Research Journal of Agriculture and Biological Sciences, 3(4): 200-213, 2007

Physiological Aspects of Mungbean Plant (*Vigna radiata* L.wilczek) in Response to Salt Stress and Gibberellic Acid Treatment

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Abstract: Pot experiment was carried out to determine the effect of salt stress (NaCl 0.0, 100, 200 and 300 mM) on growth and metabolic activities of mungbean plants. Pre-soaking the seeds in GA_3 (200 mg/L) was shown to a meliorate the deleterious effects of salinity in the majority of cases. The results revealed that, NaCl treatment induced drastic reduction in growth characteristics of mungbean plant through decreasing the shoot and root lengths, number of lateral roots and number of leaves, total area of leaves as well as fresh and dry weights of shoot and root of mungbean plants. Furthermore, this treatment markedly decreased the pigment contents, photosynthetic activity, reducing sugars and sucrose contents as well as total soluble-N, total-N and protein-N contents, nucleic acids content, peroxidase and catalase activities and rate of respiration as O_2 uptake and CO_2 evolution. On the other hand, salt stress appeared to increase polyamine (putrescine PUT, spermidine Spd, spermine Spm and total polyamines, RNase and polyphenol oxidase activity. Moreover, salt stress increased sodium and chlorine contents and decreased potassium, calcium and magnesium levels in root and shoot of mungbean plants. In the majority of cases pre-soaking the seeds in 200 mg/L GA_3 caused partial decrease in the deleterious effects of salinity in all parameters of this study.

Key words: Munbgean, NaCl, Gibberellic acid, Polyamine.

INTRODUCTION

Mungbean crop (*Vigna radiata* (L) Wilczek) is considered as one of the new crop in Egypt, it is important legume crop characterized by a relative high content of protein and short summer season crop, also it can be used as food in similar manner as faba bean and lentil. Moreover, mungbean can be used as a crop with export potential[12].

One of the approaches to increase legume production is to expand the cultivation of mungbean in the newly reclaimed sandy soils. However, these newly reclaimed sandy soils face some stress conditions (i-e) unfertility, shortage of water as well as salinity. Saline water is considered the major factor affecting field crops production under desert condition.

The effect of salinity levels on mungbean was studied by some researchers[68,80] reported that salt stress (NaCl) caused decreases in germination, shoot and root lengths and fresh mass in mungbean. Raptan *et al*[84] and Yupsanis *et al*[110] found that salinity decrease dry matter and biomass reduction and decreased root, stem and leaf weights, plant height of mungbean plants. Neelam Misra and Dwivedi[76] found that the increasing levels of salinity remarkably decreased seed germination and cauced pronounced decrease in seedling vigor. Rabie,[81] found that salinity caused decrease in mungbean growth and plant height.

Pigment content and photosynthesis in various plant types are generally reduced by salinity irrespective of the types of salinity agent used, but the salt concentration which bring about severe reduction in pigments and photosynthesis varies greatly between plant types[85,5,99]. Chlorophyll a and b contents in leaves of mungbean decreased with increasing salinity[96,36,111]. Moreover, Sahu *et al*[88] and Misra *et al*[67] found that salinity caused decreases in phototsynthetic pigment content and photosystem II electron transport activity in mungbean plants. Haroun[42] recorded a marked reduction in pigments content and photosynthetic activity on fenugreek growth under sea water stress. Abdul-Wahid *et al*[2] reported that the salt induced injury symptom on mungbean such as enhanced chlorosis and necrosis and decreased content of chlorophyll a, b and carotenoid.

Furthermore, salt stress reduced total soluble sugars, proteins, free amino acids,[27] sugars and soluble protein[111]. Also, Promila[80] Rajan *et al*[82] and Rabie[81] found that salinity reduced sugar and protein contents in mungbean plants.

Raptan *et al*[83] reported that salinity decreased total-N[42], Hassan *et al*[44] found that salinity decreased total-N and protein-N contents, but

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increased total soluble-N and total amino-N in fenugreek mungbean and tomato plants. However, Anthroper and Dubois[9], Chakrabarti and Mukherji[21a,22b], Nandini and Mukherjee[73] found that NaCl concentrations caused greatest reductions in growth, tissue nitrogen, N_2 fixation, total-N contents, amino acid and protein contents.

Proline content increased with increasing salinity and accumulated in different organs of mungbean plants under salt effect as described[19,96,10,36,75,74]. Different stresses may influence polyamine metabolism in different manners and specific function under the stress condition[112]. Salt stress induced increases in putrescine, spermidine, spermine and total polyamine contents has been reported in various plant species[33,13,31].

Growth changes in salinized plant appear to be associated with high electrolyte levels contributing to toxicity and or osmotic adjustment and turgor maintenance[69]. In this respect the patterns of distribution of Na, K, Ca, Mg, Fe, P and chlorine within the plants under salinity conditions have been described [106,86,19,20,47,88,75,83,74,42,77].

