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Physiological Effects of Nicotinamide and Ascorbic Acid on *Zea mays* Plant Grown Under Salinity Stress II-Changes in Nitrogen Constituents, Protein Profiles, Protease Enzyme and Certain Inorganic Cations

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Abstract: Adverse effects of salinity (Tap water, 50, 100 and 200 mM NaCl) on some physiological responses of *Zea mays* plant were studied. Salt stress induced the accumulation of the osmoprotectants, total-soluble-N, amino-N and proline concurrently with an increase in protease activity. On the other hand, protein-N and total-N contents were decreased as compared with those of the control. In addition, the content of Na^{+} increased significantly under salinity stress, while K^{+}, Ca^{+2} and Mg^{+2} contents were decreased, when compared with those of the control. Application of 100 ppm of vitamins (nicotinamide or ascorbic acid) by grain soaking or shoot spraying, counteracted the adverse effects of salinity and this accompanied by significant increases in total-nitrogen contents and amino-N, and significant decreases in proline and protease activity. Also, treatment with vitamins by any of the two methods resulted mostly in a decrease of Na^{+} accumulation and significant increases of K^{+}, Ca^{+2} and Mg^{+2} contents, when compared with those of the reference controls. Three prominent types of modifications are observed in the protein patterns, some proteins were disappeared, certain of other proteins were selectively increased and synthesis of a new set of protein was induced, some of these responses were observed under vitamins and salinity, while others were induced by either vitamin or salinity.

Key word: *Zea mays*, Vit pp, Vit. C, NaCl, Nitrogen, Protein profile, Protease, Cations

INTRODUCTION

Maize is classified as a salt-sensitive crop plant (Maas and Hoffman, 1977). The response of maize to salinity varies depending on the stage of development (Maas *et al.,* 1983; Pasternak *et al.,* 1985). Vegetative growth appears to be most sensitive to salinity, while plants are much less affected at later stages (Cramer, 1994). Salt stress affects a growing plant by causing changes in membrane chemistry, cell and plant water status, enzyme activities, protein synthesis and gene expression (Chapin, 1991 and Blomber and Alder, 1992). Salinity induced inhibitory effects on the biosyntehsis of free amino acids, but opposite effects were observed on the biosynthesis of protein and proline in *Zea mays* plants (Hashem 2000, Azooz *et al.,* 2002). The most common interpretation of proline accumulation is that it acts as a cytoplasmic osmotic solute and as a source of energy and nitrogen, so proline might play a role in the alleviation of salt stress (Ford and Wilson, 1981 and Venkatesan and Chellappan, 1998). Also salinity caused increases in the contents of both soluble nitrogen and free amino acid in the yield of wheat and broad pean (Doheem and Sharaf, 1983 and Sharaf and Youssef, 1987).

Levels of protein and nucleic acids in plants growing under saline stress are affected by salt-induced alteration in the activities of synthetic and hydrolytic enzymes (Prisco and O'Leary 1972; and Dubey, 1985). Activities of the enzymes protease and amino peptidase in seedlings of rice raised under increasing levels of NaCl salinity (Dubey and Rani, 1990). So, the breakdown of proteins in germinating seeds as well as in various parts of the plant is accomplished by the activities of protease and peptidase (Mikkonen, 1986).

Environmental stresses cause important modification in gene expresssion (Soussi *et al.,* 2001). Gene expression is manifested by the appearance of new proteins, which are not present before the stimulation.

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Salinity promotes the synthesis of salt stress-specific proteins (Hashem, 2000), many of these proteins were suggested to protect the cell against the adverse effect of salt stress. Accumulation of 26 KDa protein (Osmotin) is a common response to salt stress (Guerrier, 1998 and Hashem, 2000). Salinity stress led to the appearance of 67 and 26 KDa polypeptide (in cv. Dorado) and 45 KDa (in cv. Hagen Shandawil) (Azooz, 2004).

