Influence of Salicylic Acid on Ion Distribution, Enzymatic Activity and Some Agromorphological Characteristics of Wheat under Salt-affected Soil

E.M. Hafez[#]

Department of Agronomy, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt.

> ${f A}$ FIELD experiment carried out to examine the effects of soil salinity on morphological and physiological characters of two wheat cultivars differing in salt tolerance and the modulation of ion distribution and activities of antioxidant enzyme by exogenous application of salicylic acid under salt-affected soil. The experiment was designed in split plot design. Two wheat cultivars including Sakha93 (salt-tolerant) and Gemmiza9 (salt-sensitive) were assigned in main plots. Three levels of salicylic acid including (0, 100 and 200 mg L^{-1}) were allocated to the subplots in 2013/2014 and 2014/2015 seasons. Salt-tolerant cultivar (Sakha93) showed higher morphological and physiological characters than salt sensitive one (Gemmiza9). Exogenous application of salicylic acid maintained lower Na⁺ concentration with higher K⁺/Na⁺ ratio in flag leaf blade in Sakha93 as compared to Gemmiza9. Exogenous application of salicylic acid increased K⁺ and decreased Na⁺ in Gemmiza9 as compared to untreated plants. Side by side, the improvement of ion distribution might be due to increment in activities of antioxidant enzymes catalase (CAT) and peroxidase (POX) by exogenous higher salicylic acid level (200 mg L^{-1}) in 2013/2014 and 2014/2015 seasons. It could be concluded that exogenous application of 200 mg SA $\mathrm{L}^{\text{-1}}$ has effective impact in increasing yield-traits and grain yield under salt-affected soil condition.

Keywords: Wheat, Salicylic acid, K⁺/Na⁺, Catalase, Peroxidase

Wheat (*Triticum aestivum* L.) is one of the most outstanding cereal food which owing a central role in world's food security. It is the fundamental food crop for more than one-fifth of human populace around the globe especially in Egypt (FAO, 2014). By the end of year 2050, it is predicted that the global population will grow to reach about 10 billion. Otherwise, wheat productivity is reducing due to the impact of different abiotic stresses which lead to loss millions of dollars annually. Therefore, it is important to minimize these losses to face the increasing food requirements (Maswada & Abd El-Kader, 2016).

In arid and semi-arid areas of the world such as Egypt is suffering from abiotic stresses. Abiotic stresses such as salinity is a global issue which adversely [#]For Correspondence: emadhafez2014@gmail.com

24

affects crop growth and decrease productivity, constraining physiological and biochemical processes in plants and ion imbalance (Munns & Tester, 2008 and Hafez & Abou El-Hassan, 2015). Munns & Tester (2008) stated that more than 45 million hectares of irrigated land have been damaged by salt worldwide and roughly 1.5 million hectares are taken out of production each year due to high salinity levels in the soil. Thus, it is worthy to enhance the performance of crop plants, especially food crops, under saline condition. In Egypt, approximately 33% of the cultivated land is salt-affected due to low rainfall (<25 mM annual rainfall) and saline water irrigation (Ghassemi *et al.*, 1995).The efficient choice to diminish the deleterious impacts of salinity on productivity is the selection of salt-resistant cultivars (Hafez & Abdelaal, 2015 and Zhu *et al.*, 2016).

Salt-affected soils lead to uptake higher rates of Na and exclude K in plants (Zhang *et al.*, 2010), resulting in poor K⁺/Na⁺ discrimination under salt stress (Shabala & Munns, 2012) which reduce yield-related traits and ultimately grain yield (Hafez *et al.*, 2015 and Zhu *et al.*, 2016). In addition to promote scavenging of reactive oxygen species (ROS) (Amjad *et al.*, 2014). Low concentrations of Na⁺ in the leaves of wheat are linked to salt tolerance (Munns & Tester, 2008). Therefore, it has been demonstrated that minimize Na⁺ uptake in plants is considered to be one of the substantial mechanisms to reduce the harmful effects of salt stress (Zhang *et al.*, 2010). Hence, alleviate detrimental impacts of salt stress on crops has received considerable attention.

Recently, many strategies are being practiced in the world to cope with salt stress; exogenous application of natural and safety substances is the one that is getting considerable attention, which regulate various physiological and biochemical processes in plants and enhance yield productivity (Rahnama *et al.*, 2011). Salicylic acid (SA) is one of safety substances which having ability of antioxidant defense system and has a major role in counteracting of abiotic stress such as salinity in wheat (Shakirova *et al.*, 2003). Exogenous application of salicylic acid alleviates deleterious impact of salt-induced owing to its ability in stomatal regulation and ion uptake in wheat (Arfan *et al.*, 2007 and Hafez *et al.*, 2014).

Although a lot of agricultural researches have been done on the response of wheat to salt stress. However, relatively little information is available regarding role of exogenous application by salicylic acid on growth, yield performance, ion balance and biochemical characters under salt-affected soil conditions in wheat cultivars which is also imperative to study. In addition, the mechanisms of this regulation under stress have not been fully elucidated yet. Thus, the present research was undertaken to explore the role of exogenous application by salicylic acid in amelioration of adverse effects of soil salinity. Furthermore, study ions distribution in leaf blade in two `bread wheat cultivars differing in salt tolerance and the activity of antioxidative enzymes.

