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Application of Salicylic Acid to Improve Seed Vigor and Yield of Some Bread Wheat Cultivars (*Triticum aestivum* L.) Under Salinity Stress

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ABSTRACT

A laboratory study was aimed to examine the influence of seed pre-sowing treatments with different concentrations of salicylic acid (distilled water, 100, 300 and 500 ppm) on germination and seedlings vigor of three wheat cultivars i.e. Giza 168, Sakha 94 and Gemmiza 9 under salinity stress conditions during 2011 season. Also, field experiments were conducted to evaluate the same previous treatments on emergence, growth and yield during 2011/13 seasons. The results indicated that salinity stress significantly decreased seed and seedlings vigor, chemical traits and yield. Giza 168 cultivar gave the highest values of seed and seedlings vigor, chemical, physiological and yield traits as compared with the other cultivars under normal conditions. But, the best results of seed and seedlings vigor produced from Sakha 94 cultivar under salinity stress conditions. While, the lowest values of all studied traits was obtained from Gemmiza 9 cultivar under normal and salinity conditions. Treating wheat seeds with 100 ppm salicylic acid improved germination criteria and seedlings characters as well as chemical and physiological traits under saline conditions as compared to untreated seed and the other treatments. While, had no effective impact on plant height, number of grains/spike and spike length under normal and salinity stress conditions compared with the other treatments. Using salicylic acid at 100 ppm had significant effect on yield through (field emergence%, number of spike/m^2) only. While, seed treated with 300 or 500 ppm salicylic acid was most weakness. It could be concluded that Giza 168 cultivar was the best in the normal condition but Sakha 94 cultivar was suitable for sownig under salintiy stress conditions and considered as plant materials which are useful to breeders for future development of salt tolerant wheat cultiavrs. Also, using 100 ppm of salicylic acid as seed treatment to improve seed germination and seedlings vigor, field emergence percent, yield and alleviation the harmful effect of salinity.

Key words: *Triticum aestivum* L., salicylic acid, salinity stress, cultivars, germination, yield

INTRODUCTION

Salinity is one of the most important obstacles that stand in the way of agricultural development in all countries of the world. Saline area increases in the world at a rate of 10% per year and this is considered extremely dangerous (Ponnamierumo, 1984). In Egypt, soil salinization is the most important and serious problems in the Egyptian land. For example, 33% of the cultivated land, which comprises only 4% of total land area, is already salinized (Ghassemi *et al.*, 1995). Salinity is an inhibitor environmental multifaceted, including the accumulation of ions to high concentrations that it becomes a toxic especially sodium and chlorine. Also, accumulation of these ions lead to a reduction in the ability to absorb the essential elements (e.g., potassium and calcium), (El-Bassiouny and Bekheta, 2001). Salinity caused poor germination and negatively affects seedling development. It is an enormous problem adversely affecting growth and development of crop plants and results to low agricultural production (Garg and Gupta, 1997).

Many researchers have demonstrated that the seedling stage must be taken into consideration when an examination of varieties and lines salinity-tolerant are done (Ashraf, 1999). The relationship between the different characteristics of seedling growth and yield components under saline conditions is very important when developing a salinity cultivar tolerant of production in saline conditions (Mujeeb-ur-Rahman *et al.*, 2008).

Salicylic acid is seen as a hormone or growth regulator and its role in the defense mechanisms against biotic and a biotic stress has been well documented (Szalai *et al.*, 2000). Using salicylic acid significantly increased resistance to drought and salinity of plants (Tari *et al.*, 2002). The soaking of wheat seed in 0.05 mM SA also reduced the harmful effect of salinity on seedlings growth which accelerated the growth processes (Shakirova *et al.*, 2003). Exogenous application of salicylic acid can encourage salt tolerance and water stress tolerance in wheat (Arfan *et al.*, 2007). The aim of present study was to reveal whether wheat plants pre-treated with different concentrations of SA could tolerant salt stress.