In various plant types activity of enzymes were changed under the salinity effect, a marked increase in the cytochrome c oxidase, fumarase, catalase[27], acid phosphatase[74], polyphenol oxidase[74], ATP-ase[82] glycolate oxidase, hydroxy acid oxidase, superoxide dismutase[21a] and decreases of nitrate reductase[36] α-amylase and protease[111], peroxidase[82], DNase and RNase[110].

Several attempts have been adopted for improving the salt tolerance properties of plants. Application of some plant growth bioregulators has increased the salt tolerance of many crop plants[41,4,43,32,48]. In this respect, Banyal and Rai[15] and Hamed *et al*[38] noticed that the ability of GA_3 to reverse the lowering of protein level caused by water stress. Moreover, GA_3 increased the pigments content of salinized plants in grasses[104,109]. On the other hand, the effect of hormones on the mechanism of ion uptake, under stress condition, has been studied[52,8,6,74,42,22b,73] showed that all IAA, GA_3 and Kinetin used were able to overcome to variable extents the adverse effects of stress imposed by NaCl solution.

Therefore, the present investigation was carried out to study the effect of presoaking mungbean plant in GA_3 to ameliorate the inhibitory effect of salinity.

MATERIALS AND METHODS

Seeds of mungbean (*Vigna radiate* L. Wilczek) were kindly obtained from Agricultural Research Center, Giza, Cairo, Egypt.

Time Course Experiment: An experiment was carried out to determine the effect of salinity levels (100, 200 and 300 mM NaCl) and alleviation of their effects by using 200 mg/L gibberellic acid on germination and seedling growth of mungbean (*Vigna radiate* L. Wilczek). The experiment was conducted in the Faculty of Education, Ain Shams University.

Homogeneous mungbean seeds were surface sterilized by 0.01 M $HgCl_2$ solution for 3 minutes, washed thoroughly with distilled water then divided into two sets, the first set was soaked for 12 hour in distilled water and the second set was soaked for the same time in 200 mg/L gibberellic acid.

The seeds were germinated in plastic pots contain 800g of washed sandy soil (saltless). The plastic pots divided into two groups each group composed of 100 pots and filled with equall amounts of sand soil.

The sandy soil was brought to filled capacity with the appropriate sodium chloride solutions to achieve the above mentioned salinity levels. The soil moisture was maintained at field capacity by weighing the empty pot and re-weighing it after filled with sand and re-weighed again after saturated the soil with water to determine the amount of water or salt solution required for irrigation.

The pots of the 1st, 2nd, 3rd and 4th groups were used for sowing the seeds which were previously soaked in distilled water. The 1st group was irrigated with tap water and used as control the 2nd, 3rd and 4th groups were irrigated with aqueous solutions of 100, 200 and 300 mM of NaCl, respectively. The pots of 5th, 6ix and 7th groups were used for sowing the seeds which were previously soaked in GA_3 (200mg/L) solution and irrigated with aqueous solutions of 100, 200 and 300 mM of NaCl respectively. All pots were kept inside an open air wire house exposed to normal day length and natural illumination. Each pot was irrigated by tap water or salt solution and ½ Hogland solution once per week to maintain the soil moisture at 80% of the total saturation capacity of the soil.

After week from sowing, thinning was done to leave five uniform seedlings in each pot for experimentation. Growth measurments were carried out at 21 days after sowing. Ten replicates for each growth parameter and triplicates for pigments, reducing sugars, sucrose content, total-N, total soluble-N, proline protein, enzymes; catalase, peroxidase and polyphenol oxidase, rate of respiration were taken for determination. Other samples were taken for determination of certain mineral ions, polyamine and protein electrophoresis.

The photosynthetic pigments (chl. a, chl. b and carotenoids) contents were determined by the spectrophotometer method[66]. Carbohydrates were extracted and clarified according to the method adopted[90]. The direct reducing value (D.R.V.) was determined[100] as a described in A.O.A.C.[1]. Total reducing value (T.R.V.) was estimated after sucrose hydrolysis by invertase. The sucrose content was calculated from the difference between T.R.V. and D.R.V. The total-N and total soluble-N contents were extracted and estimated using the conventional micro-kjeldahl according to the methods of Chibnall *et al*,[24] and Pirie[78] as described by Hassanein[45]. Free proline was determined[14].

DNA and RNA were extracted and estimated according to the method described[40]. Photocolorimetric estimations were done using diphenylamine reaction for DNA[18] and orcinol test for RNA[40].

For the assay of catalase, peroxidase and polyphenol oxidase the plant material was extracted[53]. Catalase activity was expressed as µ mole H_2O_2 destroyed/gram fresh weight/hour, peroxidase and polyphenol oxidase activity was expressed as the change in the optical density/gram fresh weight/hour. For the assay of RN-ase activity, phosphorus content was determined[87].

In case of assay of each enzyme, value at zero time was taken as blank and the activity of each enzyme was expressed as ($\Delta A \times TV$)/ t×v, where A is the absorbance or titration figure of the sample after incubation minus the absorbance or titration figure at zero time. TV is the total volume of the filtrate, t is the time (in minutes) of incubation with substrate and v is the volume of the filtrate taken for incubation[34].