Material and Methods

Plant material and experimental site

Two wheat (*Triticum aestivum* L.) cultivars, Sakha-93 as salt-tolerant and Gemmiza-9 as salt sensitive, were selected based on the previous researches of (El-Lethy *et al.*, 2013). The tested cultivars were obtained from the Wheat Research Department, Field Crops Research Institute, Egypt. The Experiment was carried out at Water Requirements Research Station (El-Karada), Water Management Research Institute, National Water Research Centre, Kafrelsheikh Governorate (North Delta, Latitude: 31°6' N/Longitude: 30° 56' E), Egypt, during the winter growing seasons of 2013/2014 and 2014/2015. Meteorological data at El-Karada Station during two growing seasons (2013/2014 and 2014/2015) are presented in Table 1. Before seedbed preparation, the experimental soil was ploughed twice and randomized three soil samples (0 to 30 cm depth) were taken for analysis, physical and chemical properties in the experimental soil are shown in Table 2.

TABLE 1. Meteorological data	for El-Karada Station	during growing seasons.

			013/2014		2014/2015				
Year Month	Temperature (°C)				Temperature (°C)		Precipitation (mm)	RH (%)	
max	min	(11111)	(70)	max	min	(IIIII)	(70)		
Dec	26.9	11.4	0.80	30.7	24.9	10.9	0.60	32.6	
Jan	23.5	9.4	3.50	41.4	22.2	9.7	3.30	43.1	
Feb	22.0	11.0	7.00	45.1	20.8	12.7	7.60	44.2	
Mar	24.0	16.2	0.00	47.9	22.5	14.7	0.50	45.4	
April	27.4	17.2	0.00	55.4	25.7	16.1	0.00	53.1	
May	31.9	17.8	0.00	64.1	30.3	15.4	0.00	65.8	
mov _ movimu		• •	DU 1		. 1.4				

max = maximum, min = minimum, RH = relative humidity

 TABLE 2. Chemical properties of soil used in 2013/2014 and 2014/2015 growing seasons.

Year	Cations (meq L ⁻¹)				Anions (meq L ⁻¹)				
rear	(ds m ⁻¹)	pН	Na ⁺	\mathbf{K}^{+}	Mg^{++}	Ca ⁺⁺	Cľ	HCo ₃	So4
2013/2014	3.04	8.05	12.78	0.73	4.76	6.54	13.56	4.22	1.54
2014/2015	2.89	7.98	10.65	0.86	5.23	7.29	13.21	3.67	1.98

Soil analysis for pH based on soil: water extract (1: 2.5), while EC and ions based on soil: water extract (1: 5). Soil texture was clayey.

Experimental design and treatments

The experiment was designed in a randomized complete block design (RCBD) of a split plot arrangement with three replicates. Two wheat cultivars including (Sakha 93 and Gemmiza 9) were assigned in main plots. Three levels of salicylic acid including (0, 100 and 200 mg L^{-1}) were allocated to the subplots.

Salicylic acid (SA) had foliar sprayed twice using hand atomizer and wetting agent at 30 and 60 days after sowing (DAS). The preceding crop was Maize (*Zea mays* L.) during the growing seasons. Calcium superphosphate (15.5% P_2O_5) was added at the rate of 250 kg ha⁻¹ during seedbed preparation, N fertilizer was applied at rate of 170 kg N ha⁻¹ as ammonium nitrate (33.5 % N) to each plot at three splits; 20% as basal dose at sowing stage, 40% at jointing stage and the remaining 40% at booting stage.

The experiment was planted on November 20^{th} during 2014/2015 and November 28^{th} during 2015/2016 with row spacing of 15 cm by drilling machine and a seeding rate 125 kg ha⁻¹. Weeds were controlled by Topik 15% WP herbicide. The net experimental unit size (plot) was 10.5 m² (3 m width x 3.5 m long). Wheat grain yield obtained by harvesting the center (2 m x 2 m) of the experimental unit, but yield components were determined from two outer rows within each plot. Plots were separated by 0.5 m allays.

Morphological and physiological measurements

Flag leaf area (cm²) measurement

Ten flag leaves from each plot were collected at heading stage, when the flag leaf was fully expanded to measure flag leaf area by Leaf Area Meter (Li-Cor 3100, Lambda Instruments Co., USA).

Chlorophyll content (SPAD)

At heading stage, chlorophyll content was measured by portable chlorophyll meter (SPAD-502; Minolta Sensing Co., Ltd, Japan) at three different positions of fully expanded leaves (Markwell *et al.*, 1995) to measure leaf greenness of the plants.

Analysis of sodium and potassium ion concentrations

At heading stage, oven- dried samples of leaves from each plot in oven at 70 °C for 48 h. Fine ground leaves (0.5 g) into fine powder were digested with concentrated H_2SO_4 (5 ml) and 80 % perchloric acid (1 ml). Digested material diluted by distilled deionized water brought up to 100 ml final volume. The concentrations of Na⁺ and K⁺ were determined using PFP7 Flame photometer (Temminghoff & Houba, 2004).