MATERIALS AND METHODS

Laboratory experiment was carried out at Seed Technology Research Unit Mansoura, Dakahlia Governorate, Seed Technology Research Department, Field Crop Research Institute, Agricultural Research Center during 2011 season to evaluate the effect of seed treatments with different concentrations of salicylic acid (0, 100, 300 and 500 ppm) on inducing seed germination behavior and seedlings vigor of three wheat cultivars (Giza 168, Sakha 94 and Gemmiza 9) under salinity stress conditions (2 and 10 dS m^{-1}). A factorial in completely randomized design with four replicates was used. Also, field experiments were conducted at the experimental Farm of Tag AL-Ezz, Agric. Res. Station, ARC, Dakahlia Governorate, Egypt during 2011-2012 and 2012-2013 seasons. A factorial with three factors (salinity stress, cultivars and salicylic acid treatments) in split split plot design, the main plots for salinity levels, sub plots for cultivars and sub-sub plots for seed treatments with salicylic acid concentrations with four replications were used in each season and combining analysis for two seasons was done to investigate the impact of seed treatments with different concentrations of salicylic acid (0, 100, 300 and 500 ppm) under salinity stress conditions (1-2 and 9-10 dS m^{-1}) on emergence, growth and yield of the same three introduced wheat cultivars under laboratory conditions.

Wheat cultivars: Three wheat cultivars (*Triticum aestivum* L.) were obtained from Central Administration of Seed (CAS).

Seed treatments: Seeds were surface-sterilized in 1.5% sodium hypochlorite solution (NaOCl) for 5 min to avoid fungal invasion. Then, sterilized seeds were soaked in different concentration of salicylic acid solution for 2 h according to each treatment. Seed soaking treatments were: 0 (soaking seeds in distilled water), 100, 300 and 500 ppm of salicylic acid.

Laboratory experiment: Fifty seeds from each treatment were placed in sterilized perti dishes (15×1.5 cm) on Whatman No. 1 filter paper that was moistened with 10 mL of salinity solution of rashid salt (2 and 10 dS m^{-1}). Each petri-dish kept close together and incubated at 20±2°C, with a 12 h photoperiod and 70% relative humidity, then, four replications were used to evaluate subjected to standard germination test as the rules of International Seed Testing Association (ISTA, 1999).

Seed and seedlings vigor characters: Counts of germinating seeds were taken daily up to eight days after the start of germination. Speed Germination Index (SGI), Germination Rate (GR), co-efficient of germination, germination percentage, seedlings length (cm) and seedlings dry weight (g) were determined in this experiment. SGI was calculated as described in the Association of Official Seed Analysis (AOSA, 1983) by following equation:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \ldots + \ldots + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

Seeds were considered germinated when the radicle was at least 2 mm long.

Germination rate (GR) was defined according to the following formula of Bartlett, 1937:

$$GR = \frac{a + (a+b) + (a+b+c) + \ldots + (a+b+c+m)}{n\,(a+b+c+m)}$$

Where a, b, c are number of seedlings in the first, second and third count, m is number of seedlings in final count, n is the number of counts.

Co-efficient of germination (CG) was calculated using the following formula (Copeland, 1976):

$$\text{Co-efficient of germination} = \frac{100\,(A_1 + A_2 + \ldots\ldots\ldots A_n)}{A_1T_1 + A_2T_2 + \ldots\ldots\ldots A_nT_n}$$

Where:

A = No. of seed germinated

T = Time (days) corresponding to A

n = No. of days to final count

Germination% and seedlings characters after eight days. Germination percentage was defined as the total number of normal seedlings at the end of the test. Seedlings length (cm) were determined from 10 normal seedlings and then dried in a forced air oven at 70°C for 48 h to obtain seedlings dry weight (g) under laboratory conditions according to Krishnasamy and Seshu (1990).

Chemical and physiological characters: Chlorophyll concentrations (mg/g fresh weight) were determined at 645, 663 and 450 nm for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids, respectively and estimated by the equations of Witham *et al.* (1971). Dried seedlings samples were finely ground in a stainless steel miller. Shoot samples were wet digested with a HNO3 and $HClO_4$ acid mixture and analyzed by a flame photometer (Jackson, 1973) to determine K^+ and Na^+ (mg dry^{-1} weight).

