



Amelioration the adverse effect of salt stress in two barley cultivars by using gibberellic acid

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Abstract: An alternative strategy to ameliorate salt stress could be to use exogenous application of plant growth regulators. The present investigation was carried out to study the effect of exogenous application of GA₃ on germination, some growth parameters, protein banding pattern, nucleic acids content and some minerals of salt stressed two cultivars of barley. The grains of both cultivars Giza 126 and 123 were individually pre emergence treated with different concentrations of NaCl or mixture of NaCl and GA₃ for 7 days. The results of germination percentage revealed that cv. Giza 123 had higher response to the interaction between salinity and GA₃ than cv. Giza 126. The morphological characters revealed inhibition in all plant growth parameters by salinity stress and this inhibition was significantly alleviated after treatment with GA₃. The protein electrophoresis of seedlings of cv. Giza 123 induced formation of unique newly protein bands including certain units may acting as protein receptors for gibberellins (M.W. 71.41 kDa). In addition, the DNA and RNA content of cv. Giza 123 showed its priority to the interaction between salinity and GA₃. Regarding to minerals the data showed that cv. Giza 123 had the ability to discard salts and prevent the accumulation of them in its tissues especially Na⁺, K⁺ and Ca²⁺ elements. cv. Giza 123 seedlings could overcome the inimical effects of salt stress whereas cv. Giza 126 seedlings appeared to have an attenuated potential to do so resulting in an efficient tolerance of cv. Giza 123 seedlings.

[Abdel-Hamid A.M.E., Mohamed H.I. **Amelioration the adverse effect of salt stress in two barley cultivars by using gibberellic acid.** *Biomedicine and Nursing* 2023;9(3):24-34]. ISSN 2379-8211 (print); ISSN 2379-8203(online) . <http://www.nbmedicine.org> 05. doi:[10.7537/marsbnj090323.05](https://doi.org/10.7537/marsbnj090323.05).

Keywords: germination, growth, *Hordeum vulgare*, minerals, nucleic acids, protein banding pattern.

1. Introduction

Salinity is a major problem that restricts yield of almost 40 million hectares of irrigated land, which is approximately one-third of the irrigated land on earth. It was estimated that about 50% of the arable land will be affected by salt stress by the year 2050 (Munns and Tester, 2008). Saline soils possess high levels of sodium and chloride contents and thus exert adverse abiotic stress on plants. Salinity induces osmotic and ionic imbalance inside plant's cell, thus affecting plant growth and metabolism (El-Beltagi et al., 2013, Mohamed and Gomaa, 2012, El-Mashad and Mohamed, 2012, Hussain et al., 2008).

Different strategies have been employed to improve crop growth under saline conditions. One of these is to produce salt tolerant genotypes in different crops (Ashraf et al., 2002, Shannon and Grieve, 1999). The effects of salinity can also be minimized with improved irrigation and drainage techniques but the cost of engineering and management is very high. Increasing costs for water and energy also emphasize the need for an alternative strategy (Shannon, 1984).

Plant hormones are active members of the signal cascade involved in the induction of plant stress responses (Pedranzani et al., 2003). The exogenous

application of gibberellic acid (GA₃) improved tolerance under abiotic stress by induction and increasing of the endogenous levels of salicylic acid (Alonso- Ramirez et al., 2009). Gibberellic acid has been reported to increase germination percentage and seedling growth and overcome the preventive effects of the salt stress on germination (Kabar and Baltepe, 1987). Also, Kaur et al. (1998) reported that gibberellic acid increases percentage of germination and seedling growth under salt stress. A few studies have, however, demonstrated the ability of foliar pretreatment with GA₃ to overcome adverse effects of NaCl stress (Chakraborti and Mukherji, 2003).

The aim of the present study was to assess the effect of different concentrations of NaCl on germination, growth, protein banding pattern, nucleic acids content and some minerals on two salt-stressed barley cultivars and to alleviate the effect of NaCl by addition of plant growth regulation GA₃.

2. Material and Methods

Grains of two cultivars of *Hordeum vulgare*; cv. Giza 126 and Giza 123 were kindly obtained from Barley Research Department, Field Crops Research Institute, Agriculture Research Center (ARC), Giza,

Egypt. The grains of both cultivars were individually pre-emergence treated with different concentrations of sodium chloride (NaCl) or mixture of sodium chloride (NaCl) and gibberellic acid (GA₃) for 7 days. The concentrations used from sodium chloride (NaCl) were: 0.0, 100 and 300 mM, and The concentrations used from gibberellic acid (GA₃) were: 0.0 and 100 μM. Such range of the concentrations was chosen after determination of the ineffective concentration and the lethal one of sodium chloride (NaCl) from the data of a preliminary experiment carried out firstly for this purpose.

Commercially produced germination paper towels of the same weight, thickness, and size were used. This method was modified from the method of Hampton and Tekrony (1995). Twenty grains were placed on a paper towel and then the mean of the twenty grains were taken as a one replicate (three replicates were used for each treatment). Plastic containers were used to soak the papers in the various treatments. Plastic bags were used to keep the towels rolls moistened. All grains were kept inside an electric incubator at 20 °C±2 for 7 days where grains germination percentage was assessed. The produced seedlings were divided into 3 sets. The first one was taken for germination percentage and some growth parameters. The second set of the produced seedlings were air dried and used for estimation of total nucleic acids content and protein banding pattern. The third one of the produced seedlings was oven dried and used to estimate some minerals.