K/Na = (% K in leaf) / (% Na in leaf)

Leaf relative water content

Leaf relative water content is a useful measure of the physiological water status of plants under salt- affected soil (Gonzalez & Gonzalez-Vilar, 2001). At heading stage, leaves detached from the stem were weighted to determine fresh weight (FW). Turgid weight (TW) was estimated after the leaves were kept floating in distilled water into a closed petri dish at 10°C in the dark for 24 h and weighted again. Dry weight (DW) was determined for leaves samples after ovendrying for 72 h at 80°C. RWC was calculated using the following equation: LRWC (%) = [(FW-DW) / (TW-DW)] × 100 (Jeon *et al.*, 2006).

Extraction and assay of antioxidant enzymes

For the determination of antioxidant enzyme activities in wheat leaves at heading stage, 0.5 g leaf material was homogenized at 0-4°C in 3 ml of 50 mM TRIS buffer (pH 7.8), containing 1 mM EDTA-Na² and 7.5% polyvinyl pyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4°C), and the total soluble enzyme activities were determined spectrophotometrically in the supernatant (Hafez *et al.*, 2012). All measurements were undertaken at 25°C, using the model UV-160A spectrophotometer (Shimadzu, Japan).The enzyme assays were tested three times. Activity of catalase (CAT) was measured spectrophotometrically based on Aebi (1984). Differences in the absorbance at 240 nm were measured every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min⁻¹ g⁻¹ fresh weight. The method used for extraction and determination of peroxidase (POX) activity was expressed as the increase intervals for 3 min. Enzyme activity was expressed as the increase in the absorbance min⁻¹ g⁻¹ fresh weight. The method used for extraction and determination of peroxidase (POX) activity was expressed as the increase in absorbance min⁻¹ g⁻¹ fresh weight as expressed as the increase intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min⁻¹ g⁻¹ fresh weight as expressed as the increase in the absorbance min⁻¹ g⁻¹ fresh weight as expressed as the increase in the absorbance min⁻¹ g⁻¹ fresh weight.

Yield and related traits

Total number of spikes m⁻² from each plot were carefully counted and averaged to determine number of spikes m⁻². Length of ten random selected spikes was taken from each experimental unit and averaged to measure spike length. Total number of spikelet's spike⁻¹ and number of grains spike⁻¹ from ten randomly selected spikes were counted carefully and averaged to record number of spikelet's and grains per spike. Five random samples each of 1000 grains were taken, weighed and averaged to measure 1000-grain weight. At harvest maturity, each plot was harvested manually, sun dried for three days and tied into bundles. These bundles were then threshed manually and grains were separated and weighed to record grain yield (14% moisture content). Left over straw was again weighed to record straw yield. Grain and straw yields were converted into kg ha⁻¹. Harvest index (HI) was determined as ratio between grain and biological yield and was expressed in percentage.

Statistical analysis

Data obtained were subjected to an analysis of variance (ANOVA) procedures according to Gomez & Gomez (1984) using the MSTAT-C Statistical Software package. Different Means were compared using (Duncan, 1955), when the ANOVA showed significant differences (P < 0.05).

Results and Discussion

Effect of treatments on flag leaf area, chlorophyll content and relative water content under salt-affected soil

Salt stress diminished flag leaf area, chlorophyll content and leaf relative water content of both cultivars; Sakha93 (salt-tolerant cultivar) and Gemmiza9 (salt-sensitive cultivar). However, exogenous application of salicylic acid has a positive impact in improving growth traits including, flag leaf area, chlorophyll

content and relative water content of the two wheat cultivars compared to control under salt-affected soil (Table 3).

Treatments	0	af area m ²)	Chlorophyll content (SPAD)		Relative water content (%)	
	2013	2014	2013	2014	2013	2014
Cultivars (C)						
Sakha93	39.45a	40.25a	48.95a	49.75a	94.52a	95.47a
Gemmiza9	33.57b	34.45b	43.37b	44.23b	91.64b	91.45b
Salicylic acid levels (SA) (mg L^{-1}))				
0	32.78c	33.24c	42.88c	43.37c	90.54b	91.23c
100	35.25b	36.46b	46.54b	47.32b	93.45a	93.78b
200	37.95a	38.85a	48.87a	49.45a	93.87a	94.56a
$\mathbf{C} \times \mathbf{S}\mathbf{A}$	ns	ns	ns	ns	ns	ns

TABLE 3. Effect of cultivars and salicylic acid levels on (flag leaf area, chlorophyll content and relative water content, respectively) in 2013/2014 and 2014/2015 seasons.

Data within columns followed by different letters are significantly different at P<0.05; ns, no significant difference.

Sakha93 significantly recorded maximum flag leaf area (39.45 and 40.25 cm²), chlorophyll content (48.95 and 49.75) and relative water content (94.52 and 95.47 %) in both seasons, respectively compared with Gemmiza9 which recorded minimum flag leaf area (33.57 and 34.45 cm²), chlorophyll content (43.37 and 44.23) and relative water content (91.64 and 91.45 %) in both seasons, respectively (Table 3). In the line of the results (Hafez & Kobata, 2012 and Maswada & Abd El-Kader, 2016) demonstrated that Sakha94 as salt tolerance with higher growth traits under salt stress and Gemmiza9 as saltsensitive with higher reduction in growth traits under salt stress. Rana et al. (2015) reported that salt-tolerance cultivars were higher growth traits may be because of higher water uptake under salt stress as compared to salt-sensitive cultivars. Singh et al. (2015) also reported that a salt tolerant cultivar had lower accumulation of salts in the leaves may be due to higher antioxidant activity, as compared to a salt-sensitive cultivar that uptake harmful ions leading to turgor loss.

