

## Physiological Effects of Nicotinamide and Ascorbic Acid on *Zea mays* Plant Grown Under Salinity Stress. I-Changes in Growth, Some Relevant Metabolic Activities and Oxidative Defense Systems.

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**Abstract:** The interactive effects of salinity water, 50, 100 and 200 mM) and 100 ppm of nicotinamide (Vit. pp) or ascorbic acid (Vit. C) either as grain soaking or shoot spraying on *Zea mays* plant were studied to study its response to salinity stress and the possible role played by vitamins (Vit. PP or Vit. C) in regulating salt-induced changes in growth, some relevant metabolic activities and oxidative defense systems of *Zea mays* plant. All growth criteria, IAA, GA<sub>3</sub>, photosynthetic pigments, insoluble sugar and total carbohydrate contents were significantly decreased with increasing salinity levels, while ABA soluble sugars and amylases activity were sharply increased as compared with the control. Applications of vitamins (Vit pp or Vit. C) as grain soaking or shoot spraying could alleviate the adverse effects of salinity on growth parameters which were accompanied by marked increases in IAA, GA<sub>3</sub>, photosynthetic pigments and carbohydrate contents, and decreases in ABA and amylases activity as compared with those of the reference controls. On the other hand, activity level of superoxide dismutase (SOD), peroxidase (POD) enzymes and lipid peroxidation (MDA) showed progressive significant increases with increasing salinity levels, while the behaviour of catalase (CAT) activity and reduced glutathione content showed an opposite response as compared with the control. Treatment with Vit. pp or Vit. C by any of the two methods (soaking or spraying) induced significant reduction in the activities of SOD, peroxidases and lipid peroxidation, and significant increases in catalase activity and reduced glutathione content under salt stress as compared with reference controls.

**Key words:** *Zea mays*, Vit pp, Vit. C, NaCl, Growth, Photosynthetic pigments, Phytohormones, Carbohydrate, Antioxidant enzymes, Glutathione, Malondialdehyde

### INTRODUCTION

Sodium and chloride are the most prominent potentially toxic ions of saline substrate. Salinity caused growth reduction primary due to the low osmotic potential of the medium and by specific ion effect as secondary cause<sup>[25,78]</sup>. Pessaraki *et al.*<sup>[58]</sup> and Mishra *et al.*<sup>[49]</sup>, Azooz *et al.*<sup>[5]</sup> and Khattab<sup>[37]</sup> found that increasing salinity level of NaCl is detrimental to plant growth and resulted in marked decreases in shoot, root length, leaf area, total dry matter production and pigment contents. Salinity stress caused disturbance in the integrity of cell membrane<sup>[46,25]</sup>. Maintenance of membrane integrity and selective uptake of minerals are the parts that confer salt tolerance<sup>[66,35]</sup>. Plant adaptation to increasing soil salinity could be obtained through osmotic adjustment either by ion uptake and / or by internal production of osmotically active solute<sup>[36]</sup>. Stress induced reduction in Chl a, b, carotenoids and

total pigments<sup>[5,9]</sup> and accumulation in soluble sugar which accompanied by a reduction in the level of starch<sup>[19,75]</sup>. Stress inhibit growth through their effect on the hormonal balance<sup>[44]</sup>. The decrease in growth regulating substances concomitantly with an increase in ABA contents in response to salinity stress were observed by El-Khawas<sup>[16]</sup> and Hashem<sup>[25]</sup>.

The enhanced production of reactive oxygen species by environmental stress may result in a significant damage to cellular constituents and even cell death if protective mechanism fail to detoxify the reactive oxygen species by antioxidative systems<sup>[55,22,50,37]</sup>. It is well known that peroxidases are important component of this defense system<sup>[60]</sup>. Salt stress enhanced the content of H<sub>2</sub>O<sub>2</sub> as well as the activities of the superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD), whereas it induced the decrease of catalase (CAT) activity and had little effect on the activity of glutathione reductase (GR)<sup>[43]</sup>.

