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Response of wheat genotypes to foliar application of amino acids as a salt stress relief agent under hydroponic conditions

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Abstract

Wheat (*Triticum aestivum* L) is a major cereal crop moderately affected by salinity. Exogenous application of amino acids can improve the crop performance under salt stress. With the purpose to induce salt tolerance, 1% amino acids mixture (containing glutamic acid 8%, arginine 6%, proline 2.5%, lysine 2%, and serine 4%) solution was foliarly applied to eight wheat varieties Punjab-11, PK-13, Glaxy-13, Ujala-15, Gold-16, Johar-16, Anaaj-17, and Gandum-1 grown under saline (S1=10 dSm^{-1}) and non-saline (S0=0.4 dSm^{-1}) hydroponic culture of half strength Hoagland solution. A treatment without any foliar application to all wheat varieties was kept as control in Completely Randomized Design (CRD). Before the initiation of booting stage, plants were harvested and the evaluation was done on the basis of various morphological (root/shoot length, root/shoot fresh weight, root/shoot dry weight, root-shoot (weight) ratio, membrane stability index and biochemical parameters (sodium, potassium and chlorophyll contents in leaf). It is confirmed that the foliar application of amino acids significantly enhanced the salt tolerance in wheat. The wheat variety Pk-13 has been selected as salinity tolerant genotype because it has obtained the maximum shoot dry weight with higher Na+/k+ ratio as compared to Anaaj-17 which was classified as salt sensitive genotype because of attaining the minimum shoot dry weight and lowest Na+/k+ ratio.

Keywords: Biochemical content, Membrane stability, Physiological parameters, Salt stress

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Introduction

Wheat is consumed as staple food by more than 36% of world population. It is grown as a major cereal crop and gives the humans more energy and carbohydrates than any other crop (Iftikhar *et al*. 2012). Salinity is a major abiotic stress which suppresses the plant growth and development leading to reduced yield (Al-Karaki 2000; Majeed *et al.* 2010). Salt stress affects crop growth in many ways like hyper osmotic effect, nutrient imbalance and ionic toxicity. In addition to it, salinity causes deleterious effects on physiological processes including enzymatic activity, photosynthesis, oxidative stress and destabilization of protein structure. There was a major decrease in photosynthesis of wheat crop due to salinity (Metwali *et al*. 2011; Aldesuquy *et al*. 2012; Ghogdi *et al*. 2012). Reactive oxygen species are produced in maize plants by increasing the salinity which causes the oxidative damage to the plant cells (Roy *et al*. 2014). When there is high salt content in the soil then sodium ions concentration will increase and cause the significant reduction in plant growth and potential productivity of plants. The economic yield is also reduced to a significant level under salinity stress (Hessini *et al*. 2015; Khalid *et al*. 2015).

Salt tolerance is a complex multigenic trait (Flowers 2004). There are lot of plant responses to salinity such as restrict ion of salts entry at root level, cellular compartmentation, exclusion of salts through leaf hairs and hydrathods, Na+ exclusion through transporters at plasma membrane and activation of antioxidant enzymes. The salt tolerance mechanism of different genotypes work at their different growth stages. Approximately 8% of all genes in plants are affected by salt stress at transcriptional level (Tester and Davenport 2003). Control of xylem ion loading and regulation of ion exchange at xylem-parenchyma boundary are considered major physiological characteristics causing salt tolerance (Munns and Tester, 2008; Tester and Davenport 2003; Wenger *et al*. 2011). The salt tolerance can be measured by different plant growth functions like root and shoot length, root and shoot dry mass. It is evident that the role of antioxidant enzymes is an important component of the plant tolerance mechanism to salinity stress (Esfandiari *et al*. 2007; Jaleel *et al*. 2007).

There are several ways to tackle salt stress issues, such as use of those varieties which can tolerate salt stress, chemical strategies and nutrient management (Akram *et al*. 2010). Foliar application of micronutrient increases plant's tolerance under salinity stress (Van Bockhaven *et al*. 2013). Plants efficiency is increased with the uptake of amino acids (Persson *et al*. 2003; Gioseffi *et al*. 2012). In this context, the application of amino acids can result in a better plant development. Amino acid is promising factor in plants (Abdel-Mawgoud *et al*. 2011; Sadak *et al*. 2014) that act as stress inducing mediator, nitrogen resource and hormone precursor (De Lille *et al*. 2011). Amino acid is also signaling factor of different physiological processes in plants and it can improve photosynthetic rate, chlorophyll contents and gas exchange capacity of wheat seedling under salt stress condition. Amino acids can promote the salt tolerance of cotton plants by manipulating the Na^+ uptake. Amino acids can increase the initial vigor of plants, and resistance against diseases or other types of stresses, ultimately yield production will be increased (Lucini *et al*. 2015).