Relative water content (RWC)

Fresh weight of seedlings was recorded immediately after collecting the seedlings. The turgid weight (TW) was measured after 24 hrs saturation with deionized water in the dark. Dry weight was determined after drying the leaves for 48 hrs in the hot air oven at 70 °C. The RWC was calculated as:

$$[(FW-DW)/(TW-DW)] \times 100$$

Table 1. Changes in germination percentage of grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to their pre-emergence treatment with different concentrations of NaCl with or without GA₃ for 7 days. Means followed by * and ** are highly significant change and significant different at $P \leq 0.01$, according to least significant difference (LSD) test.

Treatments NaCl/GA ₃ mM/ μM	Germination (%)	
	cv. Giza 126	cv. Giza 123
0 /0	100.00	100.00
100 /0	77.50 *	100.00
300 /0	67.80 *	97.20 *
0 /100	100.00	100.00
100 /100	88.87 **	100.00
300 /100	80.29 *	97.23 *
LSD at 1%	1.60	0.33

Protein electrophoresis

Gel electrophoresis SDS-PAGE was carried out with gel slabs according to the method of Laemmli (1970). Protein subunit bands were stained with Coomassie blue R-250 by standard techniques. The gel was scanned using Gel pro-Analyzer.

Determination of nucleic acids

DNA and RNA were extracted following the method was similar to that described by Morse and Carter (1949). RNA was estimated colorimetrically by the orcinol reaction as described by Dische (1953) while DNA was estimated by diphenylamine (DPA) color reaction as described by Burton (1956).

Determination of minerals

The method of extraction adopted was that of Chapman and Pratt (1982). Sodium, potassium and calcium were determined photometrically by using flame photometer (B-7-E). Magnesium, iron, manganese, zinc and copper estimated by atomic absorption spectrometry (FMD3).

Statistical analysis

The statistical analysis of the obtained data was done using the Least Significance Difference test (L.S.D.) at 1% and 5% levels of probability (Snedecor and Cochran, 1973).

3. Results

Changes in germination percentage

It is obvious from Table 1 that there is a difference between the two cultivars in their response to salt stress. The results indicated that cv. Giza 123 had the ability to tolerate the stress of salts than the cv. Giza 126. Also, the results showed that, GA₃ at concentration of 100 mM NaCl had eliminated the inhibitory effect of salts by increasing the germination percentages of the tested seeds, especially in case of cv. Giza 126.

Changes in some growth parameters

The length and dry weight of roots were significantly inhibited by salinity stress and effect of treatment with GA₃ is not clearly obvious in the two cultivars. The combined effect of salinity and

Also, the results of fresh root weight and fresh shoot weight of Giza 123 increased significantly with treatment of 100 µM of GA₃ while cv. Giza 126 showed non significant effect. The fresh shoot weight of cv. Giza 126 significantly decreased at 100 and 300

gibberellic acid appeared in Table 2 in which the shoot length and the dry shoot weight of both cultivars highly significant increased when the plants treated with 100 µM of GA₃ and 100 mM salinity.

mM salinity and 100 µM of GA₃ but in cv. Giza 123 showed reduction at 100 µM of GA₃ and 300 mM salinity only. On the other hand, the fresh root weight of treated plants at 100 µM of GA₃ and 100 mM salinity significantly increased.

Table 2. Changes in some growth parameters of the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to their pre-emergence treatment with different concentrations of NaCl with or without GA₃ for 7 days. Means of measurements on each ten replications. Means followed by * and ** are highly significant change and significant different at $P \leq 0.01$, according to least significant difference (LSD) test.

Cultivars	Treatments NaCl / GA ₃	Root length (cm)	Shoot length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)	Relative water content in root (%)	Relative water content in shoot (%)
Giza 126	0 / 0	18.64	5.99	2.217	1.55	0.162	0.158	92.68	89.51
	100 / 0	13.68*	4.73*	0.667*	0.21*	0.353*	0.131	33.32*	37.28*
	300 / 0	6.16*	0.95*	1.000*	1.38*	0.034*	0.028*	96.60*	97.97*
	0 / 100	17.69**	8.10*	1.247*	0.96*	0.161	0.165*	85.88*	72.10*
	100 / 100	16.44**	6.97*	1.489*	1.25*	0.102*	0.162*	93.17	85.73**
	300 / 100	6.95*	0.45*	0.232*	0.44*	0.053*	0.032*	79.10*	91.50
LSD at 1% I		1.25	0.73	0.16	0.12	0.026	0.015	5.46	5.10
Giza 123	0 / 0	18.86	6.53	1.172	0.68	0.175	0.163	85.48	80.13
	100 / 0	17.77**	6.70	1.322*	1.44*	0.125*	0.188*	77.56*	84.50*
	300 / 0	5.53 **	0.73 *	0.549*	1.32*	0.066*	0.018*	78.14*	98.60*
	0 / 100	18.89	10.08*	0.732*	1.39*	0.155*	0.230*	78.50*	83.12*
	100 / 100	17.75**	7.43 *	1.745*	1.32*	0.120*	0.188*	77.24*	82.37*
	300 / 100	5.49 *	0.58 *	0.385*	0.29*	0.045*	0.055*	85.04	75.54*
LSD at 1%		1.52	0.88	0.12	0.11	0.012	0.019	0.88	1.79

Changes in protein banding patterns

The protein banding pattern extracted from the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to their pre-emergence treatment with 100 and 300 mM of sodium chloride (NaCl) or mixture of sodium chloride (NaCl) and 100 µM gibberellic acid (GA₃) showed clear differences in the number of bands and their molecular weights and also the intensity of

bands by the appearance of new bands or the disappearance of others (Table 3 and Figure 1).