Salinity stress caused a considerable increase in both Na^+ and Cl^- ions while potassium ion decreased leading to decreased K^+ / Na^+ ion ratio (Saha and Gupta, 1998). Azooz *et al.* (2002) showed that the content of Ca^{+2} decreased significantly under salinity stress, Na^+ and K^+ contents were intensively accumulated. Lynch and Lauchi (1984) found that excess NaCl inhibit uptake and transport of K^+ to the xylem. Evlagon *et al.* (1990) and Cramer, (1992) showed that supplemental Ca^{+2} alleviate the effect of NaCl salinity on maize.

Vitamins are required in trace amount to maintain normal growth and proper development of all organisms, these compounds act as coenzymes systems and thus take essential part in the regulation of metabolism. Plant would respond to exogenous supply of the vitamins only if its endogenous vitamins level was low (Bonner and Green, 1939). Presoaking of seeds with optimal concentration of vitamins has been shown to be beneficial in seedling growth under saline condition by increasing physiological availability of water and nutrient (Azooz *et al.,* 2002; Barakat, 2003, El-Bassiouny and Bekheta, 2005).

The aim of this work is to study the influence of grain soaking in or shoot spraying with nicotinamide (Vit pp) or ascorbic acid (Vit. C) on counteracting the deleterious effect of salinity on nitrogenous constituents, protein profile and mineral composition of *Zea mays* plant.

MATERIALS AND METHODS

Pure strain of *Zea mays* (single cross 10) were obtained from the Agriculture Research Center, Giza, Egypt. Grains were sown in the plastic pots (25 cm in diameter) containing a mixture of clay and sand soil (2:1 w/w). Seedling (15 days from sowing) were subjected to different concentrations of NaCl (Tap water, 50, 100 and 200 mM NaCl) and/or vitamins (nicotinamide or ascorbic acid) solution (100 ppm) either by spraying of shoots, for 4 times at intervals of 8 days, or from the beginning by soaking the grains in 100 ppm with any of the two vitamins (Vit. pp or Vit. C) for 12 hours, and the tested plants were left to grow until the end of the experimental period (40 days).

Determination of Nitrogen Fractions:

Nitrogenous constituents were extracted as described by Yemm and Willis (1956). T.S.N. (from the extract) and T.N. (from dry powdered tissue) were determined by the conventional semimicro-modification of Kjeldahl method (Chibnall *et al.,* 1943 and Pirie, 1955). Subtracting the T.S.N. from T.N gave the value of protein-N.

Amino-N was extracted as described by Yemm and Willis (1956) and amino-N contents were then determined colourimetrically according to the method of Muting and Kaiser (1963). Free proline was determined according to the methods described by Bates *et al.* (1973).

Protein Electrophoresis:

Electrophoretic determination of total protein was estimated according to their molecular weight by denatured sodium dodcyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (1970) as modified by Studier (1973).

Determination of Protease Enzyme:

Extraction of protease in maize plants were carried out by the method described by Mukherjee and Choudhurri (1983), and the activity was determined according to the method of Ong and Gaucher (1973).

Determination of Certain Minerals:

Inorganic cations Na^+, K^+, Mg^{+2} and Ca^{+2} ions were extracted from dried plant material according to Chapman and Pratt (1978). Sodium and potassium were estimated by flame emission technique as adopted by Ranganna (1977). Magnesium and calcium were determined simultaneously by ICP spectroscopy according to the method of Soltanapour (1985).

The result were statistical analysis using L.S.D. at 5% and 1% levels, of probability according to SAS program (1982). Three replicates were used in each parameters.