Materials and Methods

Experimental site: A hydroponic experiment was conducted at experimental site, Ghazi University, Dera Ghazi Khan during the month of November, 2018. Geographical location of Dera Ghazi Khan is 30.06ºN latitude and 70.63ºE longitude with an elevation of 129 meter above the sea level. During the research study, the monthly mean temperature data are given in Figure 1

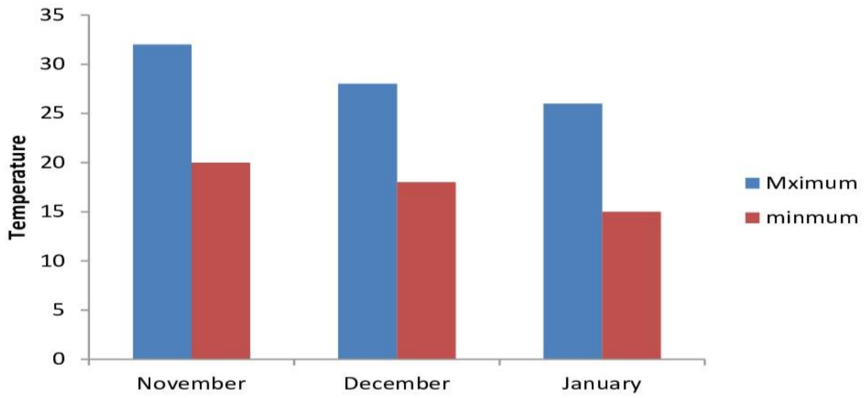

Figure 1. Minimum and maximum temperature (ºC) throughout the study trials

Treatments and growing conditions: The experimental treatments comprised of two amino acids applications [A0: Control (no foliar application); A1: 01% amino acids solution (foliar application)] to eight wheat varieties (Punjab-11, PK-13, Glaxy-13, Ujala-15, Gold-16, Johar-16, Anaaj-17 and Gandum-1) grown under saline (S1=10 dSm-1) and non-saline (S0=0.4 dSm-1) hydroponic culture. To fulfill the nutrient requirement, half strength Hoagland solution (Hoagland and Arnon 1950) was used as hydroponic culture and pH of the solution was maintained 6±0.5 by adding dilute H_2SO_4 and NaOHon daily basis. Essential nutrients were used to provide the proper growth and development. The experiment was laid out in Completely Randomized Design.

In order to develop the nursery, first trays were filled with inert sand then wheat was sown. Uniform seedlings at two leaf stage were transplanted eight days after sowing nursery. Two iron tubs having the capacity of 100 liter volume of water filled with water and Hoagland solution were used. Three days after transplanting the nursery, NaCl was used to maintain the required EC level i.e.10 dSm^{-1} (100 mM NaCl). Salt was applied in two equal split doses to secure the plant from sudden shock. Foliar application of 1% amino acids solution was done 10 days after transplanting both in saline and non-saline conditions, thereafter, 2nd foliar spray was applied 25 days after transplanting. Plants were harvested before the initiation of booting stage.

Amino acids composition: Amino acid mixture contained five types of amino acids (glutamic acid 8%, arginine 6%, proline 2.5%, lysine 2%, and serine 4%) whose 1N solution was made for foliar application. These amino acids are selected on the basis of bio-stimulating and growth regulating activity under salt stress conditions.

Procedure for recording observations

Parameters: Vegetative growth parameters were recorded by measuring root length (cm), shoot length (cm), fresh weight of root/shoot, dry weight of root/shoot and root/shoot ratio.

Biochemical parameters

K^+/Na^+ contents in leaf (m mol g^- dwt^{-1}): Digestion of the plant samples was performed according to the method given by Black (1965) for the determination of potassium and sodium contents in leaf. The filtrate was used to determine potassium and sodium contents in leaf with a flame photometer (Sherwood Flame Photometer, Model 410, Sherwood Scientific, Ltd. Cambridge, England).

K+/ Na+ ratio:K^+/Na^+ ratio was determined by dividing potassium over sodium.

$$K^+/Na^+ \text{ ratio} = \text{potassium} / \text{sodium}.$$

Chlorophyll contents (SPAD value): Chlorophyll content of one leaf of each plant from all varieties was determined by chlorophyll meter (SPAD).

