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Interactive Effects of Salinity and Certain Vitamins on Gene Expression and Cell Division

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ABSTRACT

Three wheat (*Triticum aestivum* L.) cultivars (Sids 1, Sakha 69 and Gemmiza 5) were selected to study the interactive effects of salinity, vitamin B6 and ascorbic acid on protein synthesis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for the characterization of differential expression of proteins of these cultivars in response to salinity. Salinity induced a considerable variation in the protein patterns among these cultivars. These variations have been manifested in the novel expression of some polypeptides, the absence of the other and the over expression of a third class of polypeptides. Salinity also, resulted in progressive reduction in growth rates as indicated by values of roots and shoots length. On the other hand, the effect of salinity, vitamin B6 and ascorbic acid on the mitotic division and induction of chromosomal aberrations was studied using *Allium cepa* test. The higher concentrations of NaCl (200 and 180mM) have mitoclassic effect on cell division in which a complete inhibition in the mitotic activity was observed. Few types of mitotic abnormalities were induced in different treatments. Treatment the roots with vitamin B6 or ascorbic acid showed a considerable increase in the mitotic index and minimize the inhibition effect of NaCl.

Key Words: *Triticum aestivum*; Salinity Stress; SDS-PAGE; Mitotic indices; Chromosome aberrations

INTRODUCTION

Soil salinity is a major abiotic stress in plants agriculture worldwide. Information on genetic diversity of crop is essential for development of successful breeding program. The use of molecular approach for detecting genetic diversity has been recently introduced through detection of isozymes, proteins and DNA markers. This has led to research into salt tolerance with the aim of improving crop plants.

Changes in the environmental conditions affect the quantity and quality of plant protein. It is well documented that various environmental stresses cause important modifications in gene expression of plants (Afiah *et al*., 1999; Soussi *et al*., 2001). Characterization and isolation of stress-induced proteins has become a standard and important technique (Hoyos & Zhang, 2000; Majoul *et al*., 2000). A large set of genes that are transcriptional activated in plants during stress condition has been identified (Tabaeizadeh, 1998; Matos, *et al*., 2000, 2001). The tolerance of plants to salinity stress has been found to differ with the developmental stage as well as the genetic constitution (Badr *et al*., 1998; Kawasaki *et al*., 2001). The level of protein differs in salt-tolerant and salt-sensitive genotype when they are subjected to salt-stress condition (Dubey & Rani, 1989; Badr *et al*., 1998; Majoul *et al*., 2000). Therefore linking the expression of a gene to a higher degree of tolerance within a genotype provides an important argument for a role in adaptation.

Salinity show substantial changes in protein expression. These changes involve either the induction of some polypeptides, the disappearance of the other or over expression of other set of protein (Hurkman & Tanaka, 1987; Dell'Aquila & Spada, 1993; Badr *et al.,* 2000). Electrophoretic protein profile alterations induced by different agents was used by many authors as a good candidate for studying and monitoring changes in gene expression among the treated plants (Abdelsalam *et al.,* 1998; Majoul *et al*., 2000).

The root tips are often the first to be exposed to chemicals spread in soil and water. Observation of the root tip system constitutes a rapid and sensitive method for environmental monitoring. Besides growth restrictions observable at the macroscopic level, cytological studies will yield detailed information on qualitatively and quantitatively harmful effects at the microscopic level. Among the test systems suitable for toxicity monitoring, the *Allium* test is well known (Grant, 1982). Salinity induced inhibition in mitotic activity is accompanied by a reduction in growth (El-Mashad & Kamel, 2001). Genotoxicity of different chemicals can be modulated by vitamins which have antimutagenic action and reduce the frequency of chromosomal aberrations (Hoda & Sinha, 1992; Andrew, 1997; Emerit *et al*., 1997; Vaglenov *et al*., 1998; Fawzia, 2002).

When plants subjected to salt stress, they can survive and grow by adaptive processes. Salinity inhibits some of the growth parameters and causes some disturbance in some metabolic activities (Kord & Khalil, 1995; El-Mashad & Kamel, 2001; Houle *et al.,* 2001). Effect of NaCl stress is harmful at early stages of germination (Levitt, 1980). Salinity causes inhibition of germination as indicated by reduction in length of root and shoot (Kayani *et al.,* 1990). Application of vitamins is a possible approach to overcome

the environmental limitation of crops by improving their tolerance and economic yield. The interactive effect of salinity and vitamins on protein synthesis and growth rate in some crop plants was studied by some investigators (Azooze, 1990). The vitamins supplements are known to enhance the plant activities and did not have toxic or mutagenic action (Hoda *et al*., 1991; Bronzetti *et al*., 2001).