RESULTS AND DISCUSSION

Nitrogen Constituents:

The data recorded in the present study (Table 1) indicated that salt stress induced accumulated amounts of total soluble-N while protein-N and total-N were consistently decreased with rise of salinity level (Hamed and Alwakeel, 1994 and Azooz, 2004). These results can be attributed to the decrease in protein synthesis and/or to the increase in its degradation. The degradation of protein under salinity condition was supported by our results which revealed the accumulation of total amino-N and proline concurrently with the increase in protease activity. Similar conclusions were also reported by Hsiao (1973) who attributed the

Table 1: Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on nitrogen content (mg/100g D. wt.), amino-N, proline contents (mg/100g D. wt.) and protease activity (mg amino-N/100g F.wt./hour) of shoots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment		NaCl mM	Total soluble-N	Protein-N	Total-N	Amino-N	Proline	Protease activity
References	Control	Tap water	112	1904	2016	234	93.54	36.84
		50	168**	1568**	1736**	238*	116.96**	37.98
		100	224**	1464**	1688**	324**	167.88**	75.26**
		200	280**	1014**	1294**	404**	177.35**	93.47**
NaCl +100 ppm Vit.pp	Sprayed	Tap water	142**	2296**	2438**	332**	92.49**	32.57
		50	181**	1986**	2167**	352**	79.20**	34.83
		100	247**	1672**	1919**	426**	98.15**	40.85**
		200	336**	1561**	1897**	590**	104.20**	48.62**
	Soaked	Tap water	149**	2520**	2669**	396**	60.39**	31.84
		50	192**	2016**	2208**	404**	95.25**	30.01**
		100	252**	1848**	2100**	420**	97.09**	35.17**
		200	307**	1288**	1595**	714**	114.72**	49.08**
NaCl +100 ppm Vit. C	Sprayed	Tap water	112	2240**	2352**	324**	71.57**	28.37**
		50	177**	2040**	2217**	386**	84.25**	31.21*
		100	238**	1736**	1974**	500**	95.25**	35.56**
		200	291**	1692**	1983**	631**	99.33**	42.39**
	Soaked	Tap water	136**	2320**	2456**	384**	61.57**	32.36**
		50	201**	2072**	2273**	414**	66.17**	35.14
		100	275**	1825**	2100**	531**	79.59**	35.99**
		200	311**	1456**	1767**	724**	101.57**	45.52**
L.S.D. at 5 %			2.96	2.38	2.67	3.98	2.53	6.03
L.S.D. at 1 %			3.83	3.09	3.46	5.15	3.28	7.81

* Significant differences

** Highly significant differences as compared with reference controls.

decrease in protein content under water stress to water disruption of the machinery consequent to water deprivation. Also, the treatments with vitamins (nicotinamide or ascorbic acid) either grain soaking or shoot spraying under the various levels of salinity, resulted in high significant increases in the contents of nitrogen (TSN, Protein-N and TN). In addition, the marked increase in nitrogen contents in vitamins treated plants was over those of untreated salinized plants and non-salinized control plants. These positive results concerning the accumulation of nitrogen constituents are in agreement with those obtained by El-Tayeb (1991) and Azooz (1997). Thus, it can be concluded that vitamins treatments not only alleviated the inhibitory effect of salinity stress, via osmotic adjustment or by conferring some desiccation resistance to plant cell, but also stimulated the accumulation of nitrogen constituents over those in the non-salinized plants. Moreover, vitamins might act as activators of protein synthesis via significant alteration in the enzymes related to protein metabolism (Kodandaramaiah, 1983)

The data clearly show a highly significant increase in the contents of both amino-N and proline in plant with increasing salt stress (Table 1). These results are in agreement with the result observed by Rains (1979) and Hassanein *et al*. (1988), They showed that NaCl treatments were capable of acting as activators of free amino acids accumulation. Also, Hassanein (2000), Azooz *et al*. (2002) and Azooz (2004) showed that the accumulation of proline and other free amino acids offers a great promise as one of the major physiological mechanism of salt tolerance in *Zea mays* plant. Proline also can play a role as protective agent for cytoplasmic enzymes (Nikolopoulos and Manetase, 1991), a reservoir of nitrogen and carbon sources (Fukutaku and Yamada, 1984), or even as a stabilizer of the machinery for protein synthesis (Kandpal and Rao, 1985), and/or scavenging hydroxyl radicals (Smirnoff and Cumbers, 1989).

