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Response of Bean (*Phaseolus vulgaris*) to Exogenous Putrescine Treatment under Salinity Stress

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Abstract: Exogenous Put treatment (10^{-2} mM) showed a valuable results on germination and growth of bean (*Phaseolus vulgaris* L. cv. Giza 6) under normal and NaCl-induced stress. Germination percentage of Put treated seeds increased to 88% under salinity stress, may be due to the activation of amylase and protease enzymes during germination. The rise of K^+/Na^+ ratio in shoots of Put treated seedlings under NaCl-induced stress suggest that Put treatment may partially ameliorate the adverse effects of sodium and chloride ions. Put treatment improved growth of salt-stressed seedlings, may be through the partial inhibition of amylase and protease enzymes and increasing the total content of nucleic acids and photosynthetic pigments. Changes in protein banding patterns suggest that a defense-response genes could be activated by Put treatment.

Key words: Amylase, germination, K^+/Na^+ ratio, nucleic acids, photosynthetic pigments, protease, protein banding

INTRODUCTION

Plant species vary in their sensitivity and response to environmental stresses because they have various capabilities for stress perception, signaling and response[1]. Biochemical studies showed that accumulation of several nitrogen containing compounds such as amino acids and proteins is regarded as a common response of plants e.g. barley[2] and wheat[3] to salinity stress. Polyamines (PAs) biosynthesis and proline accumulation under stress could be a mechanism of formation of nitrogenous reserves in these situations[4]. Broetto *et al.*[5] suggested that polyamines play an important role in the growth and development of the cells. They also concluded that polyamine metabolism appear to be an important biochemical marker of tolerance to salinity in several plant species. Among the early markers, polyamines have an essential role in the control of cell division and elongation, cell differentiation and morphogenesis[6,7]. *In vitro* experiments showed that PAs affect many functions of nucleic acids, including transcription[8]. Bagga *et al.*[9] suggested that the putrescine aminopropyltransferase (PAPT) activity and the accumulation of the possibly protective polyamines would be favored in meristematic tissues that are needed for plant recovery following stress and are not required in more mature nonmeristematic tissues that will not be utilized or will not be reactivated for growth during plant recovery from stress. In many plant systems an increase in growth occurs in parallel to enhancement of ornithine decarboxylase (ODC) and/or arginine decarboxylase (ADC) activity[10-11]. Erdei *et al.*[12] and Santa *et al.*[13] supported the idea that the initiation of polyamine accumulation needs an osmotic signal; however, when a permeable ion is present, salt accumulation can contribute to the osmotic adjustment and thus the onset of polyamine biosynthesis is delayed or does not take place. Some enzymes in the polyamine biosynthetic pathway may be sensitive for high salinity and the biosynthetic processes shift towards oxidative degradation. Exogenous PAs stabilize the protoplasts, increase the frequency of mitosis and delay senescence[14]. PAs retard or prevent the increase in RNase and protease in detached oat and radish leaves[15,16]. PA treatment may retard chlorophyll loss in detached leaves incubated in darkness, but accelerates it during light incubation[15]. It has been proposed that PAs may retard senescence by stabilizing nucleic acids and cell membranes against enzymatic degradation and solute leakage[17]. These polycationic compounds may interact with anionic macromolecules such as DNA and RNA[8]. Therefore, stabilization of the double helical-structure of mRNA, rRNA and tRNA via their binding to specific sites is affected by PA level in the plant cell[18]. Structure and many types of proteins and the activities of numerous enzymes are modulated by PA binding[19,20]. PAs are suggested to have an important role in gene expression in many plant species e.g. tomato[21,22].

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From the above mentioned reviews, the present work was conducted to elucidate the mechanism by which Put treatment improve germination and growth of bean under salinity stress. Activity of the hydrolytic enzymes, amylases and protease, was studied during germination and seedling growth as affected by Put treatment and salinity. Nitrate reductase (NRase) activity was also assayed in leaves and roots of bean seedlings. Growth, chlorophyll, nucleic acids, proline, total sugars and soluble proteins content, as well as K^+/Na^+ ratio were measured under the influence of salt stress and Put treatments. The effect of Put treatment on gene expression in salt stressed bean seedlings was also studied via detecting changes in the protein banding patterns.