Vitamins are organic compounds which are required in trace amount to maintain normal growth and proper development of organism, these compounds act as coenzymes and thus take essential part in the regulation of metabolism, vitamins, can be limiting factors in the development of plant<sup>[4]</sup>. Ascorbic acid has important functions in photosynthesis, it protects the photosynthetic apparatus against the oxygen radicals and H<sub>2</sub>O<sub>2</sub> that are formed during photosynthetic activity<sup>[3]</sup>. Laboratory and field experiments have indicated that seed soaking in vitamin solution, ascorbic acid (Vit. C), nicotinamide (Vit. pp) and pyridoxine (Vit. B<sub>6</sub>) counteracted the adverse effects of salinity on seed germination, seedling growth and some relevant metabolic activities<sup>[30,2,5,15]</sup>.

The aim of this work was to determine the changes in growth and some relevant metabolic activities associated with *Zea mays* and the possible role played by vitamin C or vitamin PP in regulating salt-induced changes in these parameters.

## MATERIAL AND METHODS

**Application of NaCl and Vitamins:** Pure strain of *Zea mays* (single cross 10) obtained from Agriculture Research Center, Giza, Egypt. Grain were sterilized with 0.1 % mercuric chloride for 5 min., then thoroughly washed with water. The sterilized grains were sown in plastic pots (25 cm in diameter) containing 5 kg of a mixture of clay-sand soil (2:1 w/w). Seedling were subjected to the desired salinization levels (tap water, 50, 100 and 200 mM NaCl) after 15 days from sowing. The test plants were irrigated with water (80 % water holding capacity). Two different methods of vitamins (nicotinamide or ascorbic acid) application (0.0 and 100 ppm) have been used in the present study, soaking of grains for 12 hours or spraying of shoots which was carried out for 4 times at intervals of 8 days, then the plants were left to grow under the different salinization levels and/or vitamins treatments until the end of the experimental period (40 days). Plant samples were collected for determination of growth parameters, leaf area (cm<sup>2</sup>/plant), shoot and root length (cm/plant), number of adventitious roots, fresh and dry weight (g/plant) and the contents of certain metabolic activities.

**Determination of Metabolic Changes:** Acidic hormones (IAA, GA<sub>3</sub> and ABA) were carried out by the method described by Wasfy *et al*<sup>[76]</sup>.

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method recommended by Metzner *et al*<sup>[48]</sup>. Soluble sugar was extracted by using 80 % ethanol according to the method described by Homme *et al*<sup>[34]</sup>. and determined by the anthrone

sulphoric acid method described by Whistler *et al*<sup>[77]</sup>. Polysaccharides were determined in the dry residue left after extraction of soluble sugars<sup>[77]</sup>.

**Determination of Enzyme Activities:** Amylases, SOD, CAT and POD were extracted by the method described by Mukherjee and Choudhurri<sup>[51]</sup>. Measurement of amylases activity were performed using the alkaline dinitro-salicylic acid reagent as described by Dohler *et al*<sup>[14]</sup>.

SOD activity (EC 1.151.1) was measured according to the method of Dhindsa *et al*<sup>[12]</sup>. The activity of CAT (EC 1.11.1.6.) enzyme was estimated by the decrease of absorbance at 240 nm as a consequence of H<sub>2</sub>O<sub>2</sub> consumption and was expressed according to Havir and Mellate<sup>[29]</sup>. POD activity (EC 1.11.1.7) was determined using guaiacol according to the method of Malik and Singh<sup>[45]</sup>. The increase in absorbance due to the dehydrogenation of guaiacol was monitored at 470 nm<sup>[38]</sup>.

In case of the assay of each enzyme, value of zero time was taken as blank and the activity of each enzyme was expressed as (DA x TV)/ t x v where A is the absorbance of the sample after incubation minus the absorbance at zero time, TV is the total volume of the filtrate, t is the time (in minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation<sup>[18]</sup>.