To determine the respiration rate, the mercury method was employed for caliberating the manometers and the vessels. In all cases, Evans blue manometric fluid was employed. Oxygen uptake was determined manometrically in an atmosphere of air through Warburg techniques[103]. Carbon dioxide evolved during respiration was measured[103]. From oxygen uptake and carbon dioxide evolution, the respiratory rate was calculated.

Polyamine levels were determined using high performance liquid chromatography (HPLC)[98]. A certain weight of plant sample was homogenized in a blender in 5% trichloroacetic acid (TCA)[95]. The blended mixture was centrifuged and adjusted to a final volume of 250 ml with 5% TCA. The mixture was made alkaline with NaOH and extracted with n-butanol/chloroform (1:1 v/v). the combined organic phase was extracted with 0.02 N HCl and the aqueous extract was dried. Saturated sodium bicarbonate solution (0.5mL) was added to the residue of dansylated derivatives of the amines. Then, 1ml of dansyl chloride reagent (500 mg in 100ml acetone) was added while using a vortex mixer. They were left to stand for 1 hour thereafter, dansylamines were extracted by adding water and several portions of diethylether. The combined ether extracts were evaporated to dryness and the residue was redisolved in acetonitrile.

Mineral ions were extracted[23] by digestion of a known amount of dry mass in concentrated HNO_3 and made up to a known volume with distilled water. Sodium, potassium and calcium concentrations were measured by flame emission spectrophotometry (B-700-E)[108]. While magnesium concentration in the clear extract was estimated by atomic absorption spectrometry (FMD_3)[105]. Chloride ion concentration was measured by the silver nitrate titration method[49].

The determination, identification and characterization of different protein fractions from treated and control mungbean plants were obtained using continous polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (Cont. SDS-PAGE)[56]. Gels were stained by Coomassie blue (0.5 g/L) and destained with 5% MeOH/ acetic acid mixture. Destained gels were photographed and protein banding patterns were analysed quantitively using a laser Gel Documentation System (GDS).

The data were statistically analyzed by one-way analysis of variance and the least significant difference (LSD) was used to lest the difference between treatments.

RESULTS AND DISCUSSIONS

Growth Characteristics: Data presented in Table 1 show that salt stress induced by NaCl 100, 200 and 300mM led to a progressive gradual decrease of the percentage of germination with increasing the salt concentration as compared with the control. In addition, GA_3 treatment overcame the effect of salt stress and improved the percentage of germination. These results were similar to those of Sekhar[94]; working on mungbean Munoz *et al*.[71] working on *Prosopis alba*, Misra *et al*[68], Rajan *et al*[82] working on mungbean and Neelam-Misra and Dwivedi[76]

Table 1: Effect of NaCl and/or GA_3 on time and percentage of mungbean germination.

Treatments	Control	NaCl 100mM	NaCl 200mM	NaCl 300mM	NaCl 100mM +GA_3	NaCl 200mM +GA_3	NaCl 300mM +GA_3
Time of germination (hour)	48	72	96	144	60	84	120
% of germination	100	84	80	16	97	85	34

Table 2: Changes in growth parameters of mungbean plants raised from NaCl and GA_3 treatments after 21 days from sowing. Each value is a mean of ten replicates.

	Parameters									
Treatments	Shoot Length (cm)	Root Length (cm)	No. of Lateral roots	No. of leaves	Area of leaves/ plant (cm^2)	Shoot Fresh wt.(g)	Shoot dry wt.(g)	Root Fresh wt. (g)	Root dry wt. (g)	% of water content
Control	18.3	8.8	18	3	12.3	1.42	0.156	0.37	0.040	89.05
NaCl 100 mM	12.7 **	8.2*	15**	2**	8.4**	0.40**	0.074**	0.24**	0.036**	82.85*
NaCl 200 mM	9.3**	6.4**	12**	2**	5.2**	0.36**	0.069**	0.16**	0.030**	80.90**
NaCl 300 mM	4.4**	2.5**	5**	2**	2.5**	0.30**	0.056**	0.10**	0.028**	76.10**
NaCl 100 mM GA_3 200 mg/L	19.6**	9.1**	20**	3**	9.6*	0.82**	0.094**	0.38**	0.044**	88.80*
NaCl 200 mM GA_3 200 mg/L	13.1**	7.3**	16**	3**	6.3*	0.51**	0.082**	0.29**	0.045*	87.80*
NaCl 300 mM GA_3 200 mg/L	8.2**	3.2*	9**	2	3.4*	0.42**	0.058**	0.21**	0.028	86.40**
L.S.D. at 5%	0.62	1.11	1.34	0.28	0.87	0.04	0.006	0.03	0.003	5.56
1%	0.89	1.59	1.92	0.39	1.25	0.05	0.009	0.04	0.005	7.99

* Significant - ** Highly significant as compared with the reference controls

Fig. 1: Changes in growth characteristics of mungbean plants treated with NaCl or NaCl & GA_3 at 7 days from sowing.