MATERIALS AND METHODS

Seeds of bean (*Phaseolus vulgaris* L. cv. Giza 6) were obtained from Agricultural Research Center, Giza, Egypt. Selected seeds of uniform size were sterilized with sodium hypochlorite solution (2.5%) for 3 min. then rinsed with distilled water several times. They were germinated in plastic pots (15 cm diameter) containing equal amounts (1 Kg) of acid washed sand soil. Pots were divided into four groups, the first was irrigated once with full strength Hoagland nutrient solution to serve as control plants. The second group was treated once with 1% NaCl in the nutrient solution. The third group was supplemented with 10^{-2} m M putrescine (Put) in the nutrient solution, while the fourth one was supplied with 10^{-2} m M Put and 1% NaCl in the nutrient solution. Soil moisture was maintained 60% of the field capacity all over the experimental period. Seeds were allowed to germinate at 25°C. Growing seeds were collected at 6 d-old for assaying the activity of the hydrolytic enzymes, α-β-amylases and protease. The total content of DNA and RNA was also measured. Another experiment was continued to the seedling stage (20 d-old), but the salt treatment was applied at 13 d-old. Activity of α-β-amylases and protease enzymes were assayed in leaves, while the activity of *in vivo* nitrate reductase enzyme was assayed in leaves and roots. Growth criteria e.g., shoot length, leaf area, fresh and dry mass of leaves; as well as the leaf content of chlorophyll, nucleic acids, proline, total sugars and soluble proteins, in addition to the K^+/Na^+ ratio were determined at 20 d-old.

Enzyme activity: For enzyme assay, plant material was prepared at 0-4°C by macerating the tissues with a chilled pestle and mortar. The tissue homogenate was centrifuged at 10 000 g for 20 min and the supernatant obtained was used directly for determining enzyme activity. For assaying the activity of α-β-amylases, 3,5-dinitrosalicylic acid reagent was used as described by Rick and Stegbauer[23]. Protease activity was assayed according to Gallop *et al.*[24]. Nitrate reductase activity was assayed *in vivo* according to the method described by Hewitt[25] using sulphanilamide and N-naphthylene diamine hydrochloride (NEDH).

Chemical analyses: For extraction of nucleic acids, the method of Marmur[26] and Mohamed and Capesius[27] was followed using Tris-EDTA buffer (pH 8). DNA content was determined using diphenylamine reagent as described by Stahl[28]. RNA was measured according to Schneider[29] using orcinol reagent. Fresh leaves were extracted in 70% ethanol and completed to a known volume with distilled water and used for estimation of sugars using anthrone reagent[30] and soluble proteins[31]. Proline content was determined according to Bates *et al.*[32]. Photosynthetic pigments were estimated in 85% acetone extracted leaves according to Metzner *et al.*[33]. Potassium and sodium ions were measured photometrically in acid digested samples using a Corning-400 Flame Photometer.

Statistical analysis: Statistical analysis was carried out according to Snedecor and Cochran[34] using analysis of variance (Completely Randomized two-factors Design) and the significance was determined using LSD values at 0.05 and 0.01 levels.

SDS polyacrylamide gel electrophoresis: The applied protocol for protein banding pattern was followed according to Laemmli[35]. Total proteins were extracted overnight using 0.2 M Tris-HCl buffer, pH 3.8 containing SDS. After centrifugation at 9000 rpm for 6 min. the supernatant was collected. SDS slab gel of 12.5% acrylamide was used. The total protein was estimated and the SDS polyacrylamide gel electrophoresis was carried out according to Hames[36]. Coomassi blue (0.5 g L^{-1}) was used for staining. Destained gel were photographed while wet and the protein patterns were analyzed quantitatively using a leser Gel Documentation System (GDS) where molecular weights of the protein banding patterns of each sample were identified using gel-pro analyzer V 3.0 computer program.