In this work, we present a comparison of response to salt stress, vitamin B6 and ascorbic acid in three Egyptian wheat cultivars on the basis of the electrophoretic analysis of their protein products and their growth. The cytological effects of sodium chloride and vitamins on cell division were also studied.

MATERIALS AND METHODS

Three different cultivars of wheat (*Triticum aestivum* L.) named Sids 1, Sakha 69 and Gemmiza 5 were selected in the present work. These cultivars were kindly supplied by Agriculture Research Center, Giza, Egypt. The grains of each cultivars, previously soaked for 24 h in tap water, were divided into seven groups; each group comprises 50 seedlings, then they treated with sodium chloride (50, 150, 180 mM), vitamin B6 (100 ppm), ascorbic acid (100 ppm) and combined treatments of 180 mM NaCl with vitamin B6 or ascorbic acid respectively. Control experiments were performed by simultaneous watering of seedling using tap water. After 24 h treated samples and control (20 seedlings) were collected and crushed using liquid nitrogen. The protein was then extracted by mixing 500mg of milled tissue with 1ml of extraction buffer (0.1 MTris-HCl, pH 6.8; 2% SDS). The extract was centrifuged at 9000 rpm for 6 minutes. Characterization of proteins was carried out using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide according to Laemmli (1970). Protein samples were prepared by mixing clear supernatant with sample buffer (0.12 M Tris-HCl, pH 6.8, 10% SDS, 10% sucrose and 0.1% mercaptoethanol) and denatured by heating at 90 ºC for 3-5 min., then loaded in equal amounts. Protein bands were separated at constant current of 25 mA. Protein bands were visualized by staining the gels with 0.25% Coomassie Brilliant blue R-250. Banding patterns were photographed and the results were analyzed by the gel documentation system.

Another group of experimental grains (100 seedlings of each cultivar) were treated with (50, 150, 180, 200, 250 mM NaCl), 100 ppm Vitamin B6, 100 ppm ascorbic acid, 100ppm Vitamin B6 + 180 mM NaCl and 100ppm ascorbic acid + 180 mM NaCl. They left to grow for 10 days then the root and shoot length was measured.

For mitotic analysis, nine sets of rooted healthy and uniform *Allium cepa* bulbs were immersed in freshly prepared concentrations of (50, 150, 180, 200, 250 mM NaCl, 100 ppm Vitamin B6, 100 ppm ascorbic acid, 100ppm Vitamin B6 + 180 mM NaCl and 100ppm ascorbic acid + 180 mM NaCl) for 24 hours; control roots were treated with tap water. Roots 1: 2 cm long were fixed in (3:1) ethanol: glacial acetic acid for 24 hours then stained with basic fuchsin. Cytological preparations were carried out using Feulgen squash technique. The frequencies of the mitotic phases, the number and the type of chromosomal abnormalities and the mitotic index were recorded.

RESULTS AND DISCUSSION

The effect of applied salt stress (50, 150, 180 mM NaCl) on the protein profiles of wheat cultivars (Sids 1, Sakha 69 and Gemmiza 5) in the absence or presence of vitamin B6 and ascorbic acid are shown in Fig. 1. The soluble protein profiles of the three wheat cultivars comprise six common major bands and a number of minor bands. The main polypeptide bands are located between 10 and 82 KDa.

Electrophoretic analysis of protein patterns of the cultivar Sids 1 showed that the polypeptides with molecular weights of 10, 15, 29, 38, 48 and 58 KD were the most prominent in the control (Table I and Fig. 1a). The effect of the applied salinity treatments resulted in the induction of new bands with molecular weight of 11, 35 and 46 KD (Fig. 1) in all treatments with NaCl and 52 and 68 in seedling treated with 50 and 150 NaCl. An over accumulation for protein bands with molecular weights of 10, 15, 38, 48 and 58 KD was observed after different treatments. On the other hand, treatment cultivar Sids 1 with Vitamin B6 resulted in the induction of five new bands with molecular weight of 11, 18, 33, 52 and 68 KD and increase the intensity of most polypeptide bands while treatment this cultivars with ascorbic acid has little change and induced only two new polypeptides with molecular weight of 46 and 68 KD.