1- Control
2- NaCl 100 mM
3- NaCl 200 mM
4- NaCl 300 mM
5- NaCl 100 mM + GA_3 200 mg/L
6- NaCl 200 mM + GA_3 200 mg/L
7- NaCl 300 mM+ GA_3 200 mg/L

working on *Phaseolus aureus.*

In the present investigation, (Table 2 and Fig. 1) salt stress by NaCl at 100, 200 and 300 mM caused marked decreases in root and shoot lengths, number of lateral roots and leaves, total leaf area/plant, fresh and dry weights of shoot and roots as well as percentage of water content of mungbean plants. In accord with these results, Younis *et al*[107] showed varied reduction in growth of *Phaseolus* seedlings treated with salinity. Also progressive gradual decreases in seed germination, plant height, shoot and root length, dry matter, biomass, root, stem and leaf weights were observed with progressive increase in salinity stress maintained of mungbean plant[68,64,83,110,76,81].

The present maintained damage associated with reduction in the various growth parameters of stressed mungbean seedlings can be considered as a reflection of biochemical and hormonal imbalances leading to changes in a wide array of metabolites and minerals (see Tables 1, 2, 3, 4, 5, 6 and 7). Also the deleterious ion

Table 3: Changes in photosynthetic pigments, carbohydrate contents, nitrogen constituents and nucleic acid contents of mungbean plants raised from NaCl and GA_3 treatments after 21 days from sowing. Each value is a mean of three determinations.

	Parameters											
	Photosynthetic pigments (mg/g fresh wt.)				Soluble sugars (mg glucose/ g dry wt.)		Nitrogen constituents (mg nitrogen/g dry wt.)				Nucleic acid Contents (mg nucleic acid/ 100g.d.wt.)	
Treatments	ChL.a	ChL.b	ChL.a/b	Carotenoids	Reducing sugars	Sucrose	Total nitrogen	Total soluble nitrogen	Protein N.	Proline	DNA	RNA
Control	1.41	0.77	1.83	0.44	4.04	2.04	100.53	45.17	55.36	13.34	320	1012
NaCl 100 mM	1.02**	0.59**	1.73	0.33**	3.70	1.35**	94.47	42.82**	51.65	75.54**	275**	377**
NaCl 200 mM	0.79**	0.47**	1.68	0.28**	3.10**	1.21**	75..92**	39.57**	36.35**	89.07**	201**	672**
NaCl 300 mM	0.65**	0.39**	1.66	0.21**	2.13**	0.90**	55.95**	31.26**	24.69**	103.94**	145**	560**
NaCl 100 mM GA_3 200 mg/L	1.20**	0.71**	1.69	0.38*	3.94	1.56**	101.93	46.89**	55.04*	36.23**	302*	917
NaCl 200 mM GA_3 200 mg/L	1.04**	0.64**	1.62	0.34**	3.52*	1.36*	82.19	41.36*	40.83*	71.18**	233*	841**
NaCl 300 mM GA_3 200 mg/L	0.87**	0.57**	1.52	0.26*	2.82**	1.10*	66.15**	35.38**	30.77*	81.26**	197**	648*
L.S.D. at 5%	0.11	0.07		0.04	0.38	0.15	9.55	1.61	4.59	5.84	26.36	88.72
1%	0.16	0.10		0.05	0.55	0.22	13.74	2.31	6.60	8.40	37.91	127.59

* Significant - ** Highly significant as compared with the reference controls

effect of NaCl on plant growth could be the toxic or specific ion effect of Na^+ and Cl^- or both[57]. These results are in harmony with those obtained by Gill[37], Ashraf & Rasul[11] and Sekhar[94].

On the other hand, the inhibitory effects of salinity treatment on growth parameters of mungbean plants seemed to be decreased by GA_3 treatment, these results are in accordance with Das Gupta *et al*[25] who recorded that foliar application of plant growth regulators like IAA, GA_3 and kinetin helped the plant to restore retardation in water content in mungbean plants subjected to water stress. Also Nandini *et al*[74] noticed that IAA, GA_3 and Kinetin used to overcome the adverse effect of salinity on mungbean plants.

The role of GA_3 in overcoming the harmful effects of salinity on growth may be due to the change in the endogenous growth regulators which affects plant water balance[62] and or decreasing root resistance to water flow[29]. The decrease in water content in stressed mungbean plants, in the present work and its increase again by GA_3 application supported these views.

Pigments Content: The leaf growth and number is more sensitive to salinity than root[70]. Thus NaCl caused a marked decrease in chl. a, chl. b, carotenoids and consequently total pigments content of mungbean leaf (Table 3). This result is in hormony with the results of Singh *et al.*[96], Garg *et al.*[36], Zayed & Zeid[111], Folkard *et al*[35] working on rice, Abdeul-Wahid *et al*[2] working on mungbean plants.

This reduction in pigment contents may be due to the inhibitory effect of the accumulated ions (Na^+ & Cl^-) on the biosynthesis of the different pigment fractions and/or/on their degradation or due to the effect of NaCl on chloroplast structure. In this respect, Strogonov[102] and Bhivare *et al.*[16], concluded that salinily affects the strength of the forces binding the complex of pigment-protein yield in the chloroplast structure. Also, Salama *et al*[92] confirmed that salinity caused swelling of membranes in chloroplasts of sensitive plants which affects their chlorophyll content, or due to excess ion in leaves which induced loss of chls[2].