RESULTS AND DISCUSSION

Table 1 shows the response of bean seeds to exogenous Put treatment during germination under NaCl-induced stress. The results indicated a reduction in germination percentage to become 66% under salinization, due to the osmotic stress and the toxic effect of sodium and chloride ions, as reported by Waisel[37]. This adverse effect of salinity led to inhibition in activity of

Table 1: Effect of salinity and Put treatment on germination (%), activity of amylases and protease [μg g^{-1} (f.m.) s^{-1}] and nucleic acids content [mg g^{-1} (d.m.)] of the growing bean seeds [6 d-old]

Treatment		Germ.	α-amylase	β-amylase	Protease	DNA	RNA
Control		100	1.82	2.46	0.025	21.5	30.6
Put		100	2.25	3.15	0.04	24.6	36.1
NaCl		66	1.06	1.16	0.057	28.6	33.9
Put+NaCl		88	1.57	1.84	0.036	28.4	33.1
LSD	0.05		0.18	0.22	0.011	1.2	1.7
	0.01		0.25	0.32	0.015	1.7	2.5

Table 2: Effect of salinity stress and Put treatment on enzyme activity [μg g^{-1} (f.m.) s^{-1}], total sugars and total soluble proteins [mg g^{-1} (d.m.)], proline [μ moles g^{-1} (d.m.)] and nucleic acids contents [mg g^{-1} (d.m.)] of bean seedlings [20 d-old]

					NRase						
					---			T.soluble			
Treatment		α-amylase	β-amylase	Protease	root	leaf	T. sugars	proteins	Proline	DNA	RNA
Control		0.498	0.561	0.235	1.28	1.75	82.9	24.3	4.2	25.0	22.4
Put		0.425	0.606	0.243	1.33	1.65	90.0	42.1	5.5	30.3	25.9
NaCl		1.189	1.361	0.274	0.82	1.12	216.5	97.4	5.7	24.1	17.3
Put+NaCl		0.953	0.934	0.253	1.12	1.12	156.5	62.6	5.6	38.0	27.3
LDS	0.05	0.11	0.12	0.1	0.06	0.05	11.0	1.3	0.5	2.7	2.8
	0.01	0.15	0.17	0.14	0.08	0.07	16.0	1.8	0.7	3.9	4.0

Table 3: Effect of salinity stress and Put treatment on growth, photosynthetic pigments, and K/Na ratio of bean seedlings [20 d-old]

		Shoot	Leaf area	Leaf f.m.	Leaf d.m.	Chl a	Chl b			Chl a+b/	
Treatment		length (cm)	(cm^2)	(mg $plant^{-1}$)	(mg $plant^{-1}$)	---[mg g-1 (d.m.)]----		Car.	Chl a/b	Car.	K+/Na+
Control		23.0	44.84	75.0	9.0	16.44	11.98	15.98	1.37	1.78	12.33
Put		26.5	50.63	128.0	13.0	21.54	15.54	19.23	1.39	1.93	12.44
NaCl		19.0	36.87	56.0	7.0	14.45	10.39	42.52	1.39	0.59	2.78
Put+NaCl		20.8	42.93	64.0	8.0	17.95	13.00	30.64	1.38	1.01	9.88
LDS	0.05	1.8	2.90	11.0	1.0	1.53	0.87	1.03			
	0.01	2.8	4.20	16.0	2.0	2.20	1.25	1.48			

α-β-amylases, suggesting a reduction in the concentration and translocation of sugars into the embryo axes during germination and early growth. However, it was observed that the activity of protease enzyme markedly increased under salinity stress which may, to some extent, compensate the reduction in amylase activity during germination through the process of gluconeogenesis. Similar results have been obtained by Roy and Srivastava[38], Mandal and Singh[39]. Exogenous Put treatment activated amylases and protease enzymes in the growing seeds which in turn increased germination percentage of salt-stressed seeds to become 88%. These results support the conclusion of Tipirdamaz *et al.*[40] who reported that after 3 days of germination, polyamines (spermine, spermidine or putrescine) significantly increased α-amylase activity in barley seeds and concluded that the adverse effect of salt stress on germination can be partially rectified by polyamines. Ali[41] suggested that there is at least one mechanism by which NaCl inhibits germination and seedling growth, through reducing the endogenous level of Put which in turn adversely affects various cellular processes. It has been postulated that the increase of endogenous Put may play a specific protective roles in plants adapted to extreme environment.