Water uptake percent was calculated by the formula (Mujeeb-ur-Rahman *et al.*, 2008) given below:

$$\text{Water uptake} = \frac{W2 - W1}{W1} \%$$

W1 = Initial weight of seed
W2 = Weight of seed after absorbing water in a particular time

Osmotic potential (-MPa): Seedlings were dried in an oven at 70°C for 48 h. then, they were soaked in distilled water (1 g per 10 mL) for 24 h at ambient temperature (25°C±2). The extract was obtained by filtering the mixture through a Whattman # 42 filter paper. The electrical conductivity of these extracts were determined and converted to osmotic potential (-MPa) using the formula (Khaliq *et al.*, 2012).

$$\text{Osmotic potential (-MPa)} = \text{Ec (ds m}^{-1}) \times -0.036$$

Field experiments: The three wheat cultivars were evaluated under two salinity levels (1-2 and 9-10 dS m^{-1}) using different concentration of salicylic acid. Grains were sown on 18th November 2011-12 and 23rd November 2012-13 seasons. The experimental plot area was 3×3.5 m occupied an area of 10.5 m^2. Wheat seed at a rate of 60 kg/fed was used. The preceding summer crop was rice and after harvesting the land was well prepared and Calcium Superphosphate (15.5% P_2O_5) at a rate of 100 kg/fed was added on the dry soil before ploughing. Urea (46% N) at a rate 163 kg/fed was added at three doses.

Studied characters

Field emergence, growth and yield characters: Field emergence percent was determined after 8 days from planting 400 seed at area 1.25 m^2 and emergence index was calculated using the formula of Scott *et al.* (1984):

$$EI = \{TiNi/S\}$$

where Ti is the number of days after sowing, Ni is the number of seeds germinated on day i and S is the total number of seeds planted.

After maturity, ten plants were randomly selected from each plot to estimate plant height (cm). At harvesting time, the inner one square meters was randomly chosen from each plot to determine the number of spikes/m^2, spike length (cm) and number of grains/Spike. Grain yield was determined by harvesting whole plants in each plot in kg/square meter and then it was converted to record grain yield (ardab/fed), ardab = 150 kg per wheat.

Statistical analysis: The data were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) procedure. Means were separated using LSD test at 0.05 level of probability (Gomez and Gomez, 1984).

RESULTS

Laboratory experiment

Seed and seedlings vigor characters: Results of germination%, speed germination index, germination rate, co-efficient of germination, seedlings length and seedlings dry weight of wheat cultivars as affected by seed treatment with salicylic acid concentrations under two salinity levels are presented in Table 1. Salinity levels significantly affected the seed and seedlings vigor characters. Salinity level (2 dS m^{-1}) was lower harmful than (10 dS m^{-1}) in connection with germination%, Speed Germination Index (SGI), Germination Rate. (GR), co-efficient of germination (CG), seedlings length (cm)and seedlings dry weight (g) which were 81.8, 70.5, 69.8, 0.77, 15.5 cm and 0.244 g, respectively.

Regarding to cultivars effect, significant differences were observed among cultivars in all characters in Table 1. Giza 168 cultivar showed superiority in all traits, which were 81.8, 62.5, 0.69, 62.5, 13.9 cm, 0.254 g, respectively. On the other hand, the lowest results of the aforementioned characters were obtained from Gemmiza 9 cultivar.

In connection with salicylic acid concentrations effect as a seed treatment, the highest values of all studied characters were produced from treating wheat seed with 100 ppm salicylic acid (Table 1) which were 89.2, 67.5, 0.77, 67.3, 14.8 cm, 0.244 g, respectively. While, soaking wheat seed in salicylic acid at 300 or 500 ppm caused negative effect and gave the lowest values compared with control.

Chemical and physiological characters: Table 2, osmotic potential (MPa), water uptake%, chl a (mg g^{-1} fresh weight), chl b (mg g^{-1} fresh weight), carotenoids (mg/g fresh weight), K^+ content (mg g^{-1} dry weight) and Na^+ content (mg dry^{-1} weight) significantly affected with increasing salinity levels, values of the aforementioned characters except osmotic potential decreased when salinity level increased from 2 to 10 dS m^{-1}.

The lowest values of osmotic potential (-MPa) which was -0.017 MPa and highest water uptake%, chl a (mg g^{-1} fresh weight), chl b (mg g^{-1} fresh weight), carotenoids (mg g^{-1} fresh weight), K^+ content (mg g^{-1} dry weight) and Na^+ content (mg dry^{-1} weight) were obtained from Giza 168 cultivar which were 6.8, 1.89, 1.21, 0.63, 6.0 and 4.3, respectively. While, Gemmiza 9 cultivar gave the lowest values of the previous characters in Table 2, except osmotic potential character, Gemmiza 9 cultivar recorded the highest value of potential (-0.011 MPa).