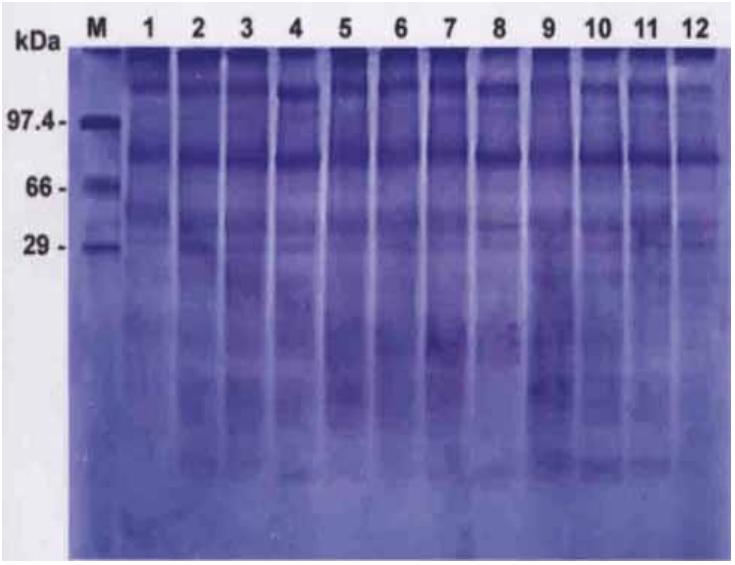

Plate 1: Electrograph of soluble protein pattern by one dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) in response to salinity and /or vitamins (vit.pp) of Zea mays plants at 40 days after sowing . Each lane contains equal amounts of protein extracted from *Zea mays* shoots.

Lane M Protein markers
Lane 1 control (H_2O)
Lane 2 50 mM NaCl
Lane 3 100 mM NaCl
Lane 4 200 mM NaCl
Lane 5 100ppm vit.pp spraying
Lane 6 50 mM NaCl + 100ppm vit.pp
Lane 7 100 mM NaCl +100ppm vit.pp
Lane 8 200 mM NaCl +100ppm vit.pp
Lane 9 100 ppm vit.pp soaking
Lane 10 50 mM NaCl+100 ppm vit.pp
Lane 11 100 mM NaCl +100ppm vit.pp
Lane 12 200 mM NaCl +100ppm vit.pp

Application of nicotinamide or ascorbic acid either grain soaking or spraying of plants induced a stimulatory effect on the accumulation of amino-N with a marked decrease of proline content as compared with those of the corresponding salinization levels. These results added support to the results obtained by Radi *et al*. (1989), El-Tayeb (1991), Azooz (1997) and Hassanein (2000). Thus, it could be suggested that salt tolerance was manifested via activated proline synthesis and hydrolysis of protein into free amino acids to act as osmoprotectants in the different organs of the test *Zea mays* plant. This means that the inhibitory effect of salt stress on the tested *Zea mays* plant was alleviated by vitamins treatments through inhibiting proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein.

Protein Profiles:

In the present work (plates 1-2 and Tables 2-3) three types of modifications are observed in the protein patterns of maize leaves; some proteins were disappeared, and certain of other proteins were selectively increased and synthesis of a new set of protein was induced, some of these responses were observed under vitamin and salinity treatments, while others were induced by either vitamin or salinity.

Table 2: Relative area (%) of each protein band of *Zea mays* in response to salinity treatment alone or in combination with different levels of vitamin pp at 40 days after sowing.