The distribution of protein bands in SDS gel of wheat cultivar Sakha 69 revealed 27 polypeptide bands (Table I and Fig. 1b). As compared with the control (Lane 1), the major polypeptide bands were not affected by salinity or vitamins treatments (Lane 2, 3, 4, 5, 6, 7 and 8). Slight modifications in polypeptide patterns are observed and three new polypeptide bands with molecular weight of 11, 52 and 68 KD (arrows) were induced under all salinity and vitamins treatments.

The effect of different NaCl treatments on the cultivar Gemmiza 5 in the absence or presence of vitamin B6 and ascorbic acid are shown in Figure 1C. This cultivar showed greater sensitivity to salt stress as indicated by the appearance of novel protein bands with molecular weights of 11, 18, 33, 35 and 46 KD under all salinity treatment. Unique band with Molecular weight of 45 KD (arrow) was observed after 180 mM Nacl treatment (Table I and Fig. 1c). Similar results were obtained by Matos *et al*. 2000 and 2001 who examined two cultivars of cowpea (*Vigna unguiculata*) differentiating in drought tolerance and identified a novel gene that stimulated by drought stress and encode a predicted 43 KD protein. They reported that the expression

Table I. Effect of different concentrations of sodium chloride, vitamin B6 and ascorbic acid on protein banding pattern of three *Triticum aestivum* cultivars using SDS-PAGE technique

Band No	M.wt	*Cultivar Sids 1* Lanes								*Cultivar Sakha 69* Lanes								*Cultivar Gemmiza 5* Lanes							
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1	82	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+++	+	++	--	+	--	+
2	80	+	+	+	+	+	--	+	--	+	+	+	+	+	+	+	+	+	+++	+	++	+	+	+	+
3	72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	68	--	--	+	+	+	+	+	+	--	+	+	+	+	+	+	+	--	--	+	+	+	+	+	+
5	64	+	--	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	--	+	+	+	+	+	+
6	62	+	+	++	++	--	++	+	+	+	+	+	+	+	+	+	+	+	--	+	+	+	+	+	+
7	58	+	++	++	++	++	+	+	+	+	++	++	++	+	++	++	++	+	++	+++	++	++	+	++	+
8	54	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	52	--	--	+	+	+	+	--	--	--	+	+	+	+	+	+	+	--	--	--	--	--	--	--	--
10	48	+	++	+	+	++	+	+	+	+	++	++	++	+	+	++	++	+	++	++	++	++	++	++	++
11	46	--	+	+	+	--	+	+	+	+	+	+	+	+	+	+	+	--	+	+	+	+	+	+	+
12	45	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	--	--	--	--	--	--
13	43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	38	+	++	++	+	+	+	+	+	+	++	++	++	+	+	++	++	+	++	++	++	+	++	+	++
15	35	--	++	+	+	--	+	--	+	+	+	+	+	+	+	+	+	--	+++	+	++	+	+	--	+
16	33	--	+	--	--	+	--	--	--	+	+	+	+	+	+	+	+	--	+++	+	++	--	++	--	+
17	30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	+	++	+	++
18	29	+	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+	++	++	++	+	++	+	++
19	27	+	+	+	+	+	--	--	--	+	+	+	+	+	+	+	+	+	+	++	++	+	+	+	++
20	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	+	++	+	++
21	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	18	--	+	+	--	++	+	--	--	+	+	+	+	--	--	+	--	--	++	+	+	--	+	--	+
23	15	+	++	++	++	++	++	+	+	+	++	++	++	+	++	++	++	+	++	+++	++	+	+++	+	+++
24	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	+	++	+	++
25	13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	11	--	+	+	+	+	+	--	--	--	+	+	+	+	+	+	+	--	+	+	+	+	+	--	+
27	10	+	++	++	+	++	++	+	+	+	++	++	++	+	+	++	++	+	+	++	+	+	++	+	+

Lane 1= Control; Lane 2= 180mM NaCl; Lane 3= 150mM NaCl; Lane 4 = 50mM NaCl; Lane 5= 100ppm Vitamin B6; Lane 6= 180mM NaCl + 100ppm Vitamin B6; Lane7= 100ppm ascorbic acid; Lane 8= 180mM NaCl + 100ppm ascorbic acid

of this gene is more pronounced in drought-sensitive cultivar than in drought-tolerant one.