It is obvious from these results that treatment with (100 mM NaCl / 100 µM GA₃) and (300 mM NaCl / 100 µM GA₃) stimulated the production of two new bands with molecular weights 205.00 and 71.41 kDa in the seedlings of the two cultivars. The treatment of grains of barley with gibberellic acid enhanced their ability to tolerate the bad effect of salinity in two treated cultivars. The cv.

Giza 123 showed to be more tolerant than cv. Giza 126 and these may be to the ability of cv. Giza 123 to produce new protein bands under treatment with GA₃ and kept it under salinity stress but the cv. Giza 126 can not to do so. In cv. Giza 123 treatment with 100 mM NaCl only lead to produce one new protein band (103.79 kDa), in case of 300 mM NaCl only three new bands appeared (103.79, 55.00, 50.50 kDa) and two bands disappeared (205.00 and 71.41 kDa). In case of cv. Giza 126, 100 mM NaCl only lead to produce one new protein band (71.41 kDa), in case of 300 mM NaCl only seven new bands appeared (205.00, 103.79, 97.00, 87.16, 71.41, 55.00, 50.50 kDa) and no bands disappeared when compared with control.

On the protein banding patterns of barley cv. Giza 123 and cv. Giza 126 seedlings were shown to respond to various concentration of NaCl treatments through induction of the appearance of distinct newly protein bands. Such protein bands were shown to characterize the seedlings of each cultivar. The difference in levels and molecular weights of such distinct protein bands in between cv. Giza 123 and cv. Giza 126 seedlings might reflect the difference in gene expression among such two cultivars. In addition, the created distinct protein bands within the tissues of each cultivar appeared to be responsible, to a great extent, for the tolerance and/or the susceptibility of such cultivars to salt stress.

Table 3. Molecular weights and relative concentrations of proteins extracted from the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to treatment with different concentrations of NaCl and GA₃ for 7 days.

Molecular weights (kDa)	cv. Giza 123						cv. Giza 126					
	0	100	300	0	100	300	0	100	300	0	100	300
	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl
	/	/	/	/	/	/	/	/	/	/	/	/
	0	0	0	100	100	100	0	0	0	100	100	100
	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃	GA ₃
205.00	4.88	3.71	-	-	4.86	5.78	-	-	4.75	-	-	4.09
116.00	-	-	-	-	-	-	-	-	-	-	-	-
103.79	-	4.18	3.34	-	3.55	4.13	-	-	2.18	-	5.45	4.42
97.00	-	-	-	-	-	-	-	-	3.06	-	-	-
87.16	-	-	-	-	-	-	-	-	2.86	-	-	-
82.56	2.37	1.53	2.72	1.87	2.74	0.718	1.95	1.86	1.42	3.45	2.15	2.78
78.63	2.49	1.22	3.31	3.79	4.06	3.49	2.64	3.57	3.15	3.12	3.57	2.29
71.41	5.28	5.22	-	-	4.9	4.71	-	4.7	4.3	2.20	4.47	5.74
55.00	-	-	1.89	-	-	2.66	-	-	1.6	-	-	1.7
50.50	-	-	3.08	-	-	2.13	-	-	2.65	-	-	3.26
45.00	-	-	-	-	-	-	-	-	-	-	-	-
43.00	5.24	5.02	5	4.73	4.03	5.53	3.99	3.66	7.5	2.86	3.91	5.93
37.80	5.36	6.87	5.95	4.35	6.02	8.92	5.46	5.38	4.42	-	6.05	6.21
32.07	3.46	7.29	3.94	4.33	3.68	-	-	-	-	-	-	-
29.00	-	-	-	-	-	-	-	-	-	-	-	-
Total number of bands	7	8	8	5	8	9	4	5	11	4	6	9

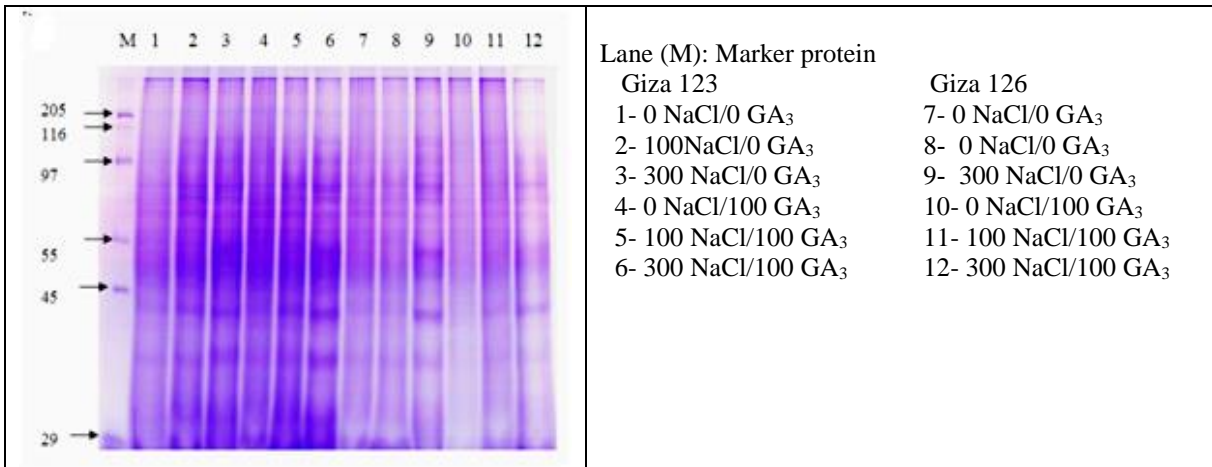


Figure 1. Electrophoretic banding profiles of protein extracted from the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to their pre-emergence treatment with different concentrations of NaCl with or without GA₃ for 7 days.