Mungbean seeds presoaked in GA_3 induced a marked increase in the above mentioned pigments in leaves of stressed mungbean plant (Table 3). The stimulative effect of GA_3 on pigments of leaves at different concentration of NaCl irrigated plants is in accordance with the results obtained by Varshney & Baijal[104]; Younis *et al*[109]; Aldesuquy[5] and El-Bastawisy[32]. Moreover, Sakr[91] found that GA_3 or kinetin partially overcome the harmful effect of NaCl on cotton cotyledonary leaves and this could eventually increase number of chloroplast in the leaf by increasing intensity of cell growth and the activity of ribosomes which consequently stimulate chlorophyll synthesis[42,73].

Carbohydrate Content: Data presented in Table (3) show that there is significant and highly significant decreases in reducing sugars and sucrose contents of

mungbean plant with increasing salinity levels. This is due to the inhibition of photosynthesis which is associated with decline in pigment contents resulted from the reduction in leaf area[27], or due to decrease in leaf organic acid with salinily[19] working on *Phaseolus vulgaris* or due to decrease in PS II electron transport activity[67] or due to increase in amylase activity and /or decrease in invertase activity[80,68], or due to les stomatal openings in leaf[111,81]. In this respect, Dua *et al*[28] recognized that under a moderate stress, the net photosynthetic rate decreased in *Brassica* pods. Moreover, Maggio *et al*[63] indicated strong down regulation of photosynthesis by stress.

In general the inhibition of photosynthesis by salt stress was partially alleviated by GA_3 application. In this connection, many reports on plant hormone stimulation of chloroplast development and whole photosynthetic machinery was reported by Aldesuquy[5]; El-Bastawisy[32]. The reduction in photosynthesis could be attributed to the more closed stomata which might be wider opened by GA_3 application[74].

Nitrogen and Proline Contents: The results obtained herein show that the salt stress (NaCl) caused a marked decrease in total nitrogen, total soluble nitrogen and protein-N in mungbean plants, such reduction was accompanied by a marked increase in proline amino acid. This results are in agreement with the results obtained by Dhingra & Sharma[27], Singh *et al*[96], Nandwal *et al*[75], Raptan *et al*[84], Nandini *et al*[72], Chakrabrti & Mukherji[22b], Nandini & Mukherjee[73], Hassan *et al*[44] and Rabie[81].

The decrease in total-N concomitantly with the decrease in soluble-N and protein-N in salinity stressed mungbean plants, can be attributed to the effect of salinity on decreasing the biosynthesis of protein and/or the decrease in nitrogen fixation and/or inhibition in nitrate reductase activity. On the other hand, the results (Table 3) show that salt stress caused marked highly significantly increases in proline contents with increasing salt concentration. These results are in conformity with those obtained by Cachorro *et al*[19], Singh *et al*[96], Arora *et al*[10], Garg *et al*[36] and Misra *et al*[68]. Moreover Singh *et al*[97] and Stewart & Larher[101] suggested that proline accumulation may be used as a sensitive index of susceptibility to water stress.

This led us to suggest that proline accumulation can be used as an indicator in selection for withstanding saline stress through the involvement in osmo-regulation, this conclusion is in agreement with Aldesuquy[6] and Haroun[42].

Seed presoaking in GA_3 alliviates the deleterious effects of NaCl on the above mentioned criteria by increasing TN, TSN, protein-N and decreasing proline content in stressed mungbean plant. This indicated that GA_3 increases the biosynthesis of protein (Table 3).

In accordance with our results, Hamed *et al*[38] indicate that GA_3 tended to increase TN and protein-N and caused accumulation of soluble N. The decrease in proline amino acids in mungbean seedling under salt stress from seeds presoaked in GA_3 may be due to the role of GA_3 in inhancing the incorporation of the free amino acids into proteins or enzymes in order to increase the salt tolerance of the seedlings as has been suggested by Ali[7], Aldesuquy[6] and Kasim & Dowidar[54].

Nucleic Acids Content: Data (Table 3) illustrated that, the decrease in nucleic acids (DNA & RNA) of mungbean shoots concurrently with the increase in RNase activity can be attributed to the increase in salinity level which might be involved in inhibiting nucleic acids biosynthsis and/or stimulating their degradation. However Yupsanis *et al*[110] showed that salt stress caused a strong reduction in acid nucleases of alfalfa and lentil plants. In this respect, Nandini and Mukherjee[73] showed that NaCl reduced RNA and soluble protein but not affected DNA content. On the other hand, they reported that IAA, GA_3 and kinetin had counteracted the adverse effect of salinity on mungbean plant.

