant	Plant height	Spike height	Number of spikelets per spike	Number of grain per spike
Genotype (G)	10	1.14**	0.462**	67.2520**	1.0774**	3.958**	254.437**
Salinity (S)	1	16.8**	3.554**	8885.3398**	0.164^{ns}	19.457**	723.334**
G×S	10	0.904**	0.168^{ns}	26.1022^{ns}	0.2854^{ns}	5.07913**	104.539**
Error	44	0.314	0.0903	16.722	0.123	0.772	37.924

Table 2 Continued.

Source	Protein	NADP-ME	Proline	Na	K	K/Na ratio
Genotype (G)	0.0482**	0.0124	2832.835**	2018.037**	111192.62**	19.864**
Salinity (S)	0.0082^{ns}	0.0736*	106999.647**	500199.749**	6586413.912**	4597.541**
G×S	0.0145^{ns}	0.0124^{ns}	2003.607**	2537.90**	65561.76**	11.992**
Error	0.0133	0.132	294.631	326.573	7306.463	2.3218

Table 3: Mean comparison of the traits measured in non-stress and salt-stress conditions.

	NADP-ME (IU mg^{-1})	Protein (mg ml^{-1})	Proline (µmol g^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})	K/Na	Plant height (cm)	Spike length (cm)	Number of spikelets per spike	Number of grains per spike	100-grain weight (g)	Grain yield per plant (g)
Non stress	0.293	0.455	14.92	124.37	3063.15	24.99	63.19	6.23	15.97	23.36	5.0	1.15
Salt stress	0.360	0.432	95.45	298.48	2431.34	8.306	55.86	6.13	14.89	16.74	4.0	0.69
LSD(0.05)	0.057	0.057	8.52	8.966	42.41	0.76	2.029	0.17	0.44	3.05	0.3	0.15
Change (%)	22.78	-4.9	539.6	139.9	-20.6	-66.77	-11.6	-1.6	-6.79	-28.35	-20.04	-40.3

Based on STI, the advanced lines AD18 and AD4 as well as the cultivar Yavarus appeared to be the salt tolerant durum wheat genotypes. The identification of the salt tolerant advanced lines is promising to develop new durum wheat cultivars with more tolerance to salinity. The yield related traits were also affected to different extents by salt stress. Number of grains per spike, grain weight and number of spikelets per spike exhibited 28.35%, 20% and 6.79% reductions due to salt stress, respectively (Table 3). The reduction of yield components due to salt stress was also reported by earlier studies (Poustini & Siosemardeh, 2004; Royo & Abio, 2003; Turki *et al*., 2014). On the other hand, no significant differences were observed for spike length between non-stress and stress conditions (Table 2). When subjected to salt stress, AD4 and AD18 genotypes had the maximum grain weight. Grain yield showed significant positive correlations with yield components including number of grains per spike and grain weight (Table 5). In addition, grain yield was significantly positively correlated with plant height and spike length. In line with the previous reports (Khan *et al.,* 2007; Turki *et al*., 2014), salt stress significantly decreased the height of the wheat genotypes. The maximum height was obtained from Seymareh cultivar in non-stress conditions.