DNA and RNA content increased in the growing seeds under salt stress, may be due to the occurrence of cell division while the cell elongation was reduced as a result of osmotic stress (Table 1). Put treatment reduced such increment of DNA and RNA content under salinity conditions, suggesting a partial recovery in early cellular growth. On the other hand, nucleic acids content was markedly reduced in leaves of salt-stressed seedlings (20 d-old), which could be attributed to growth inhibition and stimulation of catabolic processes e.g. hydrolysis (Table 2). Amylases and protease enzymes were stimulated by salinity in the seedling leaves (20 d-old). Consequently, the organic solutes e.g. sugars, soluble proteins and proline content increased as indicated in Table 2. This response may play a significant role in cellular osmoregulation[42]. However, Put treatment reduced such activation of hydrolytic enzymes in leaves of salt-stressed seedlings. Nucleic acids content also increased in leaves of Put treated seedlings under salt stress. All these effects may suggest an improvement in the anabolic processes in Put treated plants under salinity conditions (Table 2). Galston and Kaur-Sawhney[43] and Kumar *et al.*[8] postulated that PAs are polycations and thus bind readily to such important cellular macromolecules as DNA and RNA, phospholipids and acidic protein residues. Through such binding, PAs could affect the synthesis and activity of macromolecules, membrane permeability and processes of mitosis and meiosis.