The interactive effect of salinity with 100 ppm vitamin B6 or ascorbic acid on protein banding patterns in studied cultivars were shown in Table I and Fig. 1 (Lanes 6 and 8). Some new bands with molecular weight of 11, 35, 46 and 68 KD were observed in the all treated cultivars. Vitamin B6 is more effective in enhancement of protein synthesis

Fig. 1. SDS-PAGE protein profile of *Triticum aestivum* L. cultivars Sids 1(a), Sakha 69 (b) and Gemmiza 5 (c) treated with NaCl, vitamins B6 and ascorbic acid. (M = marker protein) Lane 1= Control; Lane 2= 180mM NaCl; Lane 3= 100mM NaCl; Lane 4 = 50mM NaCl; Lane 5= 100ppm Vitamin B6; Lane 6= 180mM NaCl + 100ppm Vitamin B6; Lane7= 100ppm ascorbic acid; Lane 8= 180mM NaCl + 100ppm ascorbic acid

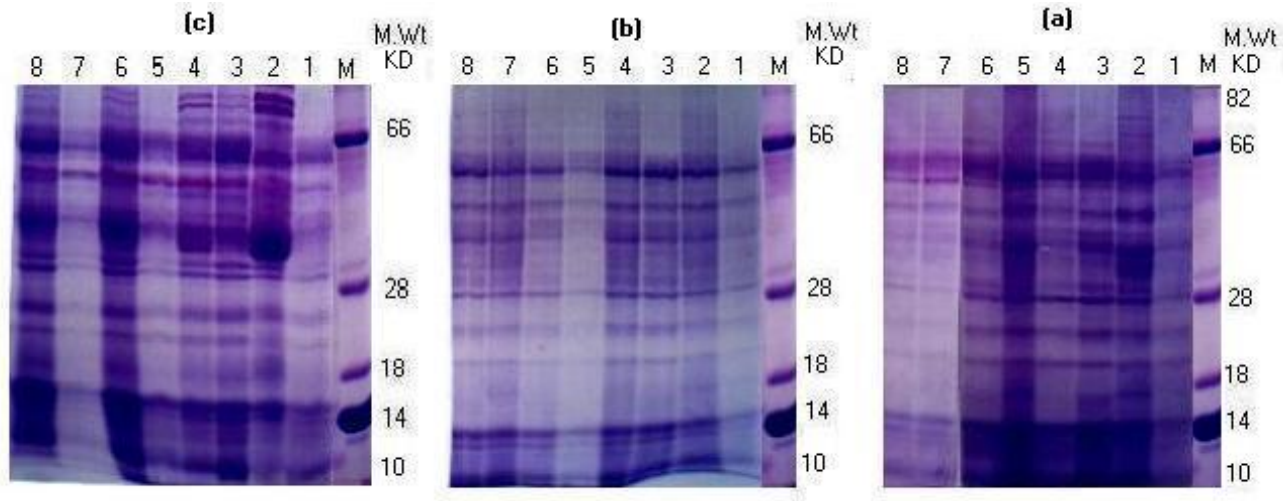

Table II. Total cell examined, total divided cells, percentage of normal and abnormal mitotic phases, different types of mitotic abnormalities and mitotic index after treating *Allium cepa* root tips with sodium chloride, vitamin B6 and ascorbic acid for 24 h

Treat-ment	*Total Cell Exam.*	*No. of Divided Cells*	*Prophase %*		*Metaphase %*		*Anaphase %*		*% of different types of abnormalities*								*Mitotic Index ±SE*
			Norm.	Abnor.	Norm.	Abnor.	Norm.	Abnor.	Sticky Met.	CM	Lagg Chrom at met.	Dist met.	Stick chrom at ana.	Bridg	Lagg. chrom at ana.	Dist. Ana.	
1	--	--	--	--	--	--	--	---	--	--	--	--	--	--	--	--	0.00
2	11782	26	--	--	53.85	100	46.15	100	100	--	--	--	100	--	--	--	0.19 ± 0.19**
3	9815	0.0	--	--	--	--	--	--	---	--	--	--	--	--	--	--	0.00
4	12920	792	39.39	--	29.04	6.09	31.56	4.40	0.87	0.87	0.87	3.48	--	0.40	0.80	3.20	6.14 ± 0.65
5	11522	785	34.94	--	23.31	2.70	30.19	1.69	--	0.55	0.55	1.64	--	0.42	0.42	0.84	6.93 ± 0.69
6	8882	998	45.89	--	27.00	--	28.06	0.70	--	--	--	--	--	--	0.35	0.35	11.28 ± 0.26**
7	9281	914	41.24	--	29.75	--	28.99	0.75	--	--	--	--	--	--	0.37	0.37	9.86 ± 0.21*
8	10791	885	42.15	--	26.67	2.54	31.18	1.45	--	0.42	0.42	1.69	--	o.36	0.36	0.72	8.27 ± 0.63
9	8772	604	45.03	--	26.98	5.52	27.98	1.78	--	--	0.61	4.91	--	--	0.59	1.18	6.92 ± 0.25
10	8954	637	44.11	--	26.05	--	29.62	0.21	--	--	--	---	--	--	--	0.21	7.20 ± 0.45