Ionic contents showed a dramatic change in response to salt stress. The sodium and potassium concentrations also significantly varied among the genotypes (Table 2). The significant genotype by salinity interaction effect (Table 2) indicated that the sodium and potassium contents altered differently among the genotypes in response to salt stress. Over two fold increase in sodium content coincident with over 20% decrease in potassium content occurred in the leaf samples under salt stress (Table 3). Consequently, K/Na ratio significantly decreased due to salt stress. The findings are in agreement with the previous reports (Chhipa & Lal. 1995; Poustini & Siosemardeh, 2004; Sharbatkhari *et al.,* 2013). Indeed, accumulation of sodium in the leaves results in the reduction of potassium which plays an essential role in plant adaptive responses (Radi *et al*., 2013). Under salt stress conditions, the lowest levels of sodium concentration were observed in the genotypes AD7, AD5 and AD4 (Table 4). Of those, AD7 showed the least increase of sodium content (63.44%) compared with non-stress conditions. The results revealed that the genotypes with lower salt tolerance had higher levels of sodium accumulation. Sodium content was significantly negatively correlated with grain yield and yield components (Table 5) indicating that salt tolerance is related with lower sodium accumulation in leaves. AD5 showed the highest potassium content in salt stress and this genotype along with the cultivar Aria had the best performance in maintaining potassium under salt stress against non-stress conditions. Similar to previous studies (Poustini & Siosemardeh, 2004; Sharbatkhari *et al*., 2013), a significant positive correlation was observed between potassium content and grain yield (Table 5). In general, salt sensitive wheat genotypes may lack efficient retention of potassium in leaves which causes potassium deficiency and in turn, adversely affects growth and production (Wu *et al*., 2014). The wheat genotypes were significantly different in terms of K/Na selectivity ratio indicating considerable variation in their relative tolerance to salt stress. High K/Na ratio is presumed to be associated with salt tolerance in wheat species (Chhipa & Lal. 1995; Munns & Tester, 2003; Poustini & Siosemardeh, 2004; Sharbatkhari *et al*., 2013). The maximum K/Na ratios were found in the genotypes AD7, AD5 and AD4 (Table 4). The significant positive correlation between grain yield and K/Na ratio (Table 5) emphasized the importance of ionic measurements to select salt tolerant durum wheat genotypes.

High accumulation of proline content occurred during salt stress (Table 3); however there were different levels of proline concentrations among the wheat genotypes. The highest proline contents were observed for AD4 and D-81-18 genotypes (Table 4) which were not salt tolerant genotypes based on K/Na ratio and STI.

Table 4: Mean values for the traits measured in the eleven wheat genotypes under non stress (NS) and salt stress (S) conditions.

Genotype	STI	100-grain weight (g)			Grain yield per plant (g)			Number of spikelets per spike			Number of Grains per spike		
		NS	S	%	NS	S	%	NS	S	%	NS	S	%
AD14	0.21	5.5	2.6	-52.14	0.82	0.22	-73.53	16.33	15.00	-8.88	14.75	8.25	-78.78
AD18	1.75	5.4	4.7	-14.44	1.25	0.19	-4.64	14.16	15.41	8.10	22.83	25.41	10.16
AD19	0.22	5.4	4.3	-19.56	0.91	0.20	-78.07	15.18	13.08	-16.05	16.87	4.83	-249.16
Chamran	0.24	5.3	4.7	-11.22	0.52	0.40	-23.12	15.25	17.91	14.88	9.66	8.41	-14.85
AD4	1.44	4.9	4.9	0.42	1.22	0.99	-18.98	15.75	14.43	-9.12	25.60	20.58	-24.37
AD5	1.06	4.8	4.2	-11.69	1.27	0.70	-44.41	16.00	14.12	-13.27	26.50	16.62	-59.39
AD7	1.09	5.5	3.9	-28.81	1.08	0.85	-21.46	15.37	14.66	-4.82	19.19	21.75	11.75
Aria	1.42	4.9	3.4	-30.16	1.62	0.74	-54.08	15.21	14.25	-6.73	33.88	23.00	-47.33
D-81-18	0.77	4.2	3.2	-24.22	1.47	0.44	-69.81	17.14	15.41	-11.20	35.05	11.16	-213.89
Seymare	1.14	4.6	4.3	-5.41	1.33	0.73	-45.43	18.87	14.33	-31.68	29.12	16.83	-73.02
Yavarus	1.54	5.0	4.1	-19.35	1.19	1.10	-7.50	16.41	15.11	-8.60	23.50	27.27	13.84
LSD (0.05)		0.72	1.13		0.54	0.48		1.45	1.52		10.22	10.63	

%: The percentage of difference between corresponding mean values of non stress and salt stress conditions.