Exogenous application of salicylic acid remarkedly increased flag leaf area, total chlorophyll content and relative water content in both seasons (Table 3). Salicylic acid enhanced flag leaf area, chlorophyll content and leaf chlorophyll content in both seasons which indicate that salicylic acid assuaged the detrimental impacts of salinity. These results are in agreement with previous studies that reported that salicylic acid application enhanced plant growth, flag leaf area, chlorophyll content and relative water content under salt-affected soils (Sakhabutdinova et al., 2003 and Hafez & Gharib, 2016). The highest flag leaf area (37.95 and 38.85 cm²), chlorophyll content (48.87 and 49.45) and relative water content (93.87 and 94.56 %) were recorded by exogenous application (200 mg L⁻¹ SA) compared to control treatment (without application SA) which recorded The lowest flag leaf area (32.78 and 33.24 cm²), chlorophyll content (42.88 and 43.37) and relative water content (90.54 and 91.23 %) in both seasons, respectively. It might be attributed to the external supply of salicylic acid on physiological processes, such as the growth regulation and promotion, metabolism and differentiation of cells under salt-affected soil and increment nutrients and water absorption (Manzoor *et al.*, 2015). Manzoor *et al.* (2015) showed that salt stress was mitigated by exogenous application of salicylic acid, which observed that salicylic acid led to decline transpiration and stomata opening and increase relative water content.

Effect of treatments on 1000-grain weight, number of grains spike⁻¹ and number of spikes m^{-2}

Wheat grain yield is a result of shared factors of some yield-related traits, *i.e.* 1000-grain weight, number of grains spike⁻¹ and number of spikes m⁻². Salt stress reduced the yield due to marked reduction in traits relevant to yield (Table 4).

TABLE 4. Effect of cultivars and salicylic acid levels on (1000-grain weight, number	
of grains spike ⁻¹ and number of spikes m ⁻² , respectively) in 2013/2014 and	
2014/2015 seasons.	

Treatments		grain ht (g)	Number of grains spike ⁻¹		Number of spikes m ⁻²	
	2013	2014	2013	2014	2013	2014
Cultivars (C)						
Sakha93	47.39a	47.88a	50.43a	51.22a	334.56a	337.34a
Gemmiza9	44.48b	44.86b	48.65b	48.82b	326.86b	329.64b
Salicylic acid levels (SA	$(mg L^{-1})$	1				
0	43.24c	44.56c	48.25c	48.38c	325.22c	328.65c
100	44.87b	45.44b	49.15b	49.85b	329.55b	332.37b
200	46.24a	46.94a	49.85a	50.19a	332.45a	335.94a
$\mathbf{C} \times \mathbf{S}\mathbf{A}$	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at P<0.05; ns, no significant difference.

Sakha 93 significantly recorded maximum 1000-grain weight (47.39 and 47.88 g), number of grains spike⁻¹ (50.43 and 51.22) and number of spikes m⁻² (334.56 and 337.34), respectively as was found in both seasons (Table 4) as compared to Gemmiza 9 which recorded minimum 1000-grain weight (44.48 and 44.86 g), number of grains spike⁻¹ (48.65 and 48.82) and number of spikes m⁻² (326.86 and 329.64) in both seasons, respectively (Table 4). It was noted from the results that Gemmiza 9 was hardly affected by salt stress whereas number of grains and spikes were more severely influenced in respect of high increase in unfilled grain number and decline in 1000-grain weight as compared to Sakha93. These results are in harmony with the findings of Maswada & Abd El-Kader (2016).

Exogenous application of salicylic acid appreciably increased 1000-grain weight, number of grains spike⁻¹ and number of spikes m⁻² in both seasons (Table 4). The highest 1000-grain weight (46.24 and 46.94 g), number of grains spike⁻¹ (49.85 and 50.19) and number of spikes m⁻² (332.45 and 335.94) were recorded by exogenous application (200 mg L⁻¹ SA) respectively as was found in both seasons compared to control treatment (without application SA) which recorded the lowest 1000-grain weight (43.24 and 44.56 g), number of grains spike⁻¹ (48.25 and 48.38) and number of spikes m⁻² (325.22 and 328.65) in both seasons, respectively. The reversal of the detrimental impacts of salt stress on yield-traits by exogenous salicylic acid application was observed in both sensitive and tolerant cultivars. The beneficial impacts of salicylic acid might be due to early application at vegetative growth leading to promotive impact on post-heading development Desoky & Merwad (2015). Desoky & Merwad (2015) who reported that number of grains, number of spikes and 1000-grain weight were positively correlated with exogenous application of salicylic acid.