Changes in nucleic acids content

The results in Table 4 showed that salinity enhanced the induction of RNA in cv. Giza 126 and the highest RNA value was recorded in the higher concentration of salts. The results of DNA reflected

the injurious effect of salts on the seedlings in both cultivars. However, cv. Giza 123 exhibited more tolerance than cv. Giza 126. This tolerance was associated with low decrease rate in DNA content in response to salinity.

Table 4. Changes in DNA and RNA contents ($\mu\text{g g}^{-1}$ dry weight) of root tips of the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to their pre-emergence treatment with different concentrations of NaCl with or without GA₃ for 7 days. Means of measurements on each three replications. Means followed by * are highly significant different at $P \leq 0.01$, according to least significant difference (LSD) test.

Treatments NaCl / GA ₃ mM / μM	Giza 126		Giza 123	
	----- DNA	RNA	----- DNA	RNA
0 / 0	6.20	133.0	5.95	128.8
100 / 0	2.41*	134.0	3.05 *	110.2 *
300 / 0	3.00 *	144.2*	3.66 *	122.3 *
0 / 100	2.65 *	132.1	6.00	109.0 *
100 / 100	2.10 *	127.3*	3.70 *	121.2 *
300 / 100	3.90 *	158.1*	4.00 *	134.0 *
LSD at 1%	0.42	2.59	0.18	2.28

Changes in minerals content

Concentration of Ca²⁺ was significantly decreased in salinity stressed wheat plants cv. Giza 123 and Giza 126. Grains presoaking in GA₃ increased Ca²⁺ but values remained lower than those of control seedlings of the two cultivars (Table 5).

Sodium ions significantly increased in barely cv. Giza 123 and 126 under salinity conditions. Treatment with GA₃ caused reduction in the accumulation of sodium ions in the two cultivars of barely (Table 5). Potassium concentration was much

lower in salinity stressed plants compared to unstressed ones (Table 5). The data also showed that GA₃ application enhanced K⁺ uptake by barley plants at 100 and 300 mM in both cultivars as compared with salt stressed plants. However, this enhancement effect of GA₃ is more pronounced in case of cv. Giza 126.

Data in Table 5 showed that Zn decreased in stressed plants especially in cv. Giza 126. Treatment with GA₃ partially restores Zn levels in cv. Giza 123 when compared with cv. Giza 126 values. All minerals such as Mn, Fe, Co, Pb, Cr and Cd content

significantly decreased in both cultivars of barely except Cd in cv. Giza 126 which showed significant increases. GA₃ application is shown to restore the levels of these minerals to be close to those of control in both cultivars.

4. Discussions

In the present investigation, salt stress caused reduction in germination percentage of seeds. These results are in accordance with El-Bakkosh (2001) reported that the exogenous applications of GA₃ enhanced the germination of many seeds under salinity stress. Also, Fu-Ping et al. (2010) demonstrated that germination energy reduced by salt but the exogenous GA₃ application significantly counteracted the inhibition of salt on germination energy. It is well known that, during the germination process, gibberellic acid is released from the embryo and activates specific genes for α -amylase mRNA transcription (Chandler et al., 1984, Taiz and Zeiger, 1991). Salinity stress reduced germination percentage may be due to It may be that NaCl reduced the rate of germination may be due to the reduced water potential and the resulting slower rate of imbibitions.

Data in Table 2 noticed that inhibition in plant growth was significantly alleviated by 100 μ M GA₃ treatments. These results are in accordance to some Patel and Saxena, (1994) who reported that fresh and dry weight was increased in seedlings raised from seeds treated with kinetin and GA₃. These results further relate with the studies of Angrish et al. (2001)

who reported that amelioration of salinity was due to enhanced N status and nitrate reductase activity through pre-sowing wheat seeds with plant growth regulators like gibberelic acid. These results were concomitant with the opinion of some plant scientists (Datta et al., 1998, Basalah and Mohammad, 1999, Hisamatsu et al., 2000). Also, Kumar and Singh (1996) reported that plant growth of wheat decreased with increasing salinity levels, but was increased by seed treatment with GA₃. NaCl inhibits growth by reducing both cell division and cell enlargement (Sobhanian et al., 2010) and the observed increased seedling growth of stressed plant seeds with GA₃ treatment could be attributed to the positive effect of gibberellic acid, which encourages cell division and cell elongation

Non-treated plants showed a relative water content (RWC) below 100%. RWC declined significantly with salinity stress treatment compared to control (Table 2). RWC reflecting the metabolic activity in tissues (Flower and Ludlow, 1986). Decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process in sugar beet (Katerji et al., 1997). Similar reports have been made for many plant species under salinity stress conditions (Srivastava et al., 1998, Nazar et al., 2011). This decrease in RWC could be because of lower water availability under stress conditions (Shalhevet, 1993), or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000).

Table 5. Changes in minerals content (mg g⁻¹ dry weight) in the seedlings produced from the grains of *Hordeum vulgare* cv. Giza 126 and Giza 123 in response to treatment with different concentrations of NaCl and GA₃ for 7 days. Means of measurements on each three replications. Means followed by * and ** are highly significant change and significant different at $P \leq 0.01$, according to least significant difference (LSD) test.