**Determination of Lipid Peroxidation and Reduced Glutathione:** The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) contents using the method of Hodges *et al.*<sup>[33]</sup>. Reduced glutathione was extracted and measured by the method adopted by Tanaka *et al.*<sup>[73]</sup>.

The result were statistical analysis using L.S.D. at 5% and 1% levels, of probability according to SAS program<sup>[65]</sup>.

## RESULTS AND DISCUSSION

**Changes in Growth Parameters:** The results of the present work (Table 1 and 2) show that, most growth parameters as height of shoot, area of leaves/plant, number of adventitious roots, fresh and dry weights of shoots and roots of *Zea mays* plants were significantly decreased with increasing the salinity level. Shoot system appeared to be more sensitive to salinity than root system, since the reductions in height of shoot, area of leaves, fresh and dry weights of shoot at 200 mM NaCl were 50%, 61%, 74.4% and 70% respectively, while the reductions in root length, fresh and dry weights of root at the same salinity level were 47.9%, 63.6% and 60% respectively below those of the control plant.

**Table 1:** Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on the height of shoots, area of leaves, fresh and dry weights of shoots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment	NaClmM	Shoot height (cm/plant)	Leave Area (cm <sup>2</sup> /plant)	Shoot weight (g/plant)	
				Fresh	Dry
Reference controls	water	28.3	48.26	7.98	1
	50	22.15**	37.50**	5.42**	0.70**
	100	20.12**	28.16**	4.31**	0.57**
	200	14.12**	18.80**	2.04**	0.30**
NaCl + 100 ppm Vit. pp	water	40.80**	57.62**	11.67**	1.42**
Sprayed	50	27.20**	50.17**	8.69**	1.03**
	100	26.76**	44.01**	7.21**	0.86**
	200	22.00**	40.82**	5.50**	0.70**
Soaked	water	34.30**	54.02**	9.91*	1.14
	50	26.70**	53.90**	8.71**	1.07**
	100	29.20**	51.77**	7.05**	0.87**
	200	23.70**	43.95**	5.13**	0.63**
NaCl + 100 ppm Vit. C	water	37.75**	53.65**	9.65	1.2
Sprayed	50	25.83**	41.57*	6.24	0.77
	100	22.33	37.26**	5.67	0.7
	200	19.83**	32.24**	4.63**	0.56*
Soaked	water	34.83**	53.87**	9.63	1.19
	50	33.50**	51.30**	7.5*	0.92
	100	31.80**	49.19**	6.6*	0.85*
	200	25.90**	41.65**	5.65**	0.62**
L.S.D. at 5 %		2.43	3.23	1.81	0.22
L.S.D. at 1 %		3.14	4.18	2.35	0.29

\* Significant differences \*\* Highly significant differences as compared with reference controls

**Table 2:** Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on the root length, number of adventitious roots, fresh and dry weight of roots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment	NaClmM	Root length (cm/plant)	Number of adventitious Roots/plant	Root weight(g/plant)	
				Fresh	Dry
Reference controls	water	22.38	18	2.8	0.5
	50	22.49	16.07**	1.80**	0.37**
	100	21.9	13.60**	1.61**	0.32**
	200	11.65**	8.00**	1.02**	0.20**
NaCl + 100 ppm Vit. pp	water	38.70**	18.6	3.11	0.55
Sprayed	50	26.44**	17	2.71**	0.46**
	100	32.56**	17.00**	2.13	0.40**
	200	15.14**	15.00**	1.82	0.30**
Soaked	water	24.83*	18.6	3.79**	0.66**
	50	24	18.00**	2.14	0.43*
	100	22	16.00**	1.92	0.38*
	200	17.83**	15.50**	1.70*	0.30**

**Table 2:** Continued.

NaCl + 100 ppm Vit. C	water	36.00**	19.40*	2.98	0.52
Sprayed	50	34.95**	17	2.14	0.42
	100	32.42**	16.40**	2.03	0.40**
	200	23.33**	16.00**	1.84**	0.35**
Soaked	water	34.90**	19	2.88	0.51
	50	33.80**	19.00**	2.72**	0.45**
	100	31.33**	17.20**	2.18*	0.43**
	200	26.50**	16.30**	1.75**	0.31**
L.S.D. at 5 %		2.3	1.28	0.56	0.06
L.S.D. at 1 %		2.98	1.66	0.72	0.08

\* Significant differences \*\* Highly significant differences as compared with reference controls.