Norm= Normal; Abnor= Abnormal; D.M.= Distributed metaphase; CM= Colchicine metaphase; Met.= Metaphase; Ana.= Anaphase; Lagg.= Laggard; Chrom= Chromosome ;Treatments 1 = 250 mM NaCl; 2 = 200 mM NaCl; 3 = 180 mM NaCl; 4 = 150 mM NaCl; 5 = 50 mM NaCl; 6 = 100ppm Vitamin B6; 7 = 100 ppm ascorbic acid; 8 = 180 mM NaCl + 100 ppm Vitamin B6; 9 = 180mM NaCl + 100ppm ascorbic acid; 10 = Control

Table III. Effect of sodium chloride, vitamin B6 and ascorbic acid on Root and shoot length of three cultivars of *Triticum aestivum*

Treatment	*Root length (% of control)*			*Shoot length (% of control)*		
	Sakha 69	Gemmiza 5	Sids 1	Sakha 69	Gemmiza 5	Sids 1
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	25.31	16.67	34.89	20.81	22.51	28.49
4	65.43	53.13	65.10	53.81	41.99	42.51
5	88.89	82.81	89.93	75.63	63.20	84.56
6	107.41	103.13	140.94	81.73	122.08	98.11
7	101.85	102.60	127.52	91.37	104.76	117.28
8	74.06	63.54	73.83	55.84	44.59	84.57
9	67..90	67.71	87.24	61.42	47.62	60.28
10	100	100	100	100	100	100

Treatments 1 = 250 mM NaCl; 2 = 200 mM NaCl; 3 = 180 mM NaCl; 4 = 150 mM NaCl; 5 = 50 mM NaCl; 6 = 100ppm Vitamin B6; 7 = 100 ppm ascorbic acid; 8 = 180 mM NaCl + 100 ppm Vitamin B6; 9 = 180mM NaCl + 100ppm ascorbic acid; 10 = Control

than ascorbic acid in salinized seedling of cultivar sids 1. Also, the polypeptides which, has disappeared in salinized seedling returned to appear when those seedlings are treated with vitamin B6 or ascorbic acid (Table I).

In the present study, salinity stress induced a considerable variation in the protein patterns among different wheat cultivars. This variation has been manifested as the novel expression of some polypeptides; the absence of others and over expression of a third class of polypeptides. Several of new proteins which are synthesized in response to environmental stress have been reported as stress-proteins in plants (Hoyos & Zhang, 2000; Patharkar & Cushman, 2000). Many of these proteins were suggested to protect the cell against the adverse effect of salt stress. Kawasaki *et al*. (2001) found that protein turnover in stressed plants were observed at early time, followed by the induction of known stressed–responsive transcripts within hours, and the induction of transcripts for defense-related function later. So, it can be suggested that the proteins with molecular weights of 11, 18, 33, 35, 43, 46, 52 and 68 KD could play an important role in triggering a system to tolerate sever stress of NaCl.

Salinity treatment was found to induce the disappearance of a protein band with a molecular weight of 64 KD in wheat cultivar sids 1 and 62 and 64 KD in wheat cultivar Gemmiza 5 under 180 mM NaCl. No protein bands were disappeared in cultivars Sakha 69 after treatment with NaCl. The disappearance of polypeptides during stress compensates the increased synthesis of the others. These polypeptides which, has disappeared in salinized seedling returned to appear when those seedlings are treated with vitamin B6 or ascorbic acid (Table I).