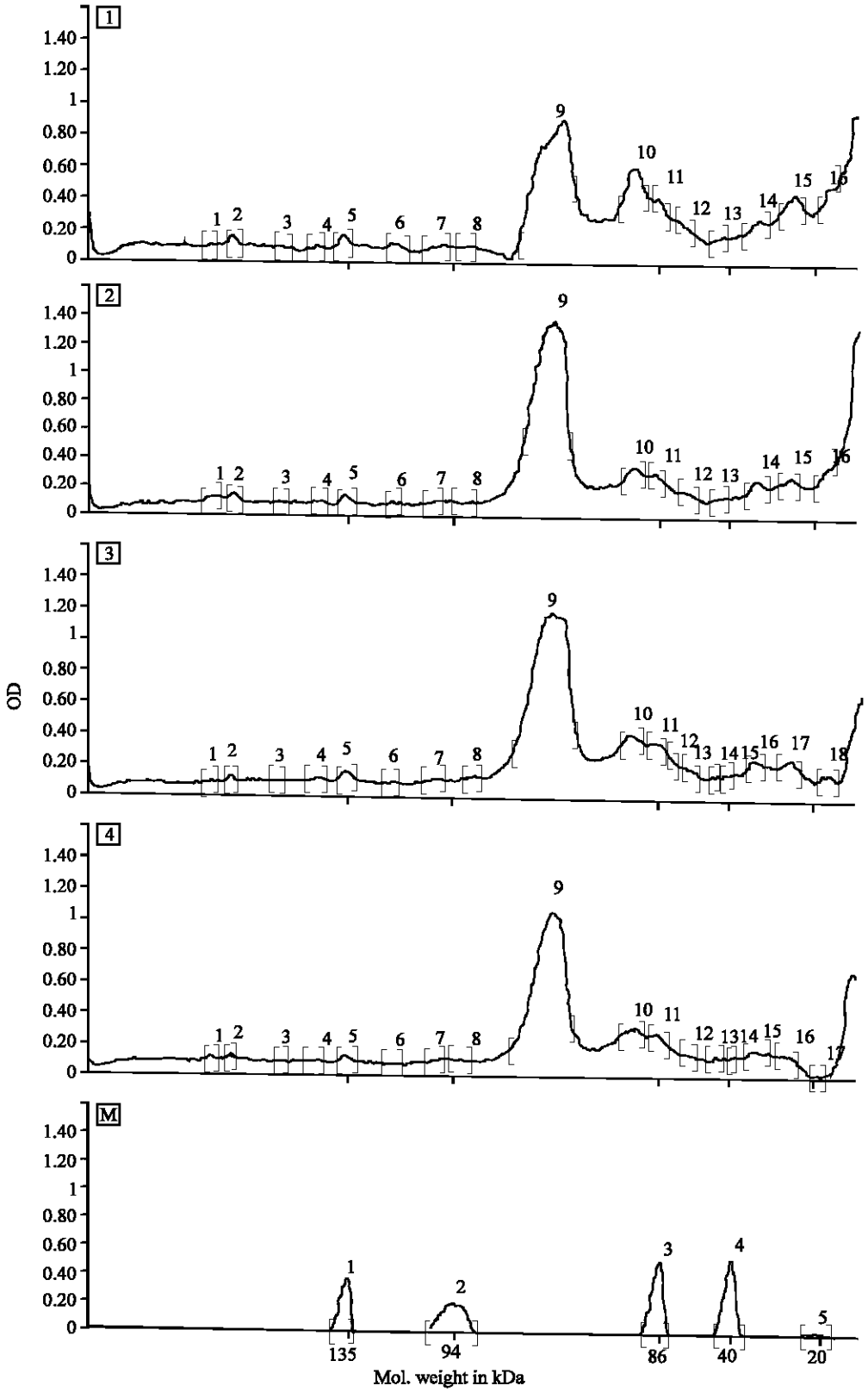

Fig. 1: Effect of salinity stress and put treatment on protein banding patterns. M: marker, lane 1: control, lane 2: Put, lane 3: NaCl + Put, lane 4: NaCl

Table 4: Effect of salinity stress and Put treatment on protein banding patterns of bean seedlings [20 d-old]

M.Wt. (KDa)	Control	Put	NaCl	Put+NaCl
218.5	0.17	0.65	0.54	0.15
203.8	1.12	0.91	0.48	0.70
172.7	0.14	0.17	0.04	0.01
149.2	0.22	0.27	0.12	0.37
137.1	1.67	1.91	1.06	2.09
135	18.16			
115.7	0.88	0.22	0.09	0.13
101.1	0.67	0.53	0.06	0.32
93.36	0.03	0.06	0.02	0.27
90.08	76.21	83.36	88.68	87.03
87.08	10.33	3.37	2.34	1.88
83.08	0.96	1.05	1.19	0.96
71.46				0.037
62.38	0.16	0.78	0.11	0.38
45.81	0.75	0.25	0.08	0.10
38.21			0.14	0.8
32.43	1.50	3.06	0.67	1.09
24.44	4.42	1.80	1.2	1.88
18.59		0.06	0.015	0.67
Total	16.00	16.00	17.00	18.00

Nitrate reductase (NRase) is the key regulatory enzyme of nitrate assimilation and its synthesis as well as activity is influenced by a variety of environmental factors, including water stress[44,45]. Salinity, in the present experiment, reduced NRase activity in leaves and roots compared to control plants (Table 2). Put treatment increased its activity in the roots under normal and salinity conditions.

Chlorophyll (Chl) a and b decreased under salinity stress, while carotenoids (Car) content increased more than 2 folds (Table 3). Application of Put (10^{-2} mM) markedly increased Chl a, b and Car content in leaves of stressed and unstressed seedlings. Chl a/b ratio did not altered under salinization, but Chl a+b/Car declined to approximately $^1/_3$ the controls. The contents of Chl a and b in seedlings treated with Put+NaCl were normalized, but not the content of Car that remained very high. Therefore, the ratio Chl a+b/Car was lower in salt stressed seedlings. K^+/Na^+ ratio was found to be greatly reduced with NaCl stress, which was paralleled with a great reduction in growth criteria (Table 3). Put treatment increased the value of K^+/Na^+ ratio in salt-stressed seedlings and increased growth criteria e.g., shoot length, fresh and dry masses of leaves and the mean leaf area. Similar results have been obtained by Krizek *et al.*[46], Hassanein and El-Shintinawy[47]. Ali[41] observed that exogenous application of Put to NaCl-stressed *Atropa belladonna* seeds reduced effectively the net accumulation of sodium and chloride ions in different organs of seedlings. The obtained results also support the suggestion of Galston and Kaur-Sawhney[43], Flores[48] who reported that senescence is extremely affected by PAs, which are commonly known as antisenescence agents and observed that PAs retained chlorophyll and inhibited RNase and protease activity. Besford *et al.*[49] attributed the positive effects of PAs on chlorophyll and carotenoid levels to preservation of the thylakoid membranes at the site of chlorophyll-protein complex.