Enzymatic Activities and Respiration Changes: Data presented in Table 4 reveal that the activity of enzymes RNase and polyphenol oxidase in shoot of mungbean plant under salt stress (NaCl) was found to be significantly and highly significantly increased with increasing NaCl concentration. However, catalase and peroxidase activities were highly significantly decreased in stressed mungbean plants. The magnitudes of increase or decreases in enzyme activities of mungbean plants due to salinity were found to be higher at high NaCl concentration. The decrease in catalase and peroxidase activity in mungbean plant under salt stress might lead to accumulation of toxic amount of H_2O_2.

In this respect, Kocsy *et al*[55] observed that catalase activity decreased in salinity stressed resistant wheat variety. Moreover, Lee *et al*[59] found that salt stress enhances the content of H_2O_2 and the activity of superoxide dismutase, ascorbic acid peroxidase while it decreases catalase activity in *Oryza sativa* plant. Nandini *et al*[74] reported that application of NaCl on mungbean caused increase in polyphenol oxidase and ascorbic acid oxidase activity in leaf and root. Chakrabarti and Mukherji[21a,22b] found that peroxidase activity increased under salt stress.

Table 4: Changes in the activities of RNase catalase, peroidase and polyphenol oxidase, after 21days from sowing. Rate of O_2 uptake and CO_2 evolved after 7 days from sowing of mungbean shoot raised from NaCl and GA_3 treatments. Each value is a mean of three determinations.

	Parameters					
	Enzymatic Activity "E. activity /g fresh wt./h"				Rate of respiration	
Treatments	Ribonuclease	Catalase	Peroxidase	Polyphenol oxidase	Rate of O_2 uptake	Rate of CO_2 evolved
Control	5.01	223	21.5	3.1	160.02	171.42
NaCl 100 mM	6.75**	178**	20.1	4.9**	152.30	156.46
NaCl 200 mM	10.13**	115**	17.2**	5.4**	134.77**	143.39*
NaCl 300 mM	13.47**	69**	6.9**	9.2**	106.62**	110.36**
NaCl 100 mM GA_3 200 mg/L	6.12	263**	23.0**	4.0**	164.91	172.49
NaCl 200 mM GA_3 200 mg/L	8.82**	214**	19.8**	4.6**	152.53*	166.28*
NaCl 300 mM GA_3 200 mg/L	11.09**	301**	10.1**	6.2**	123.38*	137.16*
L.S.D. at 5%	0.91	18.39	1.72	0.54	16.69	22.94
1%	1.30	26.45	2.48	0.77	23.99	32.99

* Significant - ** Highly significant as compared with the reference controls

Table 5: Changes in putrescine, spermidine, spermine and total polyamines of mungbean plants raised from NaCl and GA_3 treatments after 21days from sowing. Value listed are expressed as µg/g fresh weight.

	Parameters			
Treatments	Putrescine µg/g	Spermidine µg/g	Spermine µg/g	Total polyamine µg/g
Control	0.41	1.44	2.53	4.38
NaCl 100 mM	0.86**	2.53**	8.20**	11.59**
NaCl 200 mM	1.11**	3.99**	13.77**	18.87**
NaCl 300 mM	2.87**	5.48**	18.56**	26.91**
NaCl 100 mM GA_3 200 mg/L	0.61**	1.98**	5.54**	8.13**
NaCl 200 mM GA_3 200 mg/L	1.02**	3.64*	7.56**	12.22**
NaCl 300 mM GA_3 200 mg/L	1.96**	4.93**	10.32**	17.21**
L.S.D. at 5%	0.003	0.31	0.65	1.08
1%	0.004	0.45	0.93	1.55

* Significant - ** Highly significant as compared with the reference controls

Presoaking of mungbean seeds in GA_3 caused a partial alleviation in the effect of salinity on RNase, catalase, peroxidase and polyphenol oxidase at high concentration of NaCl but non significantly affected these activities at low concentration of NaCl. The alleviation effect of GA_3 on enzymatic activity of mungbean plants under salt stress is in accordance with the results obtained by Nandini *et al*[74] and Chakabarti and Mukherji[21a,22b] who concluded that IAA, GA_3 and kinetin overcome the adverse effects of salinity stress.

Rate of O_2 Uptake and CO_2 Evolution: It is apparent from Table (4) that the low NaCl concentration caused a non significant decrease in the rate of O_2 uptake and rate of CO_2 evolved, but the high concentrations caused a significantly or highly significantly decrease in the same parameters. These results indicate that the salt stress caused a marked decrease in the respiration rate especially at the high concentration of NaCl treatment. These results are in agreement with those obtained by Lapina & Bikmukhametova[58] in corn leaves

Table 6: Changes in mineral composition of shoots and roots of mungbean plants raised from NaCl and GA_3 treatments after 21days from sowing. Values listed are expressed as mg mineral ion/g dry weight. Each value is a mean of three determinations.