Changes in protein synthesis under salt stress may be due to changes in the efficiency of mRNA translation or the regulation of RNA transcription, transport and stability. The expression of salt-stress proteins is related to the adaptation process of seedlings to salinity as well as to the genetic constitution of selected salt tolerant genotypes (Dell'Aquila & Spada, 1992, 1993). The levels of proteins differ in salt-tolerant and salt-sensitive genotypes when they are subjected to salinity stress (Dubey & Rani, 1989). In the present study, the wheat cultivar Sakha 69 which is claimed to be salt-tolerant appears to be the least affected by salinity with regard to growth and protein pattern (Table I, III). Similar results were obtained by Badr *et al*. (1998) on the effect of NaCl treatments on protein expression of wheat cultivars Sakha 8. They reported that salinity treatment on this cultivar at plumule stage appearance showed no effect on SDS-PAGE patterns. Costa-Franca *et al*. (2000) also reported that two cultivars of *phaseolus vulgaris* (Carioca & Ouronegro) were characterized by having better drought tolerance mechanisms leading to a better growth under water deficits as compared with other two cultivars (A320 & Xodo). Majoul *et al.* (2000) study the effect of salt-stress on polypeptide patterns in roots of salt-tolerant and salt-sensitive cultivar of wheat by two-dimensional polyacrylamide gel electrophoresis when seedling treated with 200 mM NaCl. They reported that the protein patterns for control and salt-stressed seedlings were qualitatively similar. Plants respond to the stress in part by modulating gene expression, which eventually leads to the restoration of cellular homeostasis, detoxification of toxins and recovery of growth (Xiong & Zhu, 2002). Two protein kinases with molecular masses of 48 and 40 KD are activated in tobacco cells exposed to NaCl. The activation of the 40-KD protein Kinase is dose-dependent and is specific for hyperosmotic stress (Hoyos & Zhang, 2000).

Treatments of seeds with vitamin B6 or ascorbic acid induced the appearance of some new protein bands in seedling of the three wheat cultivar. Also, treatmenting the seed with vitamin B6 resulted in an increased intensity of most polypeptide bands which already apparent in the control (Fig. 1 a, b, c). The induction of new bands and the significant increase in the intensity of some original bands, indicate that the vitamin B6 and ascorbic acid has a profound effect on the qualitative and quantitative changes in the protein component of the plants. It is known that different B vitamins are known to be precursors of various coenzymes; acting in several mechanisms. Treatment clusterbean (*Cyamopsis tetragonoloba* L.) with vitamin B induced significant alternation in the enzymes related to protein metabolism and enhancement of the endogenous level of cytokinin (Kodandaramiah *et al.*, 1984).

The cytological effects and the data obtained from the analysis of root tips treated with NaCl and vitamin B6 or ascorbic are summarized in Table II. One of the major effects of NaCl on root tips of *Allium cepa* is its influence on the rate of cell division. Salinity caused an inhibitory effect on the process of cell division. Sodium chloride has lethal effect at the highest concentration tested, (250 mM NaCl) in which the mitotic index was recorded zero. Treatment cells for 24 h with 200, 180 mM NaCl caused significant inhibition in cell division and drastic drop in the mitotic index was recorded in roots treated with 200 mM NaCl (0.19%), while a complete inhibition in mitotic division was observed in roots treated with 180 mM NaCl. These results indicate that NaCl has mitostatic effect at these treatments. On the other hand, roots treated with 150 and 50 mM NaCl showed little effect on the mitotic index since it reached 6.14 and 6.93 % respectively as compared with control which recorded 7.20%. Mitotic inhibition is due to arrest the cells at G1phase and suppressing DNA synthesis (Schneiderman *et al.*, 1971), or arrest the cell in G2, preventing the cell from entering mitosis (Van't Hof, 1968). The entry of cells from G2 into mitosis is regulated by cyclin dependant kinase CDK/cyclin B while cyclins (D & E) can move cells from G1 into S phase (Fabian-Marwedel *et al*., 2002; Vandepolle *et al*., 2002).