					NaCl + 100 ppm Vit. pp							
Salinity					Sprayed				Soaked			
M.wt	Tap water	50	100	200	Tap water	50	100	200	Tap Water	50	100	200
149.51	16.0	10.7	8.06	6.1	14.1	12.2	14.4	7.94	12.2	10.1	13.2	14.4
140.85	5.79	3.34	3.91	2.54	5.24	6.23	3.12	2.44	3.89	1.7	1.05	2.87
128.95	6.77	2.9	4.76	6.57	6.39	5.29	5.13	5.68	5.17	3.01	3.43	2.78
121.03	3.59	6.31	4.98	6.45	4.22	4.59	5.33	6.78	5.63	3.36	3.96	2.95
112.33	2.72	3.12	4.04	5.14	4.69	3.05	4.17	4.71	3.1	2.38	2.87	2.26
97.84	3.19	2.56	1.98	2.46	3.23	2.82	1.98	-	1.94	2.42	1.39	1.8
83.58	4.68	4.2	5.42	3.65	4.64	6.23	4.77	4.88	5.14	4.42	3.62	4.43
79.10	8.92	13.6	12.4	16.9	7.36	7.84	9.22	17.2	10.6	17.1	14.4	11.4
73.24	1.33	1.76	1.45	1.51	2.77	2.76	2.21	2.1	2.68	2.63	2.81	2.62
69.37	-	0.289	0.147	0.322	0.291	0.436	0.725	0.657	0.086	1.61	1.49	2.37
61.44	1.67	1.35	1.53	0.902	0.269	1.33	0.588	0.387	2.68	0.902	1.33	1.99
34.06	0.43	4.52	2.08	1.94	1.32	1.24	0.392	1.19	1.45	1.71	3.59	2.19
28.49	0.098	0.97	4.14	2.44	0.594	0.439	0.786	1.11	1.96	1.99	1.51	0.719
25.104	0.114	0.169	1.62	1.34	0.069	0.248	1.2	0.169	-	-	-	-
19.223	0.158	0.048	0.134	0.441	-	-	-	-	-	-	-	-
15.86	-	-	-	-	0.308	0.194	0.337	1.09	0.423	0.877	0.138	0.299
10.1	1.02	0.972	0.624	0.308	1.92	1.37	2.28	3.1	0.897	2.22	0.803	0.53
6.86	-	0.427	0.233	0.211	3.17	2.58	2.2	1.68	0.335	0.733	0.289	-
4.51	-	0.177	0.096	0.393	2.3	0.617	2.7	0.554	1.11	-	0.053	0.188
3.22	0.704	0.845	1.07	1.53	1.51	2.43	1.44	0.345	2.55	1.7	0.731	1.43
2.673	-	1.01	0.75	0.942	0.684	1.15	1.38	0.952	1.41	1.67	2.45	-
2.17	1.51	1.17	1.02	0.705	-	-	0.277	1.15	3.5	2.01	2.48	3.45
1.363	1.35	1.82	2.3	3.17	0.517	1.05	1.91	3.84	1.72	4.82	4.19	4.69
1.01	-	-	-	-	-	-	-	-	-	-	-	-
Total no. of bands	18	22	22	22	21	21	22	21	21	20	21	19

Table 3: Relative area (%) of each protein band of *Zea mays* in response to salinity treatment alone or in combination with different levels of vitamin C at 40 days after sowing.