Treatmen t s NaCl/GA ₃	cv. Giza 126											
	Na	K	Ca	Mg	Mn	Cr	Co	Fe	Zn	Cd	Pb	Cu
0 / 0	27.4	100.0	11.0	7.6	0.74	1.63	1.01	4.55	0.81	1.81	4.57	0.71
100 / 0	45.2*	47.7*	8.6*	5.5*	0.36*	1.49*	0.92*	4.00*	0.56*	1.99*	4.16*	0.60*
300 / 0	54.1*	72.3*	4.1*	4.2*	0.25*	1.78*	0.92*	2.48*	0.30*	1.99*	4.16*	0.60*
0 / 100	10.7*	69.2*	9.6*	6.2*	0.61*	1.63	1.01	2.89*	0.57*	1.99*	4.16*	0.71*
100 / 100	27.4	58.4*	8.9*	6.4*	0.51*	1.63	1.01	3.58*	0.44*	1.18*	4.16*	0.66*
300 / 100	48.2*	16.9*	4.8*	3.7*	0.18*	1.34*	0.82*	1.79*	0.28*	1.81	4.16*	0.55*
LSD at 1%	3.5	6.4	0.63	0.33	0.05	0.035	0.02	0.24	0.046	0.072	0.038	0.015
	cv. Giza 123											
	Na	K	Ca	Mg	Mn	Cr	Co	Fe	Zn	Cd	Pb	Cu
0 / 0	10.7	89.2	31.0	7.45	1.13	1.63	1.19	3.17	0.49	1.99	4.16	0.66
100 / 0	29.2*	69.2*	11.0*	6.55*	0.56*	1.63	1.10	5.52*	0.56*	1.99	4.16	0.66

300 /0	49.4*	18.5*	6.2*	4.40*	0.26*	1.49*	0.92*	1.94*	0.30*	1.81*	3.74*	0.60*
0 /100	10.1*	98.4*	18.3*	7.79*	0.88*	1.78*	0.92*	3.45*	0.86*	1.99	4.57*	0.66
100 /100	23.2*	70.7*	16.2*	6.55*	0.58*	1.49*	3.31*	2.89*	0.39*	1.99	4.16	0.60*
300 /100	43.4	20.0*	8.9*	4.65*	0.26*	0.89*	0.92*	2.91**	0.74*	2.18*	4.16	1.66*
LSD at 1%	4.2	7.86	2.20	0.33	0.079	0.071	0.22	0.28	0.049	0.027	0.060	0.096

The combined effect of salinity and gibberellic acid formed two protein bands in cv. Giza 123 with molecular weights 205.00 and 71.41 kDa and the final band (71.41 kDa) similar to belonged to the gibberellic acid that is mean that this band stimulated the production of GA₃ inside tissues of the treated seedlings and this leads to increase the activity of some enzymes responsible for the growth and differentiations. The other band with molecular weight 205.00 kDa is an evidence to production of polypeptide chains that had the ability to slow the movement of the poisons of sodium cations and prevent it to reached to the growth parts of the plant and this will to increase the ability of cv. Giza 123 to tolerate the bad effect of salinity, in the other side this bands did not appear in cv. Giza 126 so, it is the reason for the susceptibility of this cultivar to salinity. This view is supported by the work of Ashraf and O'leary (1996), Hardie (2005), Ampudia and Lugo (2006).

On the protein banding patterns of barley cv. Giza 123 and cv. Giza 126 seedlings were shown to respond to various concentration of NaCl treatments through induction of the appearance of distinct newly protein bands. From such protein bands, that having molecular weight of 67.45 kDa and was documented to act as protein receptor for gibberellins (Pence and Caruso, 1987) and that having molecular weight of 75.21 kDa and was recorded to act as metal-binding protein or phytochelatin (Grill et al., 1989).

Phytohormones are essential for the ability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses (Peleg and Blumwald, 2011). They often rapidly alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin proteasome system (Santner and Estelle, 2010).

The induction of formation of such specific protein bands among the other ones in the tissues of cv. Giza 123 seedlings and their absence in the tissues of cv. Giza 126 seedlings may be the casual factor of amplifying the signal transduction pathway of the endogenous plant hormones within the tissues of cv. Giza 123 seedlings which would enhance their responsibility to auxins and gibberellin compounds synthesized *in situ*. This would enable such cultivar to

enhance its cell division machinery and operating the mechanism of gene regulation efficiently resulting in production of certain types of functional proteins having the potentiality to overcome the inimical effects of salinity (Fu-Ping et al., 2010). In addition, Sobhanian et al. (2010) reported that in tolerant crops, amino acids accumulate under salt stress, but little or no amino acid accumulation occurs in salt-sensitive crops. This suggests that amino acids may be involved in osmotic adjustment and that manipulating amino acid metabolism may be a promising means of improving the salt tolerance of Poaceae crops.

Salinity enhanced the induction of RNA in cv. Giza 126 but decrease the contents in cv. Giza 123. These results are in accordance with Rong-Chao et al. (2007) who reported that the accumulation of RNA induced in wheat plants grown under salt stress conditions. In case of cv. Giza 123 the concentration of RNA decreases with increasing the concentration of NaCl. After GA₃ application, the concentration of RNA increased in both cultivars and the highest RNA contents were found in the roots subjected to the medium contains 300 mM NaCl and 100 μM GA₃. Similar results obtained by Fujii et al. (2008) who demonstrated the role of plant hormones in regulating the expression of genes especially abscisic acid and gibberellic acid and mRNA is strongly induced by high salinity stress and the possible relation between the contents of endogenous GA₃ and acquisition of stress protection.