On the other hand, treatment of the roots with Vitamin B6 or ascorbic acid (100 ppm) showed an increase in MI (11.28 & 9.86 % respectively) as compared with the control. The addition of Vitamin B6 or ascorbic acid to 180 mM NaCl showed a considerable increase on the cell division. The mitotic index is promoted after combined treatments of NaCl with vitamin B6 or ascorbic acid and the MI reached 8.27 and 6.92 % respectively as compared with the value 0.00 obtained after treatment with 180 mM NaCl only. Antagonistic effect of Vitamin B6 or ascorbic acid was observed by other investigators (Wafers & Sies, 1988; Koul *et al.*, 1989). The previous results indicates that Vitamin B6 and ascorbic acid of concentration 100ppm when mixed with 180 mM NaCl does not only antagonize the inhibitory action of the NaCl but activates the cells to inter mitosis and induce a high mitotic activity. The antimutagenic actions of different vitamins have been reported by many investigators (Hoda *et al*., 1991; Emerit *et al*., 1997; Vaglenov *et al*., 1998; Andrew, 1997). Fawzia (2002) found that vitamin C minimize the effect of lead acetate on the mitotic activities. Also, El-Shiekh (1999) found that Vitamin B complex had the ability to minimize the incidence mitotic inhibition and clastogenicity by detoxification of bavistin fungicide.

Few types of abnormal mitotic phases are induced after different treatment with NaCl. The types and frequencies of mitotic abnormalities (Table II). In roots treated with 250 and 200 mM NaCl DNA lesions were observed. The interphase cells at these concentrations had an abnormal appearance with the chromosome material condensed into heterochromatic clumps. High concentrations lead also to the development of oblong hyaline structures formed by material extruded from the nucleus. Aliyev *et al.* (2000) found that drought stress caused quantitative changes in the genetic materials (nuclear, chloroplast and mitochondrial nucleic acids) of wheat seedling and GA_3 application alleviated this effect by

activation the synthesis of nucleic acids. On the other hand, roots treated with 150 and 50 mM NaCl showed low percentage of chromosomal abnormalities which mainly comprise disturbed metaphase or anaphase, C-metaphase and lagging chromosomes. The production of these abnormalities indicates that NaCl induced partial inhibition on mitotic apparatus. No deviations from the normal were seen in roots treated with Vitamin B6 or ascorbic acid. Similar results were observed by Bronzetti *et al*. (2001) who found that Vitamin A and E did not have toxic or mutagenic action.

The effect of the applied treatment on growth rate is documented in Table III. The inhibition effect of NaCl on mitotic activity is accompanied by a reduction in the growth rate of seedlings. This reduction appears to be concentration-dependent. This indicated by the values of root and shoot length of *Triticum* seedling after 10 days of germination in which a complete inhibition in growth was observed after treatment with 250, 200 and 180 mM NaCl (Table III). These results are in harmony with those obtained by (Kord & Khalil, 1995; Houle *et al.*, 2001) who found that seed germination was reduced in low concentrations and completely inhibited by high concentrations of NaCl and this inhibiting effect was reversible. The reduction in growth and changes in protein pattern are consistent with results of Soussi *et al.* (2001), who investigate the responses of *Mesorhizobium ciceri* to salt stress and the changes in protein profiles induced by salinity. They reported that strain (ch-191) tolerated up to 200 mM NaCl although higher salt dosages limited its growth and the protein profile showed major alteration at salinity levels which inhibited growth.

On the other hand, treatment grains with vitamin B6 or ascorbic acid showed a considerable increase in root and shoot length. Combination of NaCl (180 mM) with vitamin B6 or ascorbic acid antagonized the inhibition effect of NaCl and caused increase in the growth rats. Plant growth can be considered to consist of two components; cell division and cell expansion, it also involves metabolic events in which growth hormones play a major role. Adverse effects of salinity or any other similar stress conditions were attributed by some investigators to lower endogenous levels of cytokinins and to imbalance hormonal content (El-Mashed & Kamel, 2001). Retardation of plant growth may thus be the result of inhibition of growth regulators or the delay in mitotic division. Padmavahti *et al.* (1992) relate seedling growth retardation to germination injury and the production of chromosomal abnormalities in dividing cells. Mendhulkar (1993) attributed plant growth inhibition to disturbances in natural growth regulators and mitotic chromosomal irregularities as additional factor.

CONCLUSION

Considering all data of the present study, it can be concluded that salinity treatments induced a considerable variation in the SDS-PAGE pattern among the three wheat cultivars; and the cultivar Sakha 69 appeared to be the least affected by salinity. Also, it can be suggested that the inhibition effect of NaCl on cell division and cell growth may be due to inactivation of Kinases or cyclins activity and subsequently arrest the cells at G1 or G2 phase, so hindering the cell to enter mitosis and the treatment with Vitamin B6 or ascorbic acid have the ability to repair the genotoxic effect of the salinity.

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