The changes in protein banding patterns in leaves of bean seedlings (20 d-old) were determined as affected by Put treatment under normal and NaCl-induced stress (Fig. 1 and Table 4). Proteins extracted from control seedlings revealed the presence of 16 protein bands with molecular weights ranging from 218.5 to 18.59 KDa. A distinct band of molecular weight 135 KDa, is considered characteristic to control because it disappeared from other treatments. Proteins extracted from leaves of salinated bean seedlings indicated a reduction in band intensities of proteins having molecular weights of 203.8, 172.7, 149.2, 115.7, 101.1, 87.08, 45.81, 32.43 and 24.44 KDa, while 3 bands (218.5, 90.08 and 83.08 KDa) were intensified and 2 protein bands (32.43 and 18.59 KDa) newly appeared. These new protein bands also appeared with Put treatment under salt stress, in addition to one characteristic band at 71.46 KDa. Put treatment increased the intensities of some protein bands which decreased under salt stress e.g., 203.8, 149.2, 115.7, 101.1 and 45.81 KDa. Adaptation of seeds to salinity depends mainly on the expression of salt-induced proteins[50]. The present results suggest a possible involvement of alterations in gene expression either by the repression or induction of some new genes or their products in the mechanism of salt tolerance in Put treated bean.

REFERENCES

1. Bohnert, H., D. Nelson and R. Jenson, 1995. Adaptation to environmental stresses. The Plant Cell, 7: 1099-1111.
2. Hurkman, W.K., C.S. Fornari and C.K. Tanaka, 1989. A comparison of the effect of salt on polypeptides and translatable mRNAs in roots of a salt-tolerant and a salt sensitive cultivars of barley. Plant Physiol., 90: 1444-1456.
3. El-Shintinawy, F. and R.A. Hassanein, 2001. The role of polyamine precursors; arginine, ornithine, or methionine in ameliorating the inhibitory effect of NaCl on wheat plant. Egyptian J. Biotechnol., 9: 328-340.
4. Smith, T.A., 1985. Polyamines. Annu. Rev. Plant Physiol., 36: 117-143.
5. Broetto, F, E. Malavolta and O.G. Brasil, 1999. Effect of salt stress on polyamine metabolism in bean cell culture. J. Plant Nut., 22: 889-900.
6. Davidonis, G., 1995. Changes in polyamine distribution during cotton fiber and seed development. J. Plant Physiol., 145: 108-112.