These results showed DNA contents in both cultivars decreased by increasing salinity stress. are in harmony with Ismail (2003) reported the salt stress caused a decrease in RNA and DNA content in sorghum plants. Our results showed that, pretreatments of barley grains with GA₃ increased DNA content in grown seedlings under salt stress. It has been found exogenous application of gibberellins produces some benefit in alleviating the adverse effects of salt stress and also improves germination and growth (Afzal et al., 2005). In wheat, the expression of seven proteins related to retrotransposons, gene transcription and translation has been shown to change as a result of salt stress. For example, putative transcription factor, which plays a role in increasing the adaptation of wheat to salt

exposure, is upregulated during salt stress (Wang et al., 2008). It has been suggested that the salt inducible DDF1 gene is involved in growth responses under high salinity conditions in part through altering GA levels and improves seed germination (Kaya et al., 2009). This suggests that the exogenous application of GA₃ may help in overcoming the effect of salinity stress and improves seed germination under saline conditions. Our results are in confirmation with the previous suggestion.

Salinity stress caused reduction in calcium ions content in both cultivars. Similar results obtained by El-Beltagi et al. (2013) who found that salinity caused significant decrease in calcium ions in the roots and shoots of cowpea plants. It has been reported that sodium ions may compete with calcium ions for membrane binding sites. Therefore, it has been hypothesized that high calcium levels can protect the cell membrane from the adverse effects of salinity. It has been reported that calcium plays a vital role in maintaining membrane stability and permeability (El-Tayeb and Ahmed, 2002). Grains presoaking in GA₃ increased Ca⁺² but values remained lower than those of control seedlings of the two cultivars (Table 5). These results are in accordance with Ashraf et al. (2002) and Iqbal and Ashraf (2013) who found that GA₃ treatment enhanced Ca⁺² concentrations in shoot of wheat cultivar. This could be explain why membrane permeability is impaired in leaves of salinity stressed plants where Ca⁺² level is lower and exogenous application of GA₃ enhanced Ca⁺² levels and restored the membrane integrity in saline stressed plants (Table 5). Greater Ca⁺² concentrations in plant tissues may also help in achieving better crop survival with improved plant growth under stress conditions (Cachorro et al., 1994).

Sodium ions significantly increased in barely cv. Giza 123 and 126 under salinity conditions. Similar results obtained by Mohamed and Gomaa (2012). Treatment with GA₃ caused reduction in the accumulation of sodium ions in the two cultivars of barely. These results are in accordance with Iqbal and Ashraf (2013) who found that Treatment with GA₃ (150 mg L⁻¹) decreased Na⁺ concentrations both in the shoots and roots and increased Ca²⁺ and K⁺ concentrations in the roots of both wheat cultivars under salt stress conditions. In addition, reduction in uptake of mineral nutrients under saline conditions may occur due to Na⁺ induced blockage or reduced activity of the transporters, resulting in ionic imbalance of K⁺, Ca²⁺, and Mg²⁺ as compared to Na⁺. On the other hand treatment with GA₃ caused reduction in sodium ions contents in two cultivars.

Potassium concentration was much lower in salinity stressed plants compared to unstressed ones. Similar results obtained by Mohamed and Gomaa

(2012) who found that salinity stress caused highly significant increase in the Na⁺ and Cl⁻ content and decrease in the contents of K⁺, Mg²⁺ and Ca²⁺ in the roots and leaves of radish plants. It is widely accepted that competition exists between Na⁺ and K⁺ leading to a reduced level of internal K⁺ at high external NaCl concentration (Botella et al., 1997). This can result in high Na/K ratios that reduce plant growth and eventually become toxic (Schachtman and Lio, 1999). The data also showed that GA₃ application enhanced K⁺ uptake by barley plants at 100 and 300 mM in both cultivars as compared with salt stressed plants. However, this enhancement effect of GA₃ is more pronounced in case of cv. Giza 126. These results are similar to that obtained by Ashraf et al. (2002) reported that GA₃ stimulated the accumulation of K⁺ in shoots of a salt tolerant cultivar of wheat at 100 mM NaCl with GA₃ application.

Zn decreased in stressed plants especially in cv. Giza 126. Treatment with GA₃ partially restores Zn levels in cv. Giza 123 when compared with cv. Giza 126 values. All minerals such as Mn, Fe, Co, Pb, Cr and Cd content significantly decreased in both cultivars of barely except Cd in cv. Giza 126 which showed significant increases. GA₃ application is shown to restore the levels of these minerals to be close to those of control in both cultivars. These results are similar to the results obtained by Akman (2009) who found that highest Zn content of wheat plant was found under 0 NaCl concentrations (control) and no hormone treatment (control). For copper concentrations, the highest values were obtained under GA₃ treatment under 164 and 94 mM NaCl concentrations in barley and wheat plant, respectively.

Conclusion

Finally, it can be inferred that GA₃ counteracts with salinity by improving membrane permeability and nutrient levels in leaves which ultimately leads to better seedling growth and also GA₃ induced physiochemical changes responsible for induction of salt tolerance in barley especially cv. Giza 123. It is, therefore, possible that exogenous applications of GA₃ could be a useful tool in promoting good seedling growth and establishment under saline soil conditions.