The reduction in plant growth of saline stressed *Zea mays* plant, in the present work may be attributed to the inhibitory effect of ABA which was induced by salinity on cell division and/or cell expansion<sup>[52,27]</sup> and/or resulted from the osmotic effect of salinity which caused disturbances in water balance of stressed *Zea mays* plant leading to stomatal closure, reduction in photosynthesis and consequently a retarded growth rate<sup>[8]</sup>. The decrease in dry weight of shoots by increasing the salinity level could be ascribed to the decrease in photosynthetic output as indicated by the significant decreases of chlorophylls and total carbohydrates in saline stressed *Zea mays* plants. Other authors concluded that, reduction of dry weight may be due to a turgor limitation<sup>[47]</sup> or cell wall hardening by limited extension growth<sup>[74,10]</sup>. Furthermore, the reduced growth observed under NaCl conditions could be attributed to increasing stiffness of the cell wall probably due to altered wall structure induced by salinity<sup>[59]</sup>.

Application of vitamins nicotinamide or ascorbic acid in the present work improved growth of *Zea mays* plants by causing significant increases in the values of the above growth parameters of salt stressed maize plant. The inhibitory effects of high levels of salinity were mitigated partially or completely alleviated. This is probably by increasing the efficiency of water uptake and utilization as well as protecting the photosynthetic pigments, and the photosynthetic apparatus.

Nicotinamide or ascorbic acid may act as growth stimulants which can play a role in mitigating the adverse effect of NaCl on metabolic activities relevant to growth through enhancing cell division and/or cell enlargement. These were further corroborated by the significantly higher levels of carbohydrate and concentration observed generally in the test plant by vitamin treatment<sup>[67,6,15]</sup>.

**Changes in Endogenous Growth Hormones:** Results of the present work (Table 3) showed that salinity

caused marked significant decreases in both GA<sub>3</sub> and IAA and increased ABA content, as compared with those of the control. Similar results were obtained by Hassanein<sup>[28,25,15]</sup>. Application of vitamins (nicotinamide or ascorbic acid) in this study generally led to high significant increases in the values of GA<sub>3</sub> and IAA concurrently with decrease in ABA level<sup>[41]</sup>.

The increases in IAA and GA<sub>3</sub> in shoot tissues of *Zea mays* plant concurrently with the increase in growth rate suggest the role of the endogenous hormones in stimulating the cell division and/or the cell enlargement and subsequently growth<sup>[72,28,15]</sup>.

**Changes in Photosynthetic Pigments:** Increasing salinity levels (50, 100, and 200 mM NaCl) showed a sharp decline in the total pigment content of maize leaves such reduction was attributed to the decline in the chlorophyll a & b as well as the carotenoid contents (Table 5). The highest reduction in photosynthetic pigments was displayed at higher salinity level (200 mM NaCl). These results are in harmony with those observed by Hassanein<sup>[27,71,15,37]</sup>. The severe reduction in the photosynthetic pigments in *Zea mays* plants in the present investigation, in response to high levels of salinity may be attributed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid<sup>[63,62,25]</sup>.