	Parameters									
	Na^+		K^+		Ca^{++}		Mg^{++}		Cl^-	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	10.2	4.4	7.2	10.5	7.5	5.7	4.2	3.5	5.2	3.7
NaCl 100 mM	25.3**	7.0**	6.2**	8.8**	5.3**	4.2**	3.9	2.9**	9.6**	5.6**
NaCl 200 mM	29.4**	9.3**	5.1**	6.4**	4.8**	3.2**	3.3**	2.1**	12.8**	7.8**
NaCl 300 mM	40.7**	13.4**	3.4**	5.0**	2.1**	1.6**	2.4**	1.2**	16.2**	10.4**
NaCl 100 mM GA_3 200 mg/L	18.2**	6.0**	8.6**	12.2**	6.1**	4.7*	4.1	3.2*	4.3**	2.3**
NaCl 200 mM GA_3 200 mg/L	21.6**	7.5**	7.8**	9.4**	5.3*	3.9**	3.8*	2.7**	6.4**	3.8**
NaCl 300 mM GA_3 200 mg/L	27.4**	7.1**	5.3**	6.5**	4.0**	2.2**	7.2**	2.8**	9.8**	4.4**
L.S.D. at 5%	2.43	0.79	0.68	0.92	0.52	0.36	0.42	0.28	1.41	0.43
1%	3.49	1.13	0.97	1.33	0.75	0.51	0.60	0.41	2.03	0.61

* Significant - ** Highly significant as compared with the reference controls

and Levine & Levine[60] in pea seedlings. Generally the inhibition of respiration rate under salt stress by NaCl was partially improved by using GA_3 pretreatment.

Polyamine Content: The results reported herein show that salt stress (NaCl) treatment caused highly significantly increases in putrescine, spermidine, spermine and total polyamines in mungbean plant. Such increases were accompanied by marked increases in amino acid proline and decreases in nitrogenous content. These results agree with that of Prakash & Prathapsenan[79] and Das *et al*[26] who found that NaCl stress decreased level of PA in rice seedlings and *Brassica*. Moreover, El-Bassiouny and Bekheta[31] found that the ratio of PUT/Spd+Spm was increased in wheat (Giza 168) in response to salinity. These results are in agreement with those obtained by Santa-Cruz *et al*[93] in tomato, also, Erdei *et al*[33] and Aziz *et al*[13] who found that salt stress induced increases in the endogenous polyamine contents in various plant species.

On the other hand GA_3 treatment particularly seed presoaking in the present work, alleviates the deleterious effects of salt stress (NaCl) on the above mentioned criteria compared to control level. A highly significant increases in putrescine, spermidine, spermine and total polyamine content under salt stress were partially decreased by applying GA_3 (Table 5).

Mineral Content: The shoots and roots of NaCl stressed mungbean plants had a higher content of sodium and chlorine and a lower potassium and magnesium level as compared with the control values (Table 6). In this respect, Hernandez *et al*[46] reported that salinity increased $Na^+$and Cl^- while K^+ level decreased in leaf of mungbean. Abdus *et al*[3] studied the physiological genetics of salt tolerance in wheat plant, they concluded that K^+/Na^+ ratio could be used to screen for salt tolerance. Nandwal *et al*[75] found that salinity caused Na^+/K^+ ratio and Cl^- content increased, El-bassiouny & Bekheta[30] found that the accumulation of Na^+ and Cl^- leads to decrease the absorption availability of essential element (e.g K^+ and Ca^{++}), also Nandini *et al*[74] noticed increases in Na^+ and Cl^- in leaf and root of salinity stressed mungbean plant.

Also, the same treatment caused a marked decreases in K^+ and Ca^{++} content. Similarly, Haroun[42], Parveen-Rashid *et al*[77] reported that salt stress on mungbean plants caused Na^+ and Cl^- accumulation but K^+ content will decreased. Rabie[81] found that N, P, K and Mg decreased in mungbean under salinity effect. Moreover, it was suggested that, the effect of salinity on mineral ions was due to decrease in xylem exudation rate and leaf water potential, relative water content and water retention capacity concurrently with increased water saturation deficit and water uptake capacity[51,65].

On the other hand, a marked reduction in the accumulation of Na^+ and Cl^- concomitantly with increases in the K^+, Ca^{++} and Mg^{++} levels were observed in stressed plants resulted from seed presoaked in GA_3 (Table 6). These results in conformity with those obtained by Al-Wakeel *et al*[8] and Aldesuquy[6]. The influence of GA_3 on the mechanism of ions uptake may be related to its effect on membrane permeability and rate of ion entry through the membrane, or enhances their translocation to the shoot[17,50,8,74,42].

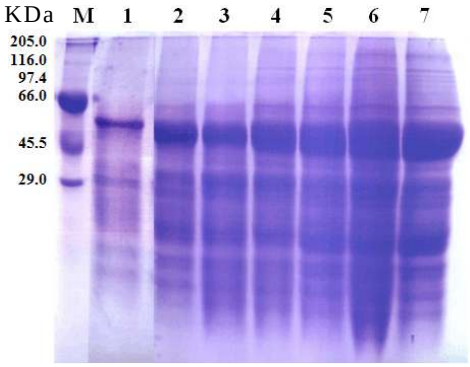

Fig. 2: SDS-PAGE profile of protein extracted from,mungbean plants raised from NaCl and GA3 treatments after 21 days from sowing.