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References

- [1]. Munns R., Tester M. Mechanisms of salinity tolerance. *Annual Review Plant Biol* 2008; 59: 651-81.
- [2]. El-Beltagi HS, Mohamed HI, Mohammed AH, Zaki LM, Mogazy AM. Physiological and biochemical effects of γ -irradiation on cowpea plants (*Vigna sinensis*) under salt stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 2013; 41(1): 104-114.
- [3]. Mohamed HI, Gomaa EZ. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress. *Photosynthetica* 2012; 50(2): 263-272.
- [4]. El-Mashad AA, Mohamed HI. Brassinolide alleviates salt stress and increases antioxidant activity of cowpea plants (*Vigna sinensis*). *Protoplasma* 2012; 249: 625-635.
- [5]. Hussain TM, Chandrasekhar T, Hazara M, Sultan Z, Saleh BK, Gopal GR. Recent advances in salt stress biology-a review. *Biotechnol Mol Biol Rev* 2008; 3: 8-13.
- [6]. Ashraf M, Karim F, Rasul E. Interactive effects of gibberellic acid (GA₃) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Plant Growth Regul* 2002; 36: 49-59.
- [7]. Shannon MC, Grieve CM. Tolerance of vegetable crops to salinity. *Scientia Hort* 1999; 78: 5-38.
- [8]. Shannon MC, Breeding, selection, and the genetics of salt tolerance. In: Staples, R.C. (Ed.), *Salinity Tolerance in Plants: Strategies for Crop Improvement*. Wiley, New York, NY 1984; 231-254.
- [9]. Pedranzani H, Racagni G, Alemano S, Miersch O, Ramírez I, Peñón-Cortés H, Taleisnik E, Machado-Domenech E, Abdala G. Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Regul* 2003; 41: 149-158.
- [10]. Alonso-Ramírez A, Rodríguez D, Reyes D, Jiménez AJ, Nicolás G, López-Climent M, Gómez-Cadenas A, Nicolás C. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in *Arabidopsis* seeds. *Plant Physiol* 2009; 150: 1335-1344.
- [11]. Kabar K, Baltepe S. Alleviation of salinity stress on germination of barley seeds by plant growth regulators. *Turkish J Biol* 1987; 11: 108-117.
- [12]. Kaur S, Gupta AK, Kaur N. Gibberellin A3 reverses the effect of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. *Plant Growth Regul* 1998; 26: 85-90.
- [13]. Chakraborti N, Mukherji S. Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. *Biologia Plant* 2003; 46: 63-66.
- [14]. Hampton JG, Tekrony DM. *Hand Book of Vigour Test Methods*. 3rd ed. The International Seed Testing Association, Zurich, Switzerland 1995; 117.
- [15]. Laemmli UK. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* 1970; 227: 680-685.
- [16]. Morse ML, Carter CF. The synthesis of nucleic acid in cultures of *Escherichia coli* strains B and B/R. *J Bacteriol* 1949; 58: 317-323.
- [17]. Dische EL. Physiological studies on herbicide (cotoran). *J Am Chem* 1953; 22: 4014-4014.
- [18]. Burton K. A study of the conditions and mechanism of the diphenyl amine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956; 62: 315-328.
- [19]. Chapman HD, Pratt PF. *Methods of Plant Analysis. I: Methods of Analysis for Soils, Plants and Water*. Chapman Publishers, Riverside, California 1982; 184-203.
- [20]. Snedecor GW, Cochran WG. "Statistical Methods". 6th ed., Iowa State University Press, Iowa, USA 1973; 593.
- [21]. El-Bakkosh A. The effect of mechanical and chemical treatments on seed germination and seedling growth of some forest trees. M. Sc. Thesis, Benghazi: Garyounis University 2001.
- [22]. Fu-Ping W, Zhao-Hui Z, Ting B, Qin X, Ying-Hong P. Proteomics reveals the effects of gibberellic acid (GA₃) on salt stressed rice (*Oryza sativa* L.) shoots. *Plant Sci* 2010; 178 (2): 170-175.
- [23]. Chandler PM, Zwar JA, Jacobsen JV, Higgins TV, Inglis AS. The effect of gibberellic acid and abscisic acid on β -amylase mRNA levels in barley leucone layer. *Plant Mol Biol* 1984; 3: 407-418.
- [24]. Taiz L, Zeiger E. *Plant physiology*. New York, The Benjamin/Cumming publishing Company Inc 1991; 349-375.
- [25]. Patel I, Saxena OP. Screening of PGRs for seed treatment in green gram and black gram. *Indian J Plant Physiol* 1994; 37: 206-208.
- [26]. Angrish R, Kumar B, Datta KS. Effect of gibberellic acid and kinetin on nitrogen content