					NaCl + 100 ppm Vit. C							
Salinity					Sprayed				Soaked			
M. Wt	Tap water	50	100	200	Tap water	50	100	200	Tap water	50	100	200
149.51	16.0	10.7	8.06	6.1	4.55	4.31	3.11	3.79	5.65	4.86	4.92	3.55
140.53	5.79	3.34	3.91	2.54	3.16	2.59	2.55	2.84	3.62	3.33	1.71	2.43
128.18	6.77	2.9	4.76	6.57	3.04	3.23	3.68	3.39	2.83	2.7	3.89	6.11
121.03	3.59	6.31	4.98	6.45	4.72	5.35	3.79	2.99	2.02	2.55	3.94	3.2
112.3	2.72	3.12	4.04	5.14	2.73	2.62	1.85	2.39	-	-	2.33	1.73
97.17	3.19	2.56	1.98	2.46	3.32	3.24	5.35	2.85	2.39	2.06	3.95	4.23
83.73	4.68	4.2	5.42	3.65	3.17	3.81	2.85	4.84	4.62	5.97	4.65	3.9
79.10	8.92	13.6	12.4	16.9	-	-	-	-	-	-	-	-
73.25	1.33	1.76	1.45	1.51	11.0	9.89	11.1	10.7	12.8	11.5	12	16.5
69.27	-	0.289	0.147	0.332	3.31	4.83	4.37	5.05	4.97	5.64	5.15	3.62
61.44	1.67	1.35	1.53	0.902	4.27	2.8	3.52	2.97	1.73	2.94	1.62	2.05
34.06	0.43	4.52	2.08	1.94	7.25	7.58	5.45	4.43	4.63	3.56	5.6	5.74
28.49	0.098	0.979	4.14	2.44	4.79	3.26	4.68	3.7	2.53	2.68	4.8	4.77
25.10	0.114	0.169	1.62	1.34	-	1.46	1.01	0.902	0.433	0.977	2.04	1.42
19.22	0.158	0.048	0.134	0.441	2.49	2.79	2.21	0.847	1.08	0.822	1.45	1.82
15.86	-	-	-	-	0.033	0.011	0.078	0.101	0.355	0.067	0.255	0.301
10.1	1.02	0.972	0.624	0.308	0.149	0.007	0.134	0.131	-	-	-	-
6.83	-	0.427	0.233	0.211	0.489	0.273	0.536	0.271	0.093	0.352	0.451	0.586
4.51	-	0.177	0.096	0.393	0.678	0.487	0.622	0.156	0.45	1.12	0.298	0.146
3.22	0.704	0.845	1.57	1.53	1.68	1.87	1.24	0.965	1.64	2.69	2.33	0.74
2.671	-	1.01	0.57	0.942	0.981	1.04	3.87	2.88	1.25	0.810	1.34	1.33
2.170	1.51	1.17	1.02	0.705	1.14	1.03	1.9	-	-	-	-	-
1.36	1.35	1.82	2.3	3.17	1.62	1.61	2.86	1.62	4.37	5.56	2.41	5.45
1.01	-	-	-	-	1.43	1.61	1.7	2.86	3.82	-	3.34	-
Total no. of bands	18	22	22	22	22	23	23	22	20	19	21	20

Four protein bands of molecular weights 69.37, 6.83, 4.51 and 2.67 KDa were de novo synthesized in *Zea mays* plant grown under salinity stress. It has been suggested that these proteins have an osmoprotection function (Dure *et al.,* 1989 and Dure, 1993) or protected cellular structures (Close and Lammers, 1993). In addition, a new unique protein band appeared at molecular weight of 15.8 KDa in salinized *Zea mays* plant treated with vitamin pp (grain soaking or shoot spraying treatments). Also, vitamin C treatments (grain soaking or shoot spraying) induced the synthesis of 2 new protein bands of molecular weights 15.86 and 1.01 KDa in salinized *Zea mays* plants, These results added support to the

Table 4: Interactive effects of salinity and vitamins (nicotinamide or ascorbic acid) on Na^+, K^+, Ca^{++} and Mg^{++} (mg/g D. Wt.) of shoots of *Zea mays* plants at 40 days from sowing. Values are the mean of 3 independent samples.

Treatment		NaCl mM	Mineral content (mg/g D. Wt.)						
			Na^+	K^+	Ca^{++}	Mg^{++}	K^+/Na^+	Ca^{++}/Na^+	Mg^{++}/Na^+
References Control		Tap water	14.6	52.13	9.03	7.69	3.57	0.618	0.526
		50	17.1**	47.68**	8.01*	6.80*	2.78	0.468	0.397
		100	24.0**	14.86**	7.2**	6.63**	1.74	0.300	0.276
		200	40.4**	30.89**	7.2**	5.80**	0.764	0.178	0.143
NaCl + 100 ppm Vit. pp	Sprayed	Tap water	12.3**	66.32**	9.84	8.55*	5.39	0.800	0.695
		50	15.3**	51.16**	9.14**	7.98**	3.34	0.597	0.521
		100	20.3**	61.43**	8.54**	7.63**	3.02	0.420	0.375
		200	36.6**	59.43**	8.58**	6.60*	1.62	0.234	0.180
	Soaked	Tap water	12.1**	61.48**	9.48	9.68**	5.08	0.783	0.800
		50	14.6**	70.16**	9.77**	8.19**	4.80	0.669	0.560
		100	19.4**	64.16**	8.88**	7.65**	3.30	0.457	0.394
		200	39.5**	50.49**	10.81**	6.88**	1.27	0.273	0.174
NaCl + 100 ppm Vit. C	Sprayed	Tap water	11.1**	55.16**	9.25	8.50*	4.96	0.833	0.765
		50	12.5**	61.59**	9.82**	7.86**	4.92	0.785	0.628
		100	16.6**	68.71**	9.90**	7.04	4.13	0.596	0.424
		200	24.7**	52.83**	10.31**	6.34	2.13	0.417	0.256
	Soaked	Tap water	10.4**	56.98**	9.98*	8.47*	5.47	0.959	0.418
		50	11.3**	51.33**	9.67**	7.10	4.54	0.855	0.628
		100	14.3**	65.78**	8.90**	7.29	4.6	0.622	0.509
		200	24.0**	66.46**	9.58**	7.86**	2.77	0.281	0.231
L.S.D. at 5 %			1.77	1.87	1.84	0.77			
L.S.D. at 1 %			2.29	2.42	1.08	1.00			