Membrane Stability index: Membrane stability index (MSI) was determined according to Saram et al. (2002). Leaf samples (0.1g) were placed in10 ml of double, distilled water in two sets. One set was kept at 40° C for 30 min and its conductivity recorded (C1) using a conductivity meter. These cond set was kept in a boiling water bath (100 °C) for 15 min and iits conductivity was also recorded(C_2). The MSI was calculated as:

$$MSI=i(1-(C_1/C_2)) \text{ x}100$$

Relative Water Contents (%): Relative water contents of plant leaf was determined according to the Schon fledi *et. al*. (1988). RWC was calculated by using the values of FW, TW and DW by the given equation.

$$RWC (\%) = [(\text{fresh weight - dry weight})/ (\text{turgid weight - dry weight})] \times 100$$

Statistical Analysis:

By using Fisher's ANOVA strategy, the observed data was statistically analyzed to recognize critical significant differences. The Least Significant Difference (LSD) test was applied ($p < 0.01$) as indicated by Steel (1997).

Results

Morphological Parameters: Significant variations ($P \leq 0.05$) were recorded among the genotypes, treatments and their interactions for morphological parameters like length of root/shoot, fresh weight of root/shoot, root/shoot dry weight and root/shoot ratio [Tables 1 (a, b)]. The trend was that all the vegetative growth parameters were significantly reduced under saline condition as compared to non-saline condition. It was observed that foliarly applied amino acids had significant influence on the plant growth, productivity related parameters of wheat crop under saline and non-saline conditions. Significantly higher values of root length (28.78 g), root fresh weight (6.67 g), shoot fresh weight (14.23 g), root dry weight (0.88 g), shoot dry weight (1.46 g) and root/shoot ratio (0.68) of wheat varieties grown under saline conditions were recorded with and without foliar application of amino acids.

Biochemical Parameters: Significant variation was recorded among the genotypes, treatments and their interaction for the biochemical parameters of wheat [Tables2 (a, b)]. The trend was that all the biochemical parameters (chlorophyll contents, relative water contents, membrane stability index, sodium in root/leaves, potassium in root/leaves and sodium/potassium ratio in root/leaves) were significantly reduced under saline condition as compared to non-saline condition. Amino acids application significantly enhanced the relative water contents, membrane stability index (MSI), sodium contents in root, potassium contents in root, sodium contents in leaf, potassium contents in leaf K^+: Na^+ in root and K^+: Na^+ in leaf of wheat varieties grown under saline as well as non-saline condition. It was observed that in saline condition with amino acids application the variety (Pk-13) has obtained the maximum chlorophyll content (34.00) and lowest content (21.00) was recorded in Anaaj-17. Amino acids application enhanced the relative water content in the presence of saline as well as non-saline condition. It was observed that under saline condition with amino acids application the variety (Punjab-11) has obtained the maximum content (92.67%) and lowest content (80.47%) was recorded in Anaaj-17. Under the non-saline condition variety (Glaxy-15) achieved maximum MSI (112.57%) with foliar application of amino acids and minimum contents (70.57%) was observed in (Gandum-1). Sodium content in root was significantly reduced under saline condition as compared to non-saline condition. Under saline condition with amino acids application the variety (Anaaj-17) has obtained the maximum sodium content in root (172.64 m mol g^{-1} dwt^{-1}) and lowest content (80.45 m mol g^{-1} dwt^{-1}) was recorded in (Punjab-11). Potassium content in root was significantly reduced under saline condition as compared to non-saline condition. Under saline condition with amino acids application the variety (Punjab-11) has obtained the maximum potassium content in root (134.27m mol g^{-1} dwt^{-1}) and lowest content (58.20 m mol g^{-1} dwt^{-1}) was recorded in (Anaaj-17).