Effect of treatments on grain yield, straw yield and harvest index

Sakha 93 significantly recorded maximum grain yield (6.88 and 7.15 ton ha⁻¹), straw yield (10.43 and 10.87 ton ha^{-1}) and harvest index (39.75 and 40.24 %), respectively as was found in both seasons (Table 5) compared with Gemmiza9 which recorded lesser grain yield (6.27 and 6.38 ton ha^{-1}), straw yield (9.82 and 9.92 ton ha⁻¹) and harvest index (38.92 and 39.14 %) in both seasons (Table 5). Abdullah et al. (2001) reported that there were many reasons of damage in grain formation of wheat under salt-affected soil, including Na^+/K^+ ratio in plant parts. The results proved that the decline in sink size was attributed to higher Na⁺ absorption and lower K^+ absorption into sink size which was most pronounced in the salt sensitive cultivar (Gemmiza9) as compared to the salt tolerant cultivar (Sakha93). Also it was observed that grain yield reduction was mainly attributed to decrease in number of grains, number of spikes, 1000-grain weight, these results might be due to the decline in fertilization through pollen viability deterioration under salt stress (Maswada & Abd El-Kader, 2016). Abdullah et al. (2001) also reported that straw yield reduction was attributed to higher Na⁺ accumulation and lower K^+ accumulation into young leaves at the late vegetative growth and the shoots, in addition inhibit translocation of assimilates from leaves to grains which was most pronounced in the salt sensitive cultivar (Gemmiza 9) as compared to the salt tolerant cultivar (Sakha 93).

It is noticeable that grain yield, straw yield and harvest index gradually increased significantly with the increase of salicylic acid levels in both seasons of study, accordingly (Amin *et al.*, 2008). External supply of salicylic acid at 200 mg L⁻¹ tended to maximize grain yield (9 and 10 %), straw yield (5 and 4 %) and harvest index (3 and 4 %) in both seasons of study respectively, as an average of salicylic acid application versus without salicylic acid application (Table 5). Amin *et al.* (2008) reported that yield reduction owing to salt-affected soil might be ameliorated by exogenous application of salicylic acid through its effect on ion balance, nutrient uptake and antioxidant enzyme activities.

Egypt. J. Agron. 38, No.3 (2016)

462

Treatments		ı yield ha ⁻¹)	Straw yield (ton ha ⁻¹)			at index 6)
	2013	2014	2013	2014	2013	2014
Cultivars (C) Sakha93 Gemmiza9	6.88a 6.27b	7.15a 6.38b	10.43a 9.82b	10.87a 9.92b	39.75a 38.92b	40.24a 39.14b
Salicylic acid levels (SA	A) (mg L ⁻¹)				
0 100 200	6.05c 6.45b 6.64a	6.23c 6.65b 6.92a	9.75c 10.07b 10.22a	9.87c 10.18b 10.28a	38.29b 39.04a 39.38a	38.70c 39.51b 40.23a
$C \times SA$	ns	ns	ns	ns	ns	ns

TABLE 5. Effect of cultivars and salicylic acid levels on (grain yield, straw yield and
harvest index, respectively) in 2013/2014 and 2014/2015 seasons.

Data within columns followed by different letters are significantly different at P<0.05; ns, no significant difference.

Effect of treatments on leaf K^+ *, leaf* Na^+ *and leaf* K^+/Na^+

Most of the physiological characters assessed showed a significantly differences between both cultivars. Under salt-affected soil, leaf K⁺/Na⁺ was still the strongest determinant of salinity tolerance. Leaf K⁺ content was significantly increased in Sakha93 as compared to Gemmiza9 in both seasons, On the contrary with leaf Na⁺ content was significantly higher in Gemmiza9 as compared to Sakha 94 in both seasons under salt-affected soil (Table 6). Sakha93 significantly recorded maximum leaf K^+ (6.85 and 7.08 mg/g LDW) compared with Gemmiza9 which recorded minimum leaf K^+ (6.05 and 6.23 mg/g LDW), respectively in both seasons. However, Sakha93 significantly recorded minimum leaf Na⁺ (7.68 and 7.72 mg/g LDW) compared with Gemmiza9 which recorded maximum leaf Na⁺ (8.74 and 8.87mg/g LDW) respectively in both seasons. While, Sakha93 significantly recorded maximumleaf K⁺/Na⁺ (0.89 and 0.92 mg/g LDW) respectively, as was found in both seasons (Table 6) as compared to Gemmiza9 which recorded minimum leaf K⁺/Na⁺ (0.69 and 0.71 mg/g LDW) in both seasons, respectively as shown in (Table 6). Sakha93 (salt-tolerant cultivar) accumulated larger K⁺ concentrations and lower Na⁺ concentrations in the leaves and higher K⁺/Na⁺ ratio which maintains high chlorophyll content and osmotic regulation for more plant growth on the contrary with Gemmiza9 (salt-sensitive cultivar). Furthermore, supply assimilates from leaves to spikes during anthesis and grains during grain filling stage and lastly increases seed set. A negative effect has been observed between Na⁺ concentration in floral parts and salt tolerance in terms of grain yield. The results of the current investigation are in full agreement with Rahnama et al. (2011) and Abu Hasan et al. (2015).