Table 1: Seed and seedlings vigor characters of wheat cultivars as affected by seed treatments with salicylic acid under salinity stress conditions

	Characters					
Treatments	Germination (%)	Seed germination index (SGI)	Germination rate (GR)	Co-efficient of germination (CG)	Seedlings length (cm)	Seedlings dry weight (g)
Salinity levels (dS m^{-1})						
2	81.8	69.8	0.77	70.5	15.5	0.244
10	75.0	47.1	0.58	47.9	10.4	0.174
F. test	**	**	**	**	**	**
Cultivars						
Giza 168	81.8	62.5	0.69	62.5	13.9	0.254
Sakha 94	77.9	59.1	0.68	61.0	13.6	0.215
Gemmiza 9	76.0	53.6	0.64	54.1	11.7	0.177
LSD at 5%	1.1	1.3	0.01	1.1	0.3	0.013
Salicylic acid (ppm)						
Distilled water(control)	87.7	64.8	0.74	66.0	14.4	0.228
100	89.2	67.5	0.77	67.3	14.8	0.244
300	70.5	51.8	0.60	52.8	11.6	0.190
500	66.8	49.5	0.57	50.3	11.0	0.183
LSD at 5%	1.3	1.5	0.02	1.3	0.3	0.014

Table 2: Physiological and chemical characters of wheat cultivars as affected by seed treatments with salicylic acid under salinity stress conditions

	Characters						
Treatments	Osmotic potential (MPa)	Water uptake (%)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	K^+ content (mg DW)	Na^+ content (mg DW)
Salinity levels (dS m^{-1})							
2	-0.016	7.1	2.05	1.28	0.68	6.0	4.4
10	-0.011	4.5	1.40	0.88	0.44	5.6	4.8
F.test	**	**	**	**	**	**	**
Cultivars							
Giza 168	-0.017	6.8	1.89	1.21	0.63	6.0	4.3
Sakha 94	-0.013	6.2	1.83	1.14	0.59	6.0	4.6
Gemmiza 9	-0.011	4.7	1.50	0.89	0.45	5.4	4.9
LSD at 5%	-0.001	0.2	0.04	0.03	0.01	0.2	0.3
Salicylic acid (ppm)							
Distilled water (control)	-0.015	6.7	1.92	1.21	0.63	6.3	4.6
100	-0.017	6.9	1.98	1.25	0.65	6.4	4.4
300	-0.012	5.2	1.56	0.98	0.50	5.3	4.7
500	-0.011	4.8	1.47	0.89	0.44	5.3	4.8
LSD at 5%	-0.002	0.2	0.04	0.03	0.01	0.2	0.4

Treating wheat seed with salicylic acid at 100 ppm had positive impact on physiological and chemical traits of seedlings, it recorded the highest results of water uptake% (6.9), chl a, chl b, carotenoids (1.98,1.25 and 0.65 mg g^{-1} fresh weight, respectively), K^+content (6.4 mg g^{-1}dry weight) and gave the lowest Na^+ content (4.4 mg dry^{-1} weight). Also, succeeded in decreasing osmotic potential from -0.015 for control to -0.017 MPa for salicylic acid (100 ppm).

Field experiments

Field emergence, growth and yield characters: Regarding to salinity levels, cultivars and salicylic acid concentrations effect on field characters. Field emergence (FE), emergence index (EI) plant height, number of spikes/m^2, spike length, number of grains/spike and grain yield (ardab/fed) showed direct relation with increase in salinity levels (Table 3). Increased salinity levels from 1-2 to 9-10 dS m^{-1} significantly decreased all previously mentioned characters (Table 3).

With respect to cultivars effect, Giza 168 cultivar demonstrated superiority as compared with the other cultivars of all field characters under study (Table 3). On the other hand, Gemmiza 9 recorded the lowest values of all previously mentioned characters.

Wheat seed treatment with salicylic acid (100 ppm) significantly affected all field characters in Table 3, except plant height, spike length and number of grains/spike, no significant differences were observed between control and seed treatment with salicylic acid (100 ppm) of three previously mentioned characters. But, wheat seed treatment with salicylic acid (300 or 500 ppm) negatively effect on all field characters in Table 3.

The interaction effect between salinity levels and cultivars on germination index, germination rate and seedlings length (cm) are shown in Fig. 1. Giza 168 cultivar under salinity level 2 dS m^{-1} recorded the highest values of germination index, germination rate and seedlings length (cm) but in salinity level 10 dS m^{-1}, Sakha 94 was the best of three traits in Fig. 1. On other hand, Gemmiza 9 cultivar produced the lowest values of previously characters.