M Marker protein
Lane 1: Control
Lane 2: NaCl 100 mM
Lane 3: NaCl 200 mM
Lane 4: NaCl 300 mM
Lane 5: NaCl 100 mM + GA_3 200 mg/L
Lane 6: NaCl 200 mM + GA_3 200 mg/L
Lane 7: NaCl 300 mM + GA_3 200 mg/L

Changes in Protein Profiles: The electrophoretic pattern of mungbean plants (Fig.2 and Table 7) was used in the present investigation to differentiate between the treatments of NaCl alone and or in combination with GA_3.

Two common polypeptide bands were observed in mungbean plants their molecular weights were 49.5 and 19.5 KDa.

The untreated control mungbean plant is characterized by the presence of 12 polypeptide bands their molecular weights ranged between 229.9 and 14.0 KDa.

NaCl treatment increased the number of protein bands to 18 instead of 12 in the control plant, while presoaking the seeds in GA_3 before NaCl treatment increased the number of bands to 21. Therefore, the maximum number of bands was 21 observed in plants presoaked in GA_3 and treated with 300 mM NaCl, whereas the minmum number was 12 bands recorded in the control plants. The highest molecular weight of the observed protein bands was 240.6 KDa recorded in samples treated with 200 and 300mM NaCl, while the lowest one was 10.5 KDa recorded in all treatments of NaCl alone or NaCl in combination with GA_3. In addition, NaCl treatment alone or in combination with presoaking the seeds in GA_3 resulted in higher intensity of the bands at molecular weights 49.5 and 19.5 KDa. The intensity of these bands was much higher in presence of GA_3. This indicated relatively higher protein concentrations in GA_3 treated seedlings than in their untreated counterparts. The protein of molecular weight 21.3 KDa started to appear in salt stressed mungbean seedlings resulted from seeds presoaked in 200 mg/L of GA_3. Similar results were reported by Lopez *et al*[61] and Hami *et al*[39].

Table 7: Comparative analysis of molecular weights and intensities of the different protein bands of the mungbesn plants treated with NaCl and GA_3 after 21 days from sowing.

No.	Mol.W KDa.	1	2	3	4	5	6	7
1	240.6			0.831	0.696			
2	238.8		0.633			0.587		
3	235.3						0.678	
4	231.7							0.541
5	229.9	1.35						
6	192.5				0.815			
7	187.2					0.831	0.975	
8	180.1	1.52						
9	171.2				0.931			
10	160.5					0.870		
11	156.9						1.19	
12	148.1							1.72
13	111.8		1.07			0.949		
14	110.1				1.0		1.08	
15	107.6			1.23				1.18
16	105.8					1.07	0.953	
17	102.5				0.629			
18	99.1					1.45	1.09	0.727
19	97.4		1.70					
20	95.5							0.895
21	92.5			0.929	2.07		0.917	
22	85.3							1.45
23	77.1							1.02
24	71.1							1.47
25	64.1		2.79	1.53	2.77	2.49	2.42	2.16
26	60.7		2.81	2.19	3.88	2.01	1.51	1.49
27	55.8	11.0						
28	51.7	4.09						
29	49.5	2.63	14.7	14.0	16.4	16.2	10.4	18.7
30	43.2		3.14					
31	41.2			2.20				
32	39.5		2.46	3.15	3.45			
33	35.1	3.74				3.2	2.77	3.35
34	31.2	7.33	7.71	8.26	9.20			
35	28.4		5.91	5.74		5.58	7.98	5.49
36	25.6	4.02			2.85			3.47
37	24.5		3.87			4.02		2.98
38	22.6	4.94		3.93	2.91			
39	21.3					4.13		4.46
40	19.5	2.63	9.97	4.19	6.62	10.7	6.85	11.5
41	18.5		4.06	4.80				
42	17.1	4.60	5.6					
43	15.5		2.29	4.28	4.43	5.30		5.35
44	14.0	4.74	5.73	4.55	3.96	3.77		3.85
45	12.2			3.90	3.50	4.89		5.27
46	10.5		3.45	4.88	3.74	4.55		4.02
Total No. of bands		12	17	17	18	18	13	21

The salt treatment is characterized by specific band, its molecular weight recorded at 39.5 KDa, while GA_3 treatment is characterized by a specific band appeared at molecular weight 99.1 KDa. These proteins might be involved in mungbean tolerance. In this connection, Kasim and Dowidar[54] detected a new unique protein band at molecular weight < 97 KDa in GA_3 primed radish seedling treated with 200 mM NaCl.

In conclusion, it may be suggested that the multiple effects of GA_3 which can be involved in alleviating the adverse effect of salinity on mungbean seedlings include the stimulation of growth parameters, the increase in photosynthetic pigments concurrently with the marked increases in reducing sugars and sucrose, increase in protein synthesis including *de novo* synthesis of new proteins and the accumulation of certain existing proteins, the increases in the activities of catalse and peroxidase and the decreases in the ribonuclease and the polypenol oxidase activities.

ACKNOWLEDGMENT

The author is much grateful to Dr.Raifa A. Hassanein Professor of Plant Physiology, Faculty of Science, Ain Shams University for her vital advise, constant encouragement and critical perusal of the mannuscript throughout this work.

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