Table1(a). Effect of amino acid application on morphological parameters of wheat varieties under saline and non-saline hydroponic conditions

Trait	Treatments		Varieties							
			Pb-11	Pak-13	Gal-13	UJ-15	Gold-16	Joh-16	AJ-17	GD-1
RL (cm)	NS	Ctrl	36ef	39cd	37ef	42b	36ef	33 g	28 jk	30 hi
		AA	38de	45 a	38c-e	45a	36 f	35f	30 ij	32 gh
	Sal	Ctrl	25l-o	27k-m	18rs	20qr	22pq	23 n-p	17 s	23 op
		AA	27j-l	29i-k	20.33 qr	22pq	24n-p	25 lmn	20 r	25m no
RFW (g)	NS	Ctrl	8de	9bc	8.80 abc	9ab	6h-l	7 ef	8 de	7 fg
		AA	9a-c	7d-f	9a-c	10a	6ghi	8.11c de	8.25 cd	7.33 def
	Sal	Ctrl	6h-l	6h-k	4 op	4 n-p	5m-o	5 m-o	3p	5k-o
		AA	6f-h	7fg	5K-o	5j-n	5j-n	5 i-m	4op	5h-j
RDW	NS	Ctrl	1h-j	1c-e	1f-h	1b	1i-k	1j-l	1-	1i-k

(g)									no	
		AA	1 f-h	1c	1cd	1a	1f-h	1h-j	1i-k	1e-g
	Sal	Ctrl	1m-o	1g-i	1o	1o	1m-o	1no	0.5q	0.73 jkl
		AA	1j-l	1d-f	1lm-o	1i-k	1jk	1k-n	0.5 p	1 g-i

Table 1(b). Effect of amino acid application on morphological parameters of wheat varieties under saline (S) and non-saline (NS) hydroponic condition

Traits	Treatments		Varieties							
			Punjb-11	Pak-13	Galaxy-13	Ujala-15	Gold-16	Johar-16	Anaaj-17	Gandum-1
SL (cm)	NS	Ctrl	50cd	54a-c	50cd	54a-c	46d-f	42f-h	39g-k	40g-j
		AA (1%)	52bc	56.00 ab	52bc	58a	49cde	44e-g	41f-i	43f-h
	SS	Ctrl	37i-l	39g-k	35j-l	37i-l	33 k-m	33 k-m	29m	34k-m
		AA (1%)	38h-l	39 g-k	37i-l	37h-l	35j-l	35kl	32lm	34k-m
SFW	NS	Ctrl	15c	15b	10j	13e	10k	13e	12h	13e
		AA(1%)	15b	16a	11i	14cd	10 j	14d	12fg	14d
	SL	Ctrl	10kl	12fg	10kl	11hi	8n	7o	6r	7q
		AA(1%)	12g	14d	10j	13f	10l	9m	7 p	8o
SDW (g)	NS	Ctrl	1jk	2b	1jk	2b	1.5i	1h	1kl	1ef
		AA(1%)	1.5bc	2a	1i	2a	1.5 de	2b	1ij	1.5cd
	SL	Ctrl	1kl	2ij	1jk	2gh	1no	1no	1o	1lm
		AA (%)	1jk	1fg	1i	1.5 d-f	1lm	1mn	1no	1kl
S:R	NS	Ctrl	1 f-h	1f-h	1gh	1 e-h	1e-h	1b	1hi	1b-d
		AA (1%)	1 d-g	1e-g	1c-f	1 c-e	1bc	1a	1gh	1b
	SL	Ctrl	0.50 op	0.55 n-p	0.50 op	0.55 no	0.66 jk	1 m	0.5q	0.63l
		AA (1%)	0.5p	1mn	1no	1mn	1ij	1l	0.5pq	1.0k

Sodium content in leaf was significantly reduced in saline condition as compared to non-saline condition. In saline condition with amino acids application the variety (Anaaj-17) has obtained the maximum sodium content in leaf (159.03m mol g^{-1} dwt^{-1}) and lowest content (108.00 m mol g^{-1} dwt^{-1}) was recorded in (Glaxy-15). Same results were observed under saline condition with no foliar amino acids application and the variety (Anaaj-17) obtained maximum sodium content (164.77m mol g^{-1} dwt^{-1}) and lowest sodium content (110.87 m mol g^{-1} dwt^{-1}) was recorded in Glaxy-15. Potassium content in leaf was significantly reduced under saline condition as compared to non-saline condition. In saline condition with amino acids application the variety (Pk-13) has obtained the maximum potassium content in leaf (87.27 m mol g^{-1} dwt^{-1}) and lowest content (69.50 m mol g^{-1} dwt^{-1}) was recorded in (Punjab-11). K^+: Na^+ in root was significantly reduced in saline condition as compared to non-saline condition. In saline condition with amino acids application the variety (Ujala-15) has obtained the maximum K^+: Na^+ in root (1.39) and lowest ratio (0.25) was recorded in (Anaaj-17). K^+: Na^+ in leaf was significantly reduced in saline condition as compared to non-saline condition. Under saline condition with amino acids application the variety (Pk-13) has obtained the maximum K^+: Na^+ ratio in leaf (0.59) and lowest ratio (0.49) was recorded in Anaaj-17. Same results were observed under saline condition with no foliar amino acids application and the variety (Pk-13) obtained maximum K^+: Na^+ ratio (0.57) and lowest ratio (0.46) was recorded in Anaaj-17.