Table 3: Field emergence, growth and yield of wheat cultivars as affected by seed treatments with salicylic acid under salinity stress conditions (combined data)

	Characters						
Treatments	Field emergence (FE)	Emergence index (EI)	Plant height (cm)	No. of spike/m^2	Spike length (cm)	No. of grains/spike	Grain yield (Ardab/fed.)
Salinity levels (dS m^{-1})							
1-2	77.3	5.41	99.4	261.3	11.4	59.3	16.3
9-10	70.3	4.92	63.2	192.8	7.3	38.4	10.9
F. test	**	**	**	**	**	**	**
B. Cultivars							
Giza 168	77.5	5.43	88.5	247.6	10.6	57.1	14.5
Sakha 94	73.9	5.17	80.6	227.1	9.0	49.0	13.8
Gemmiza 9	70.1	4.91	74.7	206.0	8.3	40.4	12.7
LSD at 5%	1.4	0.05	0.5	1.6	0.1	0.3	0.4
Salicylic acid (ppm)							
Distilled water (control)	81.2	5.68	86.6	247.0	10.1	52.7	15.0
100	84.0	5.88	87.0	256.0	10.1	53.3	15.4
300	67.0	4.69	76.6	208.0	8.7	45.5	12.3
500	63.2	4.42	74.9	195.0	8.3	43.8	11.9
LSD at 5%	1.1	0.04	0.9	1.7	0.2	1.1	0.3