7. Cvikrová, M., P. Binarova, V. Cenklova, J. Eder and I. Macháèkova, 1999. Reinitiation of cell division and polyamine and aromatic monoamine levels in alfalfa explants during the induction of somatic embryogenesis. Physiol. Plant., 105: 330-337.
8. Kumar, A., T. Altabella, M.A. Taylor and A.F. Tiburcio, 1997. Recent advances in polyamine research. Trends Plant Sci., 2: 124-130.
9. Bagga, S., J. Rochford, Z. Klaene, G.D. Kuehn and G.C. Phillips, 1997. Putrescine aminopropyltransferase is responsible for biosynthesis of spermidine, spermine and multiple uncommon polyamines in osmotic stress-tolerant alfalfa. Plant Physiol., 114: 445-454.
10. Aribaud, M., M. Carre and J. Martin-Tanguy, 1994. Polyamine metabolism and *in vitro* cell multiplication and differentiation in leaf explants of *Chrysanthemum morifolium* Ramat. Plant Growth Regul., 15: 143-155.
11. Mattar, A. and G. Hunault, 1997. Effect of growth regulators on polyamine level of tissues during osmotic embryogenesis induction in fennel (*Foeniculum vulgare* Miller). Life Sci., 320: 245-251.
12. Erdei, L., Z. Szegletes, K. Barabas and A. Pestenacz, 1996. Responses in polyamine titer under salt stress in sorghum and maize seedlings. J. Plant Physiol., 147: 599-603.
13. Santa, C.A., M.T. Estan, M.C. Bolarin and M. Acosta, 1997. Effect of NaCl and mannitol iso-osmotic stress on the free polyamine level in leaf discs of tomato species differing in salt tolerance. J. Plant Physiol., 151: 754-758.
14. Altman, A., R. Kaur-Sawhney and A.W. Galston, 1977. Stabilization of oat leaf protoplasts through polyamine-mediated inhibition of senescence. Plant Physiol., 60: 570-574.
15. Kaur-Sawhney, R. and A.W. Galston, 1979. Interaction of polyamines and light on biochemical processes involved in leaf senescence. Plant Cell Environ., 2: 189-196.
16. Altman, A., 1982. Retardation of radish leaf senescence by polyamines. Physiol. Plant, 54: 189-193.
17. Kaur-Sawhney, R., A. Altman and A.W. Galston, 1978. Dual mechanisms in polyamine mediated control of ribonuclease activity in oat leaf protoplasts. Plant Physiol., 62: 158-160.
18. Heby, O. and L. Person, 1990. Molecular genetics of polyamine synthesis in eukaryotic cells. Trends in Biochem. Sci., 15: 153-158.
19. Drolet, G., E.B. Dumbroff, R.L. Legg and J.E. Thompson, 1986. Radical scavenging properties of polyamines. Phytochem., 25: 367-371.
20. Lovass, E., 1991. Antioxidative effects of polyamines. J. Am. Oil Chem. Sci., 68: 353-358.
21. Nicholass, F.J., C.J.S. Smith, W. Schuch, C.R. Bird and D. Gricrson, 1995. High levels of ripening-specific reporter gene expression directed by tomato fruit polygalacturonase gene flanking regions. Plant Mol. Biol., 28: 423-435.
22. Sitrit, Y. and A. Bennett, 1998. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: A reexamination. Plant Physiol., 16: 1145-1150.
23. Rick, W. and H.P. Stegbauer, 1974. Methods of Enzymatic Analysis. In: Bergmeyer, H.U. (Ed.), Verlag. Chemic. Weinheim and Academic Press. New York, London, pp: 885.
24. Gallop, P.M., S. Seifter and E. Meilman, 1957. The partial purification and mode of bacterial collagenesis. J. Biol. Chem., 227: 891-906.
25. Hewitt, E.J., 1974. Aspects of trace element requirements in plants and micro-organisms. In: Northcote, D.H., (Ed.), The metaloenzymes of nitrate and nitrite reduction. MTP International Review of Science, Vol. 11. Butterworths, London.
26. Marmur, J., 1961. A procedure for the isolation of deoxyribonucleic acids from microorganisms. J. Mol. Biol., 3: 208-218.
27. Mohamed, Y. and I. Capesius, 1980. Wirkung von Gibberellin-Säure und Fd Urd auf Menge und die Zusammensetzung der DNA wahrend des Streckungswachstum von *Pisum sativum*. Z. Pflanzen Physiol., 98: 15-23.
28. Stahl, E., 1937. Thinlayer Chromatography. 2nd Ed. Springer Verlag, Berlin.
29. Schneider, W.C., 1957. Colorimetric Analysis of Sugars. In: Grossmann and Moldave (Eds.), Methods in Enzymology, XII (Part A), pp: 557.
30. Umbreit, W.W., R.H. Burris, J.F. Stauffer, P.P. Cohen, W. Johnse, W.J. Johnse, G.A. Lee Page, V.R. Potter and W.C. Schneider, 1959. Manometric Technique, a Manual Describing Method, Applicable to the study of Describing Metabolism. Burgress Publishing Company.
31. Lowry, O.H., J. Rosenbrough, A.C. Fan and R.J. Randal, 1951. Protein measurements with Folin phenol reagent. J. Biol. Chem., 193: 265-270.
32. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant Soil., 39: 205-207.
33. Metzner, H., H. Rau and H. Senger, 1965. Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel Mutanten von *Chlorella*. Planta, 65: 186-193.
34. Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods. 6th Ed., Iowa, State University Press. Ames.
35. Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head bacteriophage T_4. Nature., 227: 680-685.