Application of Vit. pp or Vit C. (shoot spray or grain soaking), in most cases, did not only alleviate the inhibitory effect of salinity stress on the biosynthesis of photosynthetic pigments, but also induced a significant stimulatory effect greater than observed in the corresponding controls, a response which may contribute directly to the effectiveness on photosynthetic apparatus and in some way can alter plant productivity. Evidence to support this suggestion can be obtained from the data herein which indicated that application of the two vitamins activated the dry matter accumulation of the salinized plants and

**Table 3:** Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on hormone contents (mg/100g F.Wt) of shoots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment	NaClmM	GA <sub>3</sub>	IAA	ABA	
Reference controls	water	411.59	2.776	0.835	
	50	115.02**	0.882**	0.552*	
	100	106.31**	0.693**	1.003	
	200	88.11**	0.373**	1.188**	
NaCl + 100 ppm Vit. pp	water	418.75**	3.567**	0.305**	
	Sprayed	50	218.11**	2.168**	0.299*
		100	132.85**	1.524**	0.585**
		200	114.6**	1.057**	0.908*
Soaked	water	463.18**	2.989	0.668	
	50	210.54**	2.430**	0.371	
	100	174.63**	1.833**	0.499**	
	200	115.95**	1.453**	0.879*	
NaCl + 100 ppm Vit. C	water	419.24**	3.04	0.340**	
	Sprayed	50	207.73**	1.753**	0.374
		100	188.20**	1.660**	0.593**
		200	112.00**	0.879*	0.946
Soaked	water	463.68**	3.388**	0.746	
	50	240.42**	2.310**	0.351	
	100	163.47**	1.137*	0.644**	
	200	100.93**	0.815*	0.815**	
L.S.D. at 5 %		2.71	0.43	0.25	
L.S.D. at 1 %		3.51	0.5	0.32	

\* Significant differences    \*\* Highly significant differences  
as compared with reference controls

completely alleviated the inhibitory effect of salt stress on fresh and dry matter yields<sup>[30,2,15]</sup>. Also, Vit. pp or Vit. C may interfere with the protection of chloroplasts and their membrane against NaCl toxicity and thus maintaining their integrity<sup>[20,11]</sup>.

**Changes in Carbohydrates Contents:** In the present investigation (Table 4), the low concentration of NaCl induced significant increases in soluble sugars (50 and 100 mM), polysaccharides and total carbohydrates (50 mM) of shoots of *Zea mays* plants, while the higher concentrations decreased these contents as compared with those of the control plants.

The significant increase in carbohydrate fractions in shoots of salt stressed *Zea mays* plants (50 mM) concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to low concentrations of NaCl (50 mM) and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of *Zea mays* plants<sup>[56,39]</sup>.

The reduction in total carbohydrates of salt stressed *Zea mays* plant concomitantly with arrested growth rate and reduction in the leaf photosynthetic pigments led to the conclusion that sodium chloride may inhibit the photosynthetic activity and/or increased partial utilization of carbohydrates into other metabolic pathways<sup>[7,68,27]</sup>.

Vitamin pp or vitamin C application generally stimulated the accumulation of carbohydrates in the salt-affected *Zea mays* plant, either via increasing endogenous levels of certain phytohormones<sup>[42]</sup> or by acting as activators of carbohydrates synthesis<sup>[40]</sup>. Moreover, accumulation of carbohydrate play a key role in alleviating the salinity stress, either via osmotic adjustment<sup>[1]</sup> or by conferring some desiccation resistance to plant cells<sup>[69]</sup>.

**Changes in Enzymes Activities, Reduced Glutathione and Lipid Peroxidation:** Salinity caused highly significant increases in amylase activity (Table 4). This result is in agreement with the observations of Rather