Treatments	Lea (mg/g	f K ⁺ LDW)		af Na ⁺ Leaf K ⁺ /g LDW) (mg /g L		
	2013	2014	2013	2014	2013	2014
Cultivars (C)						
Sakha93	6.85a	7.08a	7.68b	7.72b	0.89a	0.92a
Gemmiza9	6.05b	6.23b	8.74a	8.87a	0.69b	0.71b
Salicylic acid levels (SA) (mg L^{-1})					
0	6.22c	6.46c	8.68a	8.95a	0.72c	0.72c
100	6.53b	6.78b	8.25b	8.07b	0.79b	0.84b
200	6.95a	7.14a	7.85c	7.66c	0.88a	0.93a
$\mathbf{C} \times \mathbf{S}\mathbf{A}$	ns	ns	ns	ns	ns	ns

 TABLE 6. Effect of cultivars and salicylic acid levels on (leaf K⁺, leaf Na⁺ and leaf K⁺/Na⁺, respectively) in 2013/2014 and 2014/2015 seasons.

Data within columns followed by different letters are significantly different at P<0.05; ns, no significant difference.

A positive response from exogenous application of salicylic acid was recorded for both cultivars. Whereas exogenous application of salicylic acid tended to increase leaf K⁺ content and decrease leaf Na⁺ content under saltaffected soil in both seasons. Exogenous application of salicylic acid significantly increased leaf K⁺ and leaf K⁺/Na⁺ and decreased leaf Na in both seasons (Table 6). Application 200 mg L^{-1} SA led to maximize leaf K⁺ (6.95 and 7.15 mg/g LDW) and leaf K⁺/Na⁺ (0.88 and 0.93 mg/g LDW) and minimize leaf Na^+ (7.85 and 7.66 mg/g LDW) respectively in both seasons (Table 6) as compared to control treatment (without application). In salt-affected soils, the reduction in growth traits and leaf chlorophyll might be due to higher Na⁺ content and lower K^+ content in the leaves and finally the yield reduction (Meneguzzo et al., 1999). These circumstances led to many restrictions such as ion imbalance, lower water absorption, high osmotic potential, restraint of biochemical processes and more reactive oxygen species. Salicylic acid application had a protective role under salt stress and could regulate of ion balance and transport (Amin et al., 2008). SA could alleviate of salt stress impacts and enhance water potential. The disturbance in stomatal conductance, transpiration rate and photosynthesis under salt stress could be reversed by external supply of salicylic acid which capable to prevent the destructive effect of ROS (Abu Hasan et al., 2015).

Effect of treatments on of antioxidant enzyme activities:

The results in Fig. 1 showed CAT and POX activities in the leaves of wheat under salt-affected soil in 2013/2014 and 2014/2015 winter seasons. In the current study, it was found from the data that the increase in salicylic acid level up to 200 mg L^{-1} resulted in more catalase and peroxidase activities in both seasons (Fig.1). The activities of CAT and POX were much higher in Sakha93 (tolerant cultivar) than Gemmiza9 (sensitive cultivar) in both seasons under salt-affected soil by exogenous application of 200 mg SA L^{-1} (Fig.1).

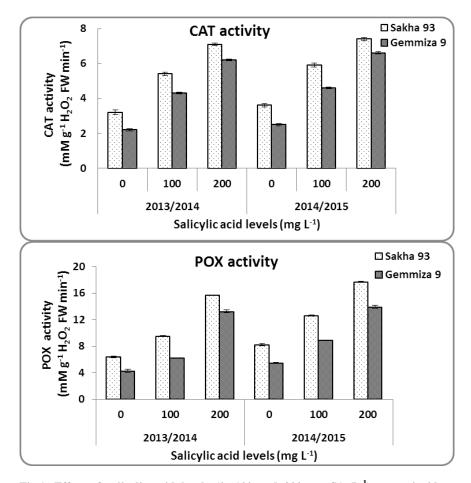


Fig.1. Effect of salicylic acid levels (0, 100 and 200 mg SA L^{-1}) on antioxidant enzymes activities (CAT and POX) of two wheat cultivars in 2013/2014 and 2014/2015 seasons. Data are the mean ± SE of three replicates.

These higher levels of antioxidant enzymes could be regarded to ability of antioxidant enzymes to promote salinity tolerance against oxidative deterioration. Exogenous application of salicylic acid had increased antioxidant enzyme activities in wheat plants, which could maintain growth traits, metabolic processes, oxidative stress resistance, osmotic balance and increase the stability of membranes and CO₂ fixation in salt-affected soil (Meneguzzo *et al.*, 1999 and Maswada & Abd El-Kader, 2016). Hence the increase in activities of antioxidant enzymes of wheat plants owing to salicylic acid application had a crucial role in scavenging of ROS and closely related with reduction of oxidative deterioration (Hafez & Hafez, 2016).

Acknowledgements: The author is grateful to the Water Management Research Institute, for support of this research. Moreover, the author would like to express

a special thanks to Dr Waleed Abou El-Hassan (Head of Farm Irrigation Management Department) for his help in this study. The author is also appreciated of Plant Pathology and Biotechnology Laboratory, Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh University, Egypt.