36. Hames, B.D., 1981. An introduction to polyacrylamide gel electrophoresis. In: Hames, B.D., Richwool, D. (Eds.), Gell Electrophoresis of Protein. IRL Press, Oxford Ltd, Pub. UK., pp: 1-91.
37. Waisel, Y., 1989. Screening for salt resistance. In: Laudelout, H. (Ed.), Methods of K Research in Plants. Proc. Zist. Collog. Int. Potash Inst. Bern., pp: 143-155.
38. Roy, N.K. and A.K. Srivastava, 1999. Effect of presoaking seed treatment on germination and amylase activity of wheat (*Triticum aestivum* L.) under salt stress conditions. Rachis., 18: 46-51.
39. Mandal, M.P. and R.A. Singh, 2000. Effect of salt stress on amylase, peroxidase and protease activity in rice (*Oryza sativa* L.) seedlings. Indian J. Plant Physiol., 5: 183-185.
40. Tipirdamaz, R., M. Durusoy and S. Bozuck, 1995. Effect of exogenous polyamines on alpha-amylase activity during seed germination under salt stress. Turk. J. Bot., 19: 411-416.
41. Ali, R.M., 2000. Role of putrescine in salt tolerance of *Atropa belladonna* plant. Plant Sci., 152: 173-179.
42. Zeid, I.M. and N.A. El-Semary, 2001. Response of two differentially drought tolerant varieties of maize to drought stress. Pak. J. Biol. Sci., 4: 779-784.
43. Galston, A.W. and R. Kaur-Sawhney, 1988. Polyamines as endogenous growth regulators. In: Davies, P.J. (Ed.), Plant Hormones and their Role in Plant Growth and Development. Kluwer Academic Pub. Dordrecht, the Netherlands, pp: 280-295.
44. Munjal, N., S.K. Sawhney and V. Sawhney, 1997. Ferricyanide restores nitrate reductase activity in leaf extracts of water stressed wheat seedlings. J. Plant Physiol., 152: 577-579.
45. Ramanjulu, S. and C. Sudhakar, 1997. Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: a comparative study in two mulberry genotypes differing in drought sensitivity. J. Plant Physiol., 150: 345-550.
46. Krizek, D.T., G.F. Kramer and R.M. Mirecki, 1997. Influence of UV-β radiation and putrescine on shoot and root growth of cucumber seedlings grown in nutrient solution. J. Plant Nut., 20: 613-623.
47. Hassanein, R. and F. El-Shintinawy, 2001. Changes in growth, protein patterns and DNA fingerprints of NaCl stressed wheat seedlings treated with arginine, putrescine or phenylenediamine. Egyptian J. Biotechnol., 10: 405-415.
48. Flores, H., 1991. Changes in polyamine metabolism in response to abiotic stress. In: Slocum, R.D., Flores, H.E. (Eds.), Biochemistry and Physiology of Polyamines in Plants. CRC Press, Boca Raton, pp: 213-228.
49. Besford, R.T., C.M. Richadson, J.L. Campos and A.S. Tiburcio, 1993. Effect of polyamines on stabilization of molecular complex in thylakoid membranes of osmotically stressed oat leaves. Planta, 189: 201-206.
50. DellAquila, A. and P. Spada, 1992. Regulation of protein synthesis in germinating wheat embryos under polyethylene glycol and salt stress. Seed Sci. Res., 2: 75-80.