* Significant differences

** Highly significant differences as compared with reference controls.

result obtained by Gomez *et al*. (1988) and Hashem (2000), who recorded a protein band of more or less the same molecular weight (15.4 kDa) which was newly synthesized in maize embryo under stress or maize plant treated with 100 mM NaCl.

Protein bands having molecular weights of 73.24 and 69.73 KDa showed a detectable increase in their intensities in response to grain soaking or shoot spraying with vitamin pp of salinized *Zea mays* plant. Also, other 2 protein bands (M.wts: 2.17 and 1.36 KDa) were intensified in salinized maize plants resulted from grain soaking in vitamin pp. Also, vitamin C treatment (grain soaking or shoot spraying) increased the overexpression of protein band appeared at molecular weight 73.25 KDa in salinized *Zea mays* plant.

These results suggested that these proteins may have a specific function to help maize plants to alleviate the harmful effect of salinity. Also the results of protein pattern may be reasonable to assume that one of the multiple effects of vitamins on stressed *Zea mays* plant is the *de novo* synthesis of a new proteins and the increased accumulation of certain existing proteins which may be involved in increasing the tolerance of maize plant.

Protease Enzyme:

Salinity caused a highly significant increase in protease activity (Table 1) (Sheoran and Garg 1978; Reddy and Vora, 1985). It has been suggested that salinity reduces the synthesis of macromolecules such as RNA, DNA and proteins (Prisco and O'Leary, 1972) and increases their degradation by affecting the hydrolytic enzymes, especially nucleases and proteases (Reddy and Vora, 1985).

Soaking of grains in/or spraying of plant with one of two vitamins (nicotinamide or ascorbic acid) was generally associated with marked decrease in the activity of protease enzyme concurrently with increasing the protein level indicating that vitamins could alleviate the inhibitory effects of salt stress by enhancing protein synthesis where vitamins might act as activators for protein synthesis (Kodandaramaiah, 1983).

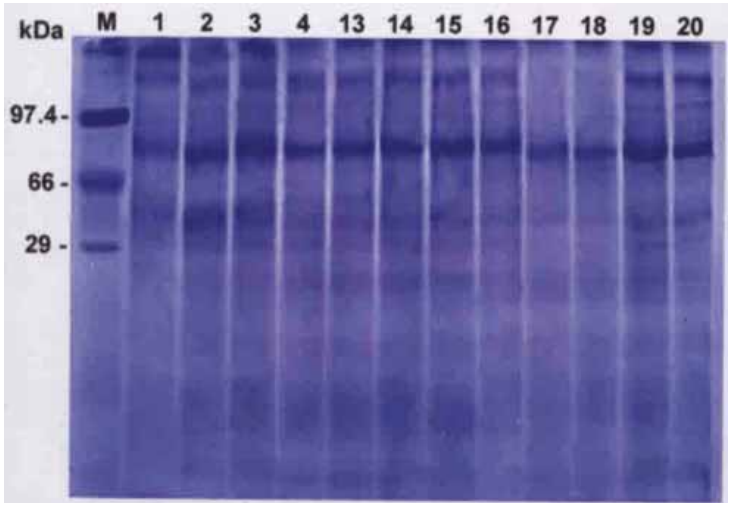

Plate 2: Electrograph of soluble protein pattern by one dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) in response to salinity and /or vitamins (vit.c) of Zea mays plants at 40 days after sowing . Each lane contains equal amounts of protein extracted from Zea mays shoots.