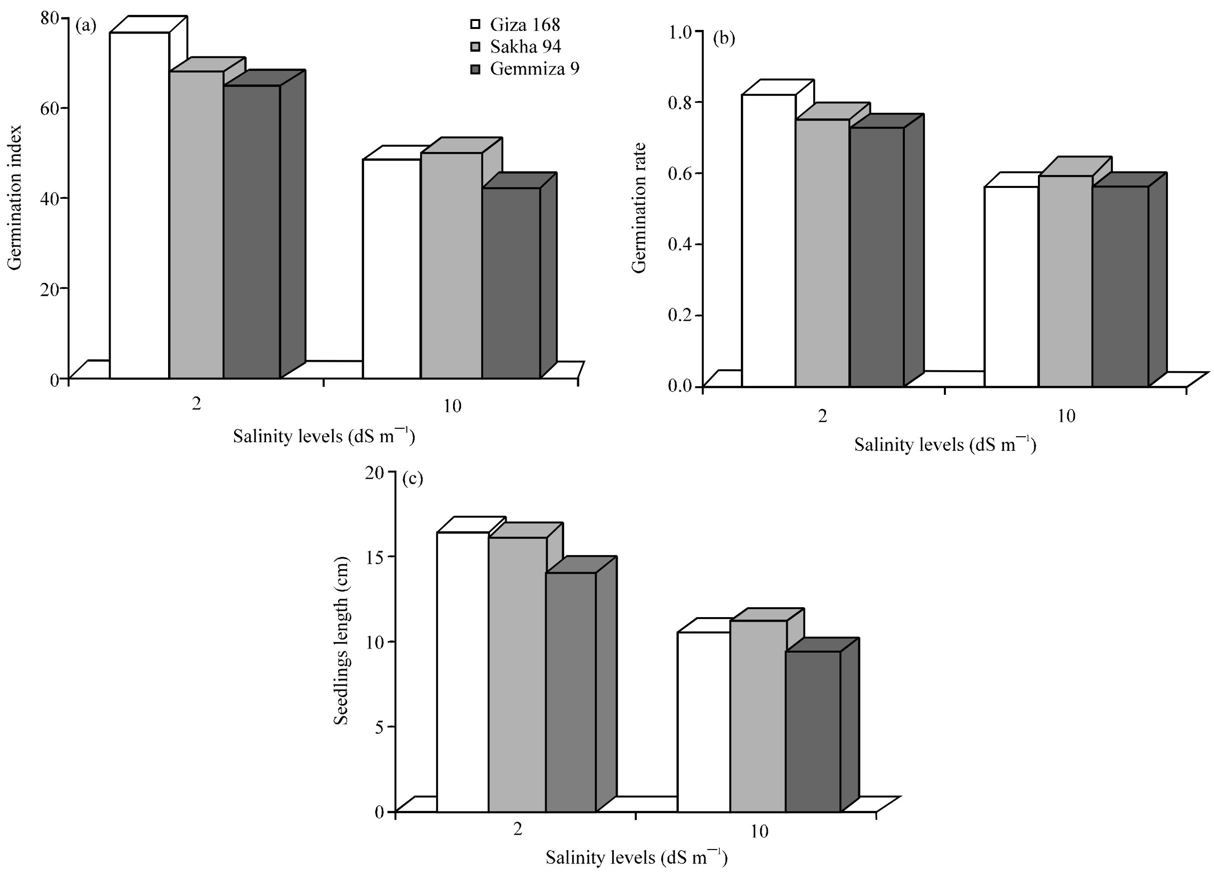

Fig. 1(a-c): Effect of interactions between salinity levels and cultivars on (a) Germination index, (b) Germination rate and (c) Seedlings length

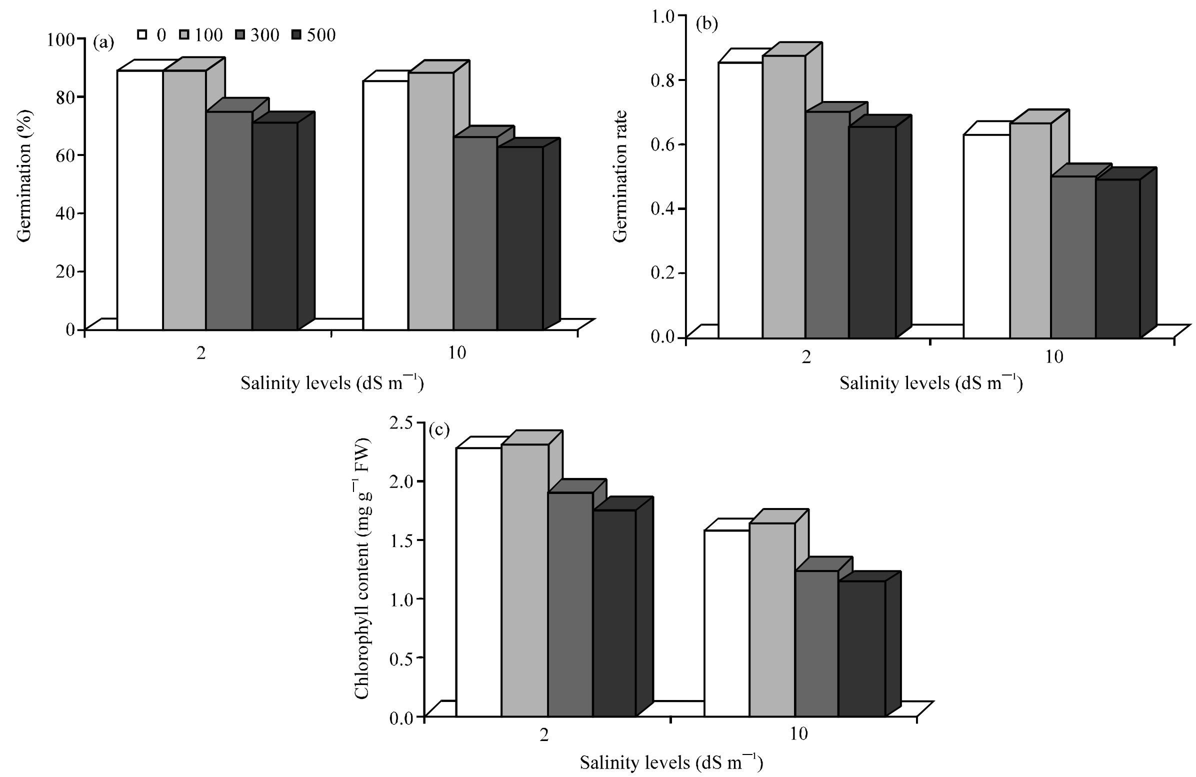

Fig. 2: Effect of interactions between salinity levels and salicylic acid concentrations on (a) Percentage germination%, (b) Germination rate and (c) Chlorophyll content (mg g^{-1} fresh/weight)