Table 4 Continued

Genotype	Na ($mgkg^{-1}$)			K ($mgkg^{-1}$)			K/Na ratio		
	NS	S	%	NS	S	%	NS	S	%
AD14	107.00	315.19	194.58	2968.24	2339.33	-21.19	27.96	7.43	-73.42
AD18	131.42	334.96	154.88	3155.89	2644.12	-16.22	24.02	7.89	-67.13
AD19	125.22	304.72	143.35	2886.35	2291.56	-20.61	2305	7.52	-67.38
Chamran	105.83	302.01	185.36	2973.92	2404.15	-19.16	28.23	7.96	-71.80
AD4	135.68	273.32	101.44	3087.65	2515.60	-18.53	22.82	9.23	-59.57
AD5	101.18	256.65	153.66	3138.83	2685.06	-14.46	31.31	10.46	-66.59
AD7	139.95	228.73	63.44	3422.01	2357.52	-31.11	24.53	10.65	-56.58
Aria	118.63	348.53	193.80	2704.39	2325.68	-14.00	22.94	6.67	-70.92
D81-18	131.42	333.41	153.70	3063.77	2589.53	-15.48	23.31	7.79	-66.57
Seymare	129.09	307.82	138.45	3262.79	2340.46	-28.27	25.56	7.60	-70.25
Yavarus	142.67	277.97	94.84	3030.79	2251.76	-25.70	21.27	8.16	-61.64
LSD (0.05)	17.08	39.76		139.76	149.56		3.44	1.20	

Table 4 Continued

Genotype	Plant height (cm)			Spike length (cm)			NADP-ME ($IUmg^{-1}$)			Proline ($\mu molg^{-1}$)		
	NS	S	%	NS	S	%	NS	S	%	NS	S	%
AD14	63.11	53.70	-17.51	5.45	5.97	8.78	0.163	0.320	96.08	23.61	158.29	570.57
AD18	63.26	61.33	-3.15	5.90	6.02	2.07	0.288	0.332	15.19	10.81	59.97	454.74
AD19	62.70	48.16	-30.18	5.80	5.66	-2.49	0.304	0.309	1.79	12.47	95.92	669.28
Chamran	60.00	59.70	-0.50	5.53	6.14	9.90	0.235	0.356	51.68	22.80	115.37	405.90
AD4	67.07	60.35	-11.13	6.90	6.72	-2.57	0.260	0.410	57.83	5.93	45.60	669.59
AD5	60.80	56.62	-7.37	6.26	6.25	-0.26	0.240	0.446	86.06	3.71	63.54	161.91
AD7	66.47	57.80	-14.99	6.10	5.74	-6.24	0.292	0.430	47.19	16.34	41.24	152.46
Aria	63.97	55.91	-14.41	6.19	6.29	1.47	0.325	0.253	-22.04	20.41	101.82	398.79
D-81-18	57.73	47.37	-21.86	7.01	6.70	-4.59	0.355	0.368	3.78	12.52	148.51	1085.82
Seymare	68.47	57.45	-19.17	6.40	5.58	-14.62	0.423	0.405	-4.16	26.24	98.91	276.95
Yavarus	61.42	56.02	-9.63	6.98	6.35	-9.97	0.338	0.325	-3.66	9.32	120.80	1196.00
LSD (0.05)	3.79	9.03		0.42	0.73		0.186	0.202		8.00	40.32	

Table 5: Correlation coefficients among the traits measured in the eleven wheat genotypes.

	GY	GW	NSS	NGS	PH	SL	Protein	Proline	NADP-ME	Na	K
GW	0.41**										
NSS	0.22	0.10									
NGS	0.93**	0.11	0.22								
PH	0.65**	0.60**	0.45**	0.52**							
SL	0.33**	-0.19	0.40**	0.44**	0.15						
Protein	-0.12	0.08	-0.20	-0.21	-0.24	-0.13					
Proline	-0.59**	-0.65**	-0.24	-0.44**	-0.66**	-0.09	0.10				
NADP-ME	0.06	-0.14	0.14	0.10	-0.04	0.27	-0.31	0.11			
Na	-0.50**	-0.61**	-0.33**	-0.34**	-0.60**	-0.03	0.03	0.84**	0.21		
K	0.49**	0.51**	0.38**	0.31*	0.55**	0.18	0.10	-0.74**	-0.19	-0.79**	
K/Na	0.47**	0.57**	0.37**	0.31*	0.57**	0.02	0.02	-0.80**	-0.27	-0.96**	0.85**