**Table 4:** Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on pigments ( $\mu\text{g/g}$  D.Wt) of leaves, carbohydrates contents ( $\text{mg glucose}/100\text{g}$  D.Wt) in shoots and amylases activity ( $\text{mg glucose}/100\text{g}$  F.Wt /hour) of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment	NaClmM	Chl. a	Chl. b	Carote-noids	Total pigments	Total soluble sugars	Insoluble sugars	Total carbo-hydrates	Amylases activity
Reference controls	water	424	132	134	691	982.85	3241.13	4223.9**	162.5
	50	304**	104**	93**	502**	1042.28**	3277.70**	4319.9**	174.3**
	100	205**	74**	67**	347**	1001.13**	2559.99**	3561.1**	193.3**
	200	101**	35**	32**	168**	978.27**	2249.13**	3227.4**	208.6**
NaCl + 100 ppm Vit. pp	water	583**	208**	152*	944**	1366.84**	3999.9**	5366.8**	136.4**
	50	528**	208**	128**	865**	1613.70**	4031.9**	5645.6**	168.2**
	100	358**	139**	85*	578**	1321.13**	4351.9**	5673.1**	182.1**
	200	269**	45	99**	415**	1165.71**	3305.13**	4470.8**	186.7**
Soaked	water	479**	141	148	769**	1065.71**	3853.7**	4918.8**	146.4**
	50	327**	107	109*	544**	1759.99**	3899.4**	5659.4**	155.4**
	100	282**	94*	88**	466**	1540.56**	3643.4**	5183.9**	179.4**
	200	196**	62**	64**	323**	1403.42**	3497.1**	4900.5**	180.2**
NaCl + 100 ppm Vit. C	water	507**	183**	150*	841**	1142.85**	3652.5**	4795.4**	133.2*
	50	410**	150**	120**	681**	1571.66**	3561.1**	5132.7**	169.8**
	100	368**	133**	107**	609**	1074.28**	3547.4**	4621.7**	178.1**
	200	215**	74**	65**	356**	1005.71**	3222.8**	4228.5**	192.5**
Soaked	water	496**	181**	158**	835**	1147.42**	4255.9**	5403.4**	155.1**
	50	396**	158**	137**	695**	1348.56**	3807.9**	5156.5**	160.8**
	100	373**	136**	118**	628**	1266.28**	3780.5**	5046.8**	181.2**
	200	199**	92**	31	322**	1138.28**	3670.8**	4809.1**	189.6**
L.S.D. at 5 %		16.3	18.3	15.9	318	3.14	3.97	3.4	4.08
L.S.D. at 1 %		21.1	23.7	20.6	412	4.07	5.14	4.4	5.28

\* Significant differences \*\* Highly significant differences as compared with reference controls.

**Table 5:** Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on antioxidant enzyme activity (activity/g. F.Wt./min.), reduced glutathione and malondialdehyde contents in shoots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment	NaClmM	Cu-ZnSOD	Catalase	Peroxidase	Glutathione	Lipid-peroxidation-MDA
Reference controls	water	73	0.2	1.9	467	3.9
	50	154**	0.12**	1.6	422**	4.3
	100	177.5**	0.02**	2.3	417.5**	5.3**
	200	268.5**	0.01**	2.9**	400.0**	5.9**
NaCl + 100 ppm Vit. pp	water	24.2**	0.26*	1.1**	674.5*	2.4
	50	140.0**	0.21**	0.6**	472**	2.6**
	100	161.8**	0.15**	1.7*	454.5**	2.9**
	200	196.2**	0.12**	1.5**	444**	3.2**
Soaked	water	43.9**	0.32**	0.6**	622**	2.5**
	50	113.6**	0.28**	1.2	432**	2.7**
	100	120.4**	0.19**	1.5**	432**	3.0**
	200	164.5**	0.16**	1.6**	428.5**	3.6**
NaCl + 100 ppm Vit. C	water	48.6**	0.41**	0.6**	627.5**	2.61
	50	142.4**	0.24**	0.6**	574.5**	3.2**
	100	171.5**	0.13**	1.2**	427**	3.4**
	200	188.1**	0.13**	1.8**	402	3.9**
Soaked	water	35.7**	0.42**	0.6**	743**	2.4**
	50	114.5**	0.25**	0.9**	516**	2.5**
	100	153.0**	0.17**	1.3**	422	2.8**
	200	197.8**	0.14**	1.8**	414.5**	2.9**
L.S.D. at 5 %		5.76	0.05	0.51	6.8	0.55
L.S.D. at 1 %		7.45	0.07	0.67	8.8	0.71