Table 2 (a). Effect of amino acid application on quality parameters of wheat varieties under saline and non saline hydroponic condition

Varieties	Chlorophyll Contents (SPAD value)				Relative Water Contents (%)				Membrane stability index (%)			
	Non-Saline		Saline		Non-Saline		Saline		Non-Saline		Saline	
	Ctrl	AA(1%)	Ctrl	AA(1%)	Ctrl	AA(1%)	Ctrl Ctrl	AA(1%)	Ctrl	AA(1%)	Ctrl	AA(1%)
Punjab-11	39 cd	41bc	32 ij	34g-j	94 b	95.37 b	91.77 f	92.67 e	100.40 f	103.53 e	84.40 n	86.53 m
Pak-13	42 ab	43a	33 h-j	34f-j	90 k	90.54 hi	82.67 r	83.43 q	105.40 d	107.53 c	80.40 p	82.53 o
Galaxy-13	32 j	33h-j	24 k-m	25k	93 e	93.42 d	89.63 k	90.30 ij	109.40 b	112.57 a	88.47 l	90.30 k
Ujala-15	34 g-j	35f-h	23 k-n	25kl	96 b	96.41 a	87.57 n	88.33 m	96.43 h	98.57 g	65.17 w	65.83 w
Gold-16	36 ef	37de	23 l-n	24kl	0.67 h	91.47 g	89.40 kl	90.51 hi	92.47 j	94.53 g	60.43 y	62.57 x
Johar-16	33 g-j	35f-h	21 m-	22.63 lmn	91.46	92.48 e	87.33 n	88.44 m	76.43 r	77.57 q	55.47	57.53 z

			o		g						a	
Anaaj-17	33 h-j	34f-i	19 o	21.00 no	85.43 p	86.30 o	79.63 t	80.47 s	72.47 t	74.53 s	48.27 e	50.57 d
Gandum-1	34 f-j	35e-g	23 k-m	24.73 kl	90 j	91.43 g	88.55m	89.30 l	68.43 v	70.57 u	51.43 c	53.50 b

Table 2(b). Effect of amino acid application on quality parameters of wheat varieties under saline (SL) and non saline (NS) hydroponic condition

Traits	Treatments		Varieties							
	Regimes	Treats	Punjab-11	Pak-13	Galaxy-13	Ujala-15	Gold-16	Johar-16	Anaaj-17	Gandum-1
Na^+L	NS	Ctrl	71a	81y	65c	89w	94u	102.37 s	108q	116n
		AA (1%)	68b	78z	61d	86x	91.33 v	99.67 t	105r	114o
	SL	Ctrl	122l	128 k	111p	133i	142.27 g	149.03 e	165a	156c
		AA (1%)	119m	123l	108q	131j	139.17 h	146.30 f	159b	153d
Na^+R	NS	Ctrl	26d	37a	27c	75u	63w	54	104k	88q
		AA (1%)	25e	36b	26d	74v	62x	53z	103l	87 r
	SL	Ctrl	82s	125 g	139e	97m	116i	163c	174a	91o
		AA (1%)	80t	125 h	138f	95n	114j	162d	173b	91p
K^+L	NS	Ctrl	144i	151 cd	148fg	151 c	146gh	153b	142j	144 ij
		AA (1%)	147g	151 c	149de	154 b	148 ef	156a	144i	145h
	SL	Ctrl	68r	82l	71p	77m	75n	75n	63q	75 n
		AA (1%)	70q	87k	73o	81 l	82l	79m	71p	77m
K^+R	NS	Ctrl	143a-c	124f g	148a	111i j	98k	85 l-n	62qr	77m-o

		AA (1%)	144ab	125 e-g	152a	112 h-j	100k	87l	64p-r	78mno
	SL	Ctrl	131 d-f	117 g-i	134de	103 jk	86 lm	75o	56r	70o-q
		AA (1%)	135cd	120 gh	135b-d	105jk	89l	77no	58r	72op