- and nitrate reductase activity in wheat under saline conditions. *Indian J Plant Physiol* 2001; 6: 172-177.
- [27]. Datta KS, Varma SK, Angrish R, Kumar B, Kumari P. Alleviation of stress by plant growth regulators in *Triticum aestivum* L. *Biologia Plant* 1998; 40: 269-275.
- [28]. Basalah MO, Mohammad S. Effect of salinity and plant growth regulators on seed germination of *Medicago sativa* L. *Pakistan J Biol Sci* 1999; 2: 651-653.
- [29]. Hisamatsu T, Koshioka M, Kubota S, Fujime Y, King RW, Mander LN. The role of gibberellin in the control of growth and flowering in *Matthiola incana*. *Physiolgia Plant* 2000; 109: 97-105.
- [30]. Kumar B, Singh B. Effect of plant hormones on growth and yield of wheat irrigated with saline water. *Annals Agri Res* 1996; 17: 209-212.
- [31]. Sobhanian H, Motamed N, Rastgar JF, Nakamura T, Komatsu S. (2010). Salt stress induced differential proteome and metabolome response in the shoots of *Aeluropus lagopoides* (Poaceae), a Halophyte C4 Plant. *J Proteomic Res* 2010; 9: 2882-2897.
- [32]. Flower DJ, Ludlow MM. Contribution of osmotic adjustment to the dehydration tolerance of water stressed pigeon pea (*Cajanus cajan* (L.) Milsp) leaves. *Plant Cell Environ* 1986; 9: 33-40.
- [33]. Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M, Mou Karzel E. Osmotic adjustment of sugarbeets in response to soil salinity and its influence on stomatal conductance, growth and yield. *Agri Water Management* 1997; 34: 57-69.
- [34]. Srivastava TP, Gupta SC, Lal P, Muralia PN, Kumar A. Effect of salt stress on physiological and biochemical parameters of wheat. *Annals Arid Zone* 1998; 27: 197-204.
- [35]. Nazar R, Iqbal N, Masood A, Syeed S, Khan NA. Understanding the significance of sulfur in improving salinity tolerance in plants. *Environ Exp Bot* 2011; 70: 80-87.
- [36]. Shalhevet J. Plants under salt and water stress. In: Fowden L, Mansfield T, Stoddart J. (Eds.), *Plant Adaptation to Environmental Stress*. Chapman and Hall, London/Glasgow/New York/Tokyo/Melbourne/Madras 1993; 133-154.
- [37]. Gadallah MAA. Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. *The J Arid Environ* 2000; 44: 451-467.
- [38]. Ashraf M, O'leary JW. Responses of some newly developed salt tolerant genotypes of spring wheat to salt stress. II: Water relations and photo synthetic capacity. *Acta Botanica Neerl* 1996; 45: 29-39.
- [39]. Hardie DG. Control of carbohydrase formation by gibberellic acid in barley endosperm. *Phytochemistry* 2005; 14 (8): 1719-1722.
- [40]. Ampudia VM, Lugo IB. Redox-sensitive target detection in gibberellic acid-induced barley aleurone layer. *Free Radical Biol Med* 2006; 40: 1362-1368.
- [41]. Pence VC, Caruso JL. Auxin and cytokinin levels in selected and temperature-induced morphologically distinct tissue lines of tobacco crown gall tumors. *Plant Sci* 1987; 46 (3): 233-237.
- [42]. Grill E, Loeffler S, Winnacker EL, Zenk MH. Phytochelatins, the heavy metal binding peptides of plants are synthesized from glutathione by a specific glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Proceedings of the National Academy Sci* 1989; 86: 6838-6842.
- [43]. Peleg Z, Blumwald E. Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin Plant Biol* 2011; 14 (3): 290-295.
- [44]. Santner A, Estelle M. The ubiquitin-proteasome system regulates plant hormone signaling. *The Plant J* 2010; 61: 1029-1040.
- [45]. Rong-Chao G, Gui-Ping C, Bao-Cun Z, Yin-Zhu S, Zhan-Jing H. Cloning and functional characterization of a wheat serine/threonine kinase gene (TaSTK) related to salt resistance. *Plant Sci* 2007; 173 (1): 55-60.
- [46]. Fujii H, Takehiko S, Aiko S, Tomoko E, Fumie N, Michiharu N, Yoshinori I, Tokuro S, Mitsuo O. Profiling gibberellin (GA₃) responsive genes in mature mandarin fruit using a citrus 22K oligoarray. *Scientia Hort* 2008; 116: 291-298.
- [47]. Ismail AM. Physiological studies on the influence of cytokinin or GA₃ in the alleviation of salt stress in sorghum plants. *Acta Agronomica Hung* 2003; 51(4): 371-380.
- [48]. Afzal I, Basra S, Iqbal A. The effect of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *J Stress Physiol Biochem* 2005; 1: 6-14.
- [49]. Wang MC, Peng ZY, Li CL, Li F, Liu C, Xia GM. (2008). Proteomic analysis on a high salt tolerance introgression strain of *Triticum aestivum/Thinopyrum ponticum*. *Proteomics* 2008; 8: 1470-1489.
- [50]. Kaya C, Tuna AL, Yoka I. The role of plant hormones in plants under salinity stress. In

- Salinity and Water Stress: Improving Crop Efficiency (eds Ashraf, M., M. Ozturk, and H. R. Arthur.), Springer 2009.
- [51]. El-Tayeb MA, Ahmed MK. EFFECT of calcium on germination, growth and accumulation of some solutes in salinity stressed *Vicia faba* and *Pisum sativum* seedlings. In Proc. 2nd Int. Conf. Biol. Sci. Fac. Sci. Tanta Univ. 27-28 April 2 2002; 468-477.
- [52]. Ashraf M, Karim F, Rasul E. Interactive effects of gibberellic acid (GA₃) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Plant Growth Regul* 2002; 36: 49-59.
- [53]. Iqbal M, Ashraf M. (2013). Gibberellic acid mediated induction of salt tolerance in wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environ Exp Bot* 2013; 86: 76-85.
- [54]. Cachorro P, Ortiz A, Cerda A. Implications of calcium on the response of *Phaseolus vulgaris* L. to salinity. *Plant Soil* 1994; 159: 205-212.
- [55]. Botella MA, Martinez V, Pardines J, Cerda A. (1997). Salinity induced potassium deficiency in maize plants. *J Plant Physiol* 1997; 150: 200-205.
- [56]. Schachtman D, Lio W. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends Plant Sci* 1999; 4: 281-287.
- [57]. Akman Z. Effects of plant growth regulators on nutrient content of young wheat and barley plants under saline conditions. *J Animal Veterinary Advances* 2009; 8(10): 2018-2021.

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