Lane M Protein markers
Lane 1 control (H2O)
Lane 2 50 mM NaCl
Lane 3 100 mM NaCl
Lane 4 200 mM NaCl
Lane 13 100ppm vit.c spraying
Lane 14 50 mM NaCl + 100ppm vit.c
Lane 15 100 mM NaCl +100ppm vit.c
Lane 16 200 mM NaCl +100ppm vit.c
Lane 17 100 ppm vit.c soaking
Lane 18 50 mM NaCl+100 ppm vit.c
Lane 19 100 mM NaCl +100ppm vit.c
Lane 20 200 mM NaCl +100ppm vit.c

Inorganic Cations:

Salinity stress caused a considerable increase in sodium content, and decrease in potassium, calcium and magnesium ions content of *Zea mays* plant (Table 4), which in turn reflected in the decrease in K^+/Na^+, Ca^{+2}/Na^+ and Mg^{+2}/Na^+ ratios (Table 4) as compared with non-salinized plants (Younis *et al.,* 1994; and Wener and Finkelstein, 1995). Lloyed *et al*. (1990) have suggested that increased accumulation of sodium (Na^+) and (Cl^-) ions in the tissues inhibits biochemical processes related to photosynthesis through direct toxicity. The promotion of Na^+ uptake by salinity was accompanied by a corresponding decline in K^+ concentration, showing an apparent antagonism between K^+ and Na^+ (Erdei *et al.* (1996). High concentration of Na^+ affects intercellular K^+ accumulation (Serrano and Gaxiola, 1994). Presumably by competing for sites through which influx of both cations occurs (Jeschke, 1984) or affecting membrane integrity and causing leakage of K^+ (Haro *et al.,* 1993). The reduction in Ca^{+2} and Mg^{+2} uptake under salt stress conditions might be due to the suppressive effect of Na^+ and K^+ on these cations or due to reduced transport of Ca^{+2} and Mg^{+2} ions (Varsheny *et al.,* 1998).

Grain soaking in/or shoot spraying with either Vit. pp or Vit. c. under the various levels of salinity caused a reduction of Na^+ accumulation and increase in the contents of K^+, Ca^{+2} and Mg^{+2}, and this lead to increase in K^+/Na^+, Ca^{+2}/Na^+ and Mg^{+2}/Na^+ ratios when compared with non-salinized plants (El-Tayeb, 1991 and El-Bassiouny, 2005). Vitamins led to increase in the contents of ions in the main organs of the stressed *Zea mays* plant through their role in increasing osmotolerance and/or through regulating various processes including

absorption of nutrients from soil solution. (Buschmann and Lichtenthaler, 1979). The antagonistic relations between Na^+ and K^+ may be taken as an indication of the role played by vitamins in modifying K^+/Na^+ selectivity under salt stress (Alpaslan and Gune, 2001 and Azooz, 2004).

In conclusion, salinity adversely affected the protein content concurrently with increasing amounts of the osmoprotectants, amino-N, and proline and higher protease activity. Also, certain modification were observed in protein pattern. Sodium ions were accumulated while K^+, Ca^{+2} and Mg^{+2} ions were significantly decreased in salt stressed *Zea mays* plant. Application of nicotinamide or ascorbic acid mitigates the adverse effects of salinity through increasing the synthesis of protein and decreasing protease activity and corrects the nutritional disorders induced by salinity by decreasing Na^+ ions and increasing K^+, Ca^{+2} and Mg^{+2} ions contents over those of control plants and salinized ones.

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