References

- Abdullah, Z., Khan, M.A. and Flowers, T.J. (2001) Causes of sterility in seed set of rice under salinity stress. J. Agro. Crop Sci. 187, 25-32.
- Abu-Hasan, H. R. H., Nurealam, S., Khatun, M., Rabiul, I. and Abdullah, M. (2015) Evaluation of wheat genotypes for salt tolerance based on some physiological traits. J. Crop Sci. Biotech. 18 (5), 333-340.
- Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105, 121-126.
- Amin, A.A., Rashad, El-Sh. and Fatma, M. (2008) Changes in morphological, physiological and reproductive characters of wheat plants as affected by foliar application with salicylic acid and ascorbic acid. Aust. J. Basic. Appl. Sci. 2,252-261.
- Amjad, M., Akhtar, J., Haq, M. A., Riaz, M.A. and Jacobsen, S.E. (2014) Understanding salt tolerance mechanism in wheat genotypes by exploring antioxidant enzymes activity. *Pak. J.Agric. Sci.* **51**, 1-8.
- Arfan, M., Athar, H. R. and Ashraf, M. (2007) "Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress?" J. Plant Physiol. 164 (6), 685-694.
- **Desoky, S.M. and Merwad, A. M. (2015)** Improving the salinity tolerance in wheat plants using salicylic and ascorbic acids. *J. Agric. Sci.* **7**(10), 203-217.
- Duncan, B.D. (1955) Multiple ranges and multiple F-test. Biometri.11, 1-42.
- El-Lethy, S.R., Abdelhamid, M.T. and Reda, F. (2013) Effect of potassium application on wheat (*Triticum aestivum* L.) cultivars grown under salinity stress. *World Appl. Sci. J.* 26, 840-850.
- FAO (2014) FAO land and plant nutrition management service http://www.fao.org/ag/agl/agll/spush [Last accessed 25 April2014].
- Ghassemi, F., Jakeman, A.J. and Nix, H.A. (1995) "Salinization of Land and Water Resources". University of New South Wales Press Ltd., Canberra, Australia, pp. 562.
- **Gomez, K.A. and Gomez, A.A. (1984)** "Statistical Procedures for Agricultural Research". John Wiley and Sons, 2nd ed. pp.180, New Yourk, U.S.A.
- Gonzalez, L. and Gonzalez -Vilar, M. (2001) Determination of relative water content. In: "Handbook of Plant Ecophysiology Techniques" Reigosa, M. J.(Ed.). pp. 207-212, Dordrecht: Kluwer Academic,.

- Hafez, E.M. and Abdelaal, Kh.A.A. (2015) Impact of nitrogen fertilization levels on morphophysiological characters and yield quality of some maize hybrids (*Zea mays* L.). *Egypt. J. Agron.* 37 (1), 35-48.
- Hafez, E.M. and Abou El-Hassan, W.H. (2015) Nitrogen and water utilization efficiency of barley subjected to desiccated conditions in moderately salt-affected soil. *Egypt. J. Agron.* **37**(2), 231 -249.
- Hafez, E.M. and Gharib, H.S. (2016) Effect of exogenous application of ascorbic acid on physiological and biochemical characteristics of wheat under water stress. *Int. J. Plant Prod.* 10(4), 579-596.
- Hafez, E.M. and Hafez, Y. M. (2016) Pivotal influence of chemical inducers on antioxidant enzyme activities and barley yield under biotic and abiotic stresses. *Egypt. J. Agron.* 38(2), 209-224.
- Hafez, E.M. and Kobata, T. (2012) The effect of different nitrogen sources from urea and ammonium sulfate on the spikelet number in Egyptian spring wheat cultivars on well watered pot soils. *Plant Prod. Sci.* 15(4), 332-338.
- Hafez, E.M, Abou El-Hassan, W.H., Gaafar, I.A. and Seleiman, M.F. (2015) Effect of gypsum application and irrigation intervals on clay saline-sodic soil characterization, rice water use efficiency, growth, and yield. J. Agric. Sci. 7 (12), 208-219.
- Hafez, E.M., Ragab, A.Y. and Kobata, T. (2014) Water-use efficiency and ammonium-N source applied of wheat under irrigated and desiccated conditions. *Int. J. Plant Soil Sci.* 3(10), 1302-1316.
- Hafez, Y. M., Bacsó, R., Király, Z., Künstler, A. and Király, L. (2012) Up-regulation of antioxidants in tobacco by low concentrations of H₂O₂ suppresses necrotic disease symptoms. *Phytopathol.* 102, 848-856.
- Hammerschmidt, R., Nuckles, E. M. and Kuć, J. (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.* 20 (1),73-82.
- Jeon, M. W., Ali, M. B., Hahn, E. J. and Paek, K. Y. (2006) Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM Doritaenopsis plantlets under relative humidity and air temperature. *Environ. Exp. Bot.* 55,183-194.
- Manzoor, K., Noshin, I., Nazima, B., Bashir, A. and Muhammad, A. (2015) Effect of salicylic acid on the growth and physiological characteristics of maize under stress conditions. J. Chem. Soc. Pak. 37(3), 588-593.
- Markwell, J., Osterman, J.C. and Mitchell, J.L. (1995) Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth Res.* 46, 467-472.
- Maswada H.F. and Abd El-Kader, N.I.K. (2016) Redox halopriming: A promising strategy for inducing salt tolerance in bread wheat. J. Agro Crop Sci. 202, 37-50.