GY: Grain Yield; GW: Grain Weight; NSS: Number of Spikelets per Spike; NGS: Number of Grains per Spike; PH: Plant eight; SL: Spike Length. *: Significant at 5% **: Significant at 1%

Salt stress caused high grain yield losses in these genotypes. Analysis of correlation coefficients also highlighted that proline content was significantly negatively correlated with grain yield and K/Na ratio (Table 5). A significant positive correlation was also observed between proline content and sodium concentration (Table 5). Several studies have already mentioned the increased proline concentration in salt-stressed plants (Gupta *et al*., 1990; Bajji *et al*., 2001; Goudarzi &Pakniyat, 2009; Poustini *et al*., 2007), although some reports did not find any relationship between proline content and the level of salt tolerance (Lacerda *et al*., 2005; Poustini *et al*., 2007; Shahbaz *et al*., 2013). As a result, proline accumulation during salt stress was not a differential indicator for screening salt tolerant durum wheat genotypes, so it could simply be considered as a common biochemical response of the wheat genotypes to salt stress.

Salt stress resulted in an increase (over 22%) in NADP-ME activity (Table 3). NADP-ME activity helps to mitigate the effect of salt stress through CO_2 production and malate regulation for stomata closure (Casati *et al*., 1999) and to synthesize antioxidant reagents such as ascorbate (Veljovic-Jovanovic, 1998). The reduced rate of photosynthesis during osmotic stress increases the formation of ROS, and causes the activity of enzymes that contribute to detoxify ROS (Munns & Tester, 2008).

No consistent response was observed among the genotypes in terms of the alteration of NADP-ME activity under salt stress (Table 4). Whilst NADP-ME activity dramatically raised in some of the genotypes such as AD14 and AD4; a decline in NADP-ME activity was observed in some other genotypes. The results showed no significant correlation between NADP-ME activity and ionic contents, grain yield and yield-related traits (Table 5). The previous studies indicated that NADP-ME activity has shown different trends in response to salinity and other stresses. The down regulation of the genes encoding NADP dependent malic enzyme was reported in wheat during the salt treatment (Fu *et al*., 2009). No significant change was reported for NADP-ME activity in Arabidopsis under heat and drought stress (Koussevitzky *et al*., 2008), in *Nicotiana benthamiana* infected by potato virus Y (Doubnerova *et al*., 2007) and in potato roots under salinity stress (Manai *et al*., 2014). On the contrary, a significant increase was detected for NADP-dehydrogenase enzymes including NADP-ME in Arabidopsis implying that they may contribute to defense mechanisms against the salinity (Leterrier *et al*, 2012). Also, the increased activity of NADP+ dependent malate dehydrogenase was observed at high salinity levels in durum wheat (Capriotti *et al*., 2014). It seems that the NADP-ME activity in response to salt stress may depend on the level of stress and the growth stage which stress is imposed on plants. Understanding photosynthesis response to salt stress is highly complex and needs to be further investigated.

In conclusion, the durum wheat genotypes significantly varied for salt tolerance. Some durum wheat genotypes appeared to be more salt tolerant than the commercial cultivars. Therefore, they can be utilized in wheat breeding programs towards salt tolerance improvement. The agronomic and biochemical attributes were significantly affected by salt stress. The results indicated that the ionic content was associated with salt tolerance and thus K/Na ratio can be used as a reliable criterion for screening salt tolerant durum wheat genotypes.

K/Na ratio had a significant correlation with grain yield. The increased activity of NADP-ME and proline accumulation occurred under salt stress, although no relationships were found between these biochemical characteristics and salt tolerance in genotypes.

ACKNOWLEDGMENT

The authors would like to thank Shiraz University for funding this research.

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