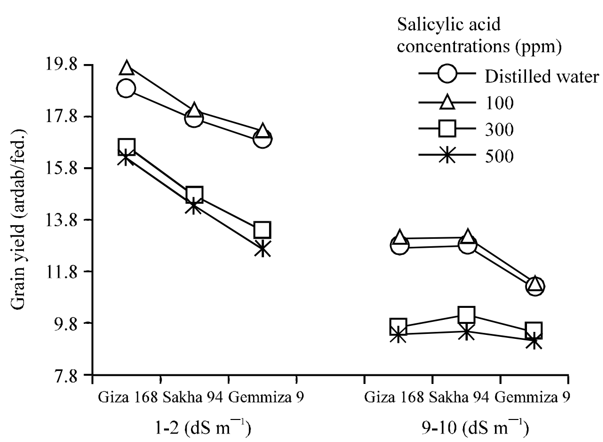

Fig. 3: Effect of the interaction between salinity levels (dS m^{-1}), cultivars and salicylic acid concentrations (ppm) on grain yield (combined data).

The interaction between salinity levels and salicylic acid concentrations significantly affected germination%, germination rate and chl a content (mg g^{-1} fresh weight) (Fig. 2). Wheat seed soaking in salicylic acid (100 ppm) under salinity level 2 dS m^{-1} was the best as compared with the other concentrations. Whilst, wheat seed treatment with salicylic acid (300 or 500 ppm) had negative effect on mentioned characters in Fig. 2.

Interaction effect between salinity levels (dS m^{-1}), cultivars and salicylic acid concentrations on grain yield (ardab/fed.) are shown in Fig. 3. Giza 168 cultivar recorded the highest value of grain yield with seed treatment salicylic acid (100 ppm) in salinity level (1-2 dS m^{-1}) compared with other treatments under study. But, Sakha 94 cultivar with seed treatment salicylic acid (100 ppm) was the best under salinity level (9-10 dS m^{-1}). The lowest value of grain yield was obtained with seed treatment salicylic acid (500 ppm) of Gemmiza 9 cultivar under salinity level (9-10 dS m^{-1}).

DISCUSSION

The negative impact of salinity is by raising the osmotic pressure of the soil surrounding the seed to the point that inhibit the absorption of water required for the mobilization of the essential nutrients necessary for the germination process. In addition to the toxic effect of some ions at high concentrations, which in turn caused damage to the embryo (Mujeeb-ur-Rahman *et al.*, 2008). Maghsoudi and Maghsoudi (2008) demonstrated that germination and emergence rates, as well as coleoptile and root length could be used as selection criteria for salt stress tolerance at early growth stages. The wheat cultivars showed different responses to salt stress at salinity level (10 dS m^{-1}). Sakha 94 cultivar appeared to be more tolerance at germination stage. It may be due to that Sakha 94 recorded highest value of water uptake as shown in Fig. 1 and higher degree of osmotic adjustment through the increasing in the uptake rate of K^+ and K/Na ratio which greatly exceeded that in the other varieties under study.

In this study, wheat seed soaking in 100 ppm salicylic acid were more effective than other treatments. These results are in consistent with those of Shakirova *et al.* (2003), who showed a promotion in seed germination with SA application. It is assumed that SA increases level of cell division in the apical meristem of seedlings root, which causes an increase in plant growth resulting in protective and growth promotion. As well as treated with SA maintain the level of plant hormones, especially IAA and cytokinin which reduced stress-induced inhibition of wheat growth. Moreover, high ABA levels were preserved in SA treated wheat seedlings, which induced the development of antistress reactions and maintenance of proline accumulation (Sakhabutdinova *et al.*, 2003). These observations are in agreement with those of Khodary (2004), who reported that SA increases the fresh and dry weights of shoot and roots of stress maize plants. Barely seeds pretreated with 10.2 M/L SA solution lead to higher chlorophyll content and significantly increased the pigment content under salt stress (El-Tayeb, 2005). Soaking seed in salicylic acid solution 100 ppm had no effective impact on plant height, number of grains/spike and spike length under normal and salinity stress as compared with the other treatments as shown in Table 3.It may be due to, that soaking has temporal effect and cannot continue for long time. So, soaking affected the yield through field emergence percentage which affected number of spike/m^2. The present experiment indicated that salicylic acid play critical roles in plant responses to salinity and it can be concluded that treating seed with 100 ppm salicylic acid increase the ability of wheat to grow successfully under saline stress conditions. Giza 168 wheat cultivar was the best in the normal condition but Sakha 94 wheat cultivar was suitable for sowing under salinity stress condition and it may be considered the best parents for salinity recovering ability and it could be considered as plant materials which are useful to breeders for future development of salt tolerant wheat cultivars to produce new crosses with desirable characters related to salinity tolerance.

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