- Meneguzzo, S., Navarri-Izzo, F. and Izzo, R. (1999) Antioxidative responses of shoot and root of wheat to increasing NaCl concentrations. J. Plant Physiol. 155, 274-80.
- Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651-681.
- Rahnama, A., Poustini, K., Tavakkol-Afshari, R., Tavakkol-Afshari, Ahmadi, A. and Alizadeh, H. (2011) Growth properties and ion distribution in different tissues of bread wheat genotypes (*Triticum aestivum* L.) differing in salt tolerance. J. Agro. Crop Sci. 197, 21-30.
- Rana, V., Ram, S., Nehra, K. and Sharma, I. (2015) Differential expression analysis of salt stress related genes TaSRG and TaRUB1 in wheat genotypes. J. Wheat Res. 7, 71-73.
- Sakhabutdinova, A. R., Fatkhutdinova, D. R., Bezrukova, M. V. and Shakirova, F. M. (2003) Salicylic acid prevents the damaging action of stress factors on wheat plants. In: Proceedings of the European Workshop on Environmental Stress and Sustainable Agriculture, 7-12 September 2002, Varna, Bulgaria, pp.314-319.
- Shabala, S. and Munns, R. (2012) Salinity stress: physiological constraints and adaptive mechanisms. In: "*Plant Stress Physiology*" S. Shabala, (Ed.). pp. 59-93, CABI Publishing, Wallingford, UK.
- Shakirova, F.M. A.R., Sakhabutdinova, M.V., Bezrukova, R.A. and Fatkhutdinova, D. R. (2003) "Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity, *Plant Science*, 164 (3), 317-322.
- Singh, A., Bhushan B., Gaikwad, K., Yadav, O.P., Kumar, S. and Rai, R.D. (2015) Induced defence response of contrasting bread wheat genotypes under differential salt stress imposition. *Indian J. Biochem. Biophys.* 52, 75-85.
- **Temminghoff, E.E.J.M. and Houba, V.J.G. (2004)** "*Plant Analysis Procedures*", 2nd ed. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Zhang, J. L., Flowers, T. J. and Wang, S. M. (2010) Mechanisms of sodium uptake by roots of higher plants. *Plant Soil*. 326, 45-60.
- Zhu, M., Shabala, S., Shabala, L., Fan, Y. and Zhou, M. X. (2016) Evaluating predictive values of various physiological indices for salinity stress tolerance in wheat. J. Agro. Crop. Sci. 202,115-124.

(*Received* 12/10/2016; *accepted* 7/12/2016)

تأثير حامض السالسيلك على التوزيع الايونى والنشاط الانزيمى وبعض الخصائص المورفولوجية المحصولية فى القمح تحت الارض المتأثرة بالملوحة

عماد الدين ماهر حافظ قسم المحاصيل – كلية الزراعة – جامعة كفر الشيخ - كفر الشيخ –مصر.

أجريت تجربة حقلية لدراسة تأثيرات ملوحة التربة على الصفات المورفولوجية والفسيولوجية لصنفين من محصول قمح الخبز والمختلفين في درجة تحملهم للملوحة وكذلك دراسة التوزيع الايونى ونشاط انزيم المضاد للاكسدة للصنفين المضاف لمهم حامض السالسيلك تحت ظروف الارض المتاثرة بالملوحة خلال المواسم الزراعية ٢٠١٤/٢٠١٣ و ٢٠١٥/٢٠١٤. تم استخدام التصميم القطاعات المنشقة مرة واحدة. والصنفين هم سخا ٩٣ وهو الصنف المتحمل للملوحة والصنف الاخر هو جميزة ٩ وهو الصنف الحساس للملوحة وتم توزيع الاصناف في القطع المنشقة الرئيسية. أما بالنسبة لمستويات حامض السالسيلك فهي (صفر ، ١٠٠ و٢٠٠ ملليجرام / لتر) وتم توزيعها في القطع المنشقة الرأسية خلال المواسم الزراعية ٢٠١٤/٢٠١٣ و ٢٠١٥/٢٠١٤ أوضحت النتائج أن الصنف المتحمل للملوحة سخا ٩٣ أعطى نتائج أعلى في الخصائص المورفولوجية والفسيولوجية عن الصنف الحساس للملوحة جميزة ٩. أدى الرش الورقى بحامض السالسيلك إلى انخفاض تركيز الصوديوم مع الزيادة في النسبة بين البوتاسيوم/الصوديوم في نصل ورقة العلم وذلك في الصنف سخا ٩٣ مقارنة ب الصنف جميزة ٩. كذلك أدى الرش الورُقى بحامض السالسيلك إلى زيادة تركيز البوتاسيوم وانخفاض تركيز الصوديوم بالصنف جميزة ٩ مقارنة باالنباتات الغير معاملة بحامض السالسيلك جنبا إلى التحسين الملحوظ في التوزيع الأيوني نتيجة اضافة حامض السالسيلك وجد ايضا زيادة نشاط الانزيمات المضادة للاكسدة (الكاتاليز و البيروكسيديز) وذلك عند اضافة المستوى الاعلى من حامض السالسيلك خلال موسمى النمو تحت الدراسة. يمكن الاستنتاج أن الرش الورقي باضافة ٢٠٠ ملليجرام / لتر من حامض السالسيلك كان له تأثير فعال على زيادة صفات المحصول ومحصول الحبوب تحت ظروف الأرض المتأثرة بالملوحة

469