\* Significant differences \*\* Highly significant differences as compared with reference controls.

and Doering<sup>[64]</sup> who found that salinity stimulates amylases and this accompanied with starch hydrolysis. Soaking of grains in/or spraying of plant with one of two vitamins (nicotinamide or ascorbic acid) was generally associated with marked decreases in the activities of amylases concurrently with increasing the soluble, insoluble and total carbohydrate contents indicating that vitamins could alleviate the inhibitory effects of salt stress by inhibiting amylase activity and/or enhancing photosynthetic mechanism<sup>[40]</sup>.

Activity levels of SOD and POD showed progressive significant increases with increasing concentration of NaCl, while the behavior of CAT enzyme showed an opposite response, when compared with unsalinized plant (Table 5). These results are in agreement with those of Fridovich<sup>[21,32,43]</sup>. They observed that salt stress has increased the activities of leaf mitochondrial Mn-SOD and chloroplastic Cu/Zn SOD, which are considered the primary scavenger in the detoxification of active oxygen species in plants and converts super oxide to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>, and offers protecting cells against super oxide-induced oxidative stress, and the increase in SOD may be due to the increase of abscisic acid, where ABA can cause an increased generation of H<sub>2</sub>O<sub>2</sub> and this induce the expression of antioxidant gene encoding Cu/Zn-superoxide dismutase (Cu/Zn-SOD)<sup>[24,57]</sup>. Our results supported the latter view that SOD activity increased concurrently with increasing salinity and endogenous ABA content. Also, our results showed a decrease in catalase activity, which led to the accumulation of toxic level of H<sub>2</sub>O<sub>2</sub><sup>[23,43]</sup>. Catalase deactivation by salt stress may be due to prevention of new enzyme synthesis<sup>[17]</sup> or catalase photo inactivation<sup>[61]</sup>.

Reduced glutathione in response to salinity was highly significantly decreased in maize as compared with non-salinized plant (Table 5)<sup>[31]</sup>. The decreased level of reduced glutathione (GSH) in maize might be due to its oxidation to oxidized glutathione (GSSG)<sup>[53]</sup>, where it can react with singlet oxygen and OH radical and protect protein thiol groups<sup>[3]</sup>.

The lipid peroxidation level as indicated by accumulated MDA increased significantly under salt stress (Table 5), this suggested that oxidative damage due to salinization of *Zea mays* plant is not controlled by the antioxidative enzymes in the present work<sup>[13,70]</sup>.

The application of vitamins (nicotinamide or ascorbic acid) either grain soaking or shoot spraying under the various levels of salinity caused marked retardation in Cu/Zn (SOD) peroxidase activities and stimulation in the catalase activity in maize as compared with the values of reference controls, also the vitamins decreased the lipid peroxidation (MDA) and stimulated the glutathione (GSH) as antioxidant defense compound. Therefore, treatment with vitamin PP or vitamin C alleviated the adverse effect of salinity

on growth and metabolic activities through decreasing the build-up of active oxygen species and thereby increasing resistance to salt stress<sup>[28]</sup>. Ascorbic acid is, water-soluble antioxidant molecules which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide, it acts directly to neutralize superoxide radicals, singlet oxygen or superoxide<sup>[54]</sup>.

In conclusion, nicotinamide or ascorbic acid can mitigate the adverse effects of salinity through increasing the content of IAA and GA<sub>3</sub> and decreasing ABA level which may be involved in protecting the photosynthetic apparatus and consequently increasing the photosynthetic pigments and the photosynthetic machinery and thereby increasing the carbohydrate contents and the growth rate. Also, nicotinamide or ascorbic acid can alleviate the adverse effect of salinity via exhibiting high antioxidant activity of catalase, preventing the toxic accumulation of H<sub>2</sub>O<sub>2</sub>.

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