Where Na+ L (Na^+ Leaves, m mol g^{-1} dwt^{-1}), Na^+R(Na^+ Roots, m mol g^{-1} dwt^{-1}), K^+ L(K^+ Leaves, m mol g^{-1} dwt^{-1}), K^+ R(K^+ Roots, m mol g^{-1} dwt^{-1})

Discussion

Sodium concentration increased due to induced NaCl stress in all wheat varieties however genotype Punjab-11 maintained the lowest Na content in saline condition with foliar application of amino acids and the maximum contents were in Anaaj-17 without foliar application of amino acids. It indicates that foliarly applied amino acids reduced the salt accumulation in roots. Furthermore, the variety Glaxy-13 attained the minimum salt contents in leaves due to exogenous application of amino acids and the genotype Anaaj-17 got the maximum Na contents without foliar spray. The results are supported by Akram *et al.* 2007 who reported that sodium is a toxic element whose higher concentration disturbs the metabolic activities in the plant cell.

It was previously reported by Azizpouer *et al*. 2010 that salt stress reduces the potassium accumulation in plant due to higher concentration of sodium in plant. Amino acids application has significantly enhanced the K substance in leaves under salt stress. The genotype Pk-13 has obtained the maximum potassium contents while the variety Punjab-11 has attained the minimum K content without amino acids application. Furthermore, potassium contents in roots were significantly increased under salinity with foliar spray. The variety Glaxy-13 has obtained the maximum K contents under saline condition with amino acids application. Meanwhile, the genotype Anaaj-17 has attained the lowest content in the leaves without exogenous application.

Na^+/ K^+ ratio was significantly reduced under the saline condition in comparison to non-saline condition. Amino acids application enhanced the Na^+/K^+ ratio to a significant level under saline and non-saline condition. K^+: Na^+ in root was higher in variety Ujala-15 with foliar application as compared to Anaaj-17 where hormonal spray was not applied under Saline condition respectively. Furthermore, similar situation were in K^+: Na^+ in leaf under saline and non-saline condition which was significantly reduced. The sodium potassium ratio in leaf was enhanced to significant level with foliar application under saline and non-saline conditions. K^+: Na^+ in leaf was higher in variety Pk-13 with foliar application of amino acids as compared to genotype Anaaj-17 which obtained lowest ratio of Na^+/K^+ ratio in leaves without the application of amino acids under saline condition.

Membrane stability index and relative water content are significant factor to evaluate the extent of salinity in wheat (Azizpouer *et al*. 2010). By increasing the stomata resistance, salt stress affects the membrane function and photosynthesis activity (Karbal*et al.* 2003; Sairam *et al*. 2002). Under the salinity or drought stress plants produces the ROS immediately which leads to membrane damage and electrolyte leakage (Kumar *et al*. 2015). Amino acids such as proline, asparagines and arginine play an important role in relative water content and membrane stability index under salt stress (Haroun 2002; Claussen 2005; Ueda 2007). In the present study, the MSI and RWC contents were higher in genotype Glaxy-13 and Punjab-11, respectively with foliar application under saline condition as compared to genotype Anaaj-17 which has obtained the lowest content under saline condition without exogenous application of amino acids.

It has been reported that sodium absorption adversely affects the chlorophyll content and also the photosynthetic process in the plants (Jiang *et al*. 2017). It is revealed that in the previous study the exogenous application of amino acids such as proline, argnine produced the resistance against saline and drought condition (Abdelhamid *et al*. 2013; Dawood *et al*. 2014a, 2014b; Semida *et al*. 2014). In the present study, it was revealed that chlorophyll was slightly decreased under saline and non-saline condition with foliar application of amino acids. It was observed that the wheat genotype Anaaj-17 has obtained the lowest chlorophyll content relative to Pk-13 because of exogenous application of amino acids enhance the chlorophyll content under saline condition. These results matched with the previous work on sunflower and wheat (Shahbaz *et al.* and Ashraf 2008; Akram *et al*. 2009).

Conclusion: The present study proved that chlorophyll substances, membrane stability index, relative water contents and K+/Na+ ratio in root, stem and leaf could serve as good criteria for selection of wheat varieties against NaCl stress with foliar application of amino acids. According to above all factors it was assumed that Pk-13 is salt bearing variety while Anaaj-17 is susceptible to NaCl stress with exogenous application of amino acids in hydroponic conditions. These findings can be a great resource for the plant breeders and plant physiologists interested in the production of salinity tolerant wheat varieties.

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