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GIBBERELLIC ACID EFFECTS ON PROTEIN PATTERN, HYDROLYTIC ENZYME ACTIVITIES AND IONIC UPTAKE DURING GERMINATION OF *VICIA FABA* IN SEA WATER

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The germination and water uptake of *Vicia faba* seeds were suppressed in response to the treatments with the different concentrations of sea water (5%, 25% and 50%). The following parameters were increased: the osmotic potential, Na^+, Cl^-, proline and protease activity. While K^+, K^+/Na^+ ratio, Ca^{2+}, amylase activity and total soluble sugars were decreased. Gibberellic acid treatments to the seeds counteracted the harmful effect which were induced by sea water treatments. In turn the germination percentage, water uptake, K^+, K^+/Na^+ ratio, Ca^{2+}, total soluble sugars, -amylase and protease activities were increased, while Na^+, Cl^-, and proline were decreased.

The changes in protein banding pattern in *Vicia faba* germinated seeds in sea water were investigated. Salinization induced *de novo* synthesis of some salt responsive proteins. The salt responsive proteins might be osmotin (M wt 23.39 and 26.86 KDa), dehydrin (36.79 and 40.63 KDa) and ubiquitin (8.81 KDa) which were apparent in *Vicia faba* seeds. The seeds priming soaking in GA and germinated in sea water induced *de novo* synthesis of some responsive proteins. This indicated that gibberellic acid had a synergistic effect on the induction of the salt gene which is responsible for the synthesis of the mentioned proteins.

Key words: gibberellic acid, hydrolytic enzymes, ionic uptake, protein pattern, sea water, *Vicia faba*

INTRODUCTION

Most studies on physiological responses of plant to stress are based on the assumption that the only possible way to enable the plants to survive under stress conditions is to express pre-existing genetic information that counteracts the harmful effects of stress (He and Cramer 1992). However, Amzallag and Lerner (1995) showed that exposure of *Sorgum bicolor* to salinity may induce one of the two following responses; either the plant copes with the stress by using its salt resistance mechanism existing prior to exposure to stress (pre-existing resistance), or the plant develops mechanisms to increase the resistance to salinity (adaptation). In relation to plant adaptation to salinity

Kumar *et al.* (1996) pointed out that salt tolerance of plants could be increased by soaking the wheat seeds in gibberellic acid, IAA or planofix (NAA).

The control of ions uptake and intracellular compartmentation are considered to be the central dogma that governs the relative salt tolerance in plants (Garg and Gupta 1996). In the presence of external salts, dramatic changes in the mineral components of the plants have been found to occur due to the high interactions between ions (Gill and Sharma 1993).

The growth response of plants to salinity could be modulated by the composition of external salt solution. Zidan and Elewa (1995) and Hajar *et al.* (1996) proved that soluble and insoluble carbohydrates and proline contents followed a parallel increase with salinity increase. On the other hand, amylase activity showed a gradual reduction with salinity increments (Chen and Zhao 1996).

Moreover, the plants could be adapted to salinity stress by rapid accumulation of several proteins. In this respect Bressan *et al.* (1987) reported that adaptation of tobacco to high levels of NaCl induced the accumulation of several proteins, particularly one with an apparent mol. wt of 26 KDa termed osmotin.

The present investigation is directed to study the gibberellic acid role in overcoming the harmful effects of sea water on *Vicia faba* seeds during germination: the changes in ionic relations, soluble sugars, proline, some enzymatic activity and the changes in protein electrophoretic pattern.

MATERIAL AND METHODS

Homogeneous *Vicia faba* seeds (Giza 461), pure strains of seeds were obtained from the Egyptian Ministry of Agriculture. *Vicia faba* seeds were surface sterilised with 0.01 M $HgCl_2$ solution for 3 min. and washed thoroughly with distilled water. These seeds were divided to four sets, which were soaked in distilled water, 25 ppm, 50 ppm and 100 ppm gibberellic acid (GA), respectively. The seeds were soaked for about 12 hours. After soaking seeds of each set were divided into four groups, which were germinated in Petri dishes (12 cm in diameter) on filter paper wetted by distilled water, 5%, 25% and 50% sea

Treatment	Distilled water	5% sea water	25% sea water	50% sea water
Distilled water	1	2	3	4
25 ppm GA	5	6	7	8
50 ppm GA	9	10	11	12
100 ppm GA	13	14	15	16

water (S) respectively, where the sea water was obtained from the Mediterranean Sea. The resulting sixteen treatments were marked as follows:

In every treatment 10 seeds were placed in every dish and then transferred to an incubator at 20±2 °C in the dark. Experiments were terminated after 24 hours and 36 hours from the time of soaking. The germination criteria and chemical analysis were estimated. Each treatment had four replicates.

The results were first subjected to an analysis of variance (ANOVA) (Snedecor and Cochran 1980).

Germination percentage, relative water contents, fresh and dry weights were estimated for ten seed from each replicates. Fresh weight was determined immediately after sampling. Then dried at 80 C until constant weights were obtained. Estimation of osmoticum by using osmometer ( ) as osmole recommended by Termaat *et al.* (1985).

Estimation of cations was determined according to the method described by Chapman and Pratt (1978). Flame spectrophotometry was used for determining potassium and sodium, while calcium was measured by atomic absorption spectrophotometry. Chloride was determined by a colourimetric method (Sigma Diagnostics).

The total soluble sugars were determined by Yemm and Willis (1954). Proline was determined according to Batls *et al.* (1973). -amylase and protease enzymes were extracted from the *Vicia faba* seed during germination by Kar and Mishra (1976). The activities of -amylase and protease were determined according the methods of Davis (1977), and Salmia *et al.* (1978), respectively.

Electrophoretic protein profile of *Vicia faba* seeds was analysed by SDS-PAGE technique (Laemmli 1970). Data were analysed and identified by gel documentation system (GDS) which compared polypeptide maps; molecular protein markers, percentages of band intensity, molecular weight of each polypeptide in relation to standard markers using gel proanalyzer version 3 MEDIA CYBERNE TICE Imaging Experts Software.

RESULTS AND DISCUSSION

Germination criteria

Data presented in Figure 1 show that the different levels of sea water (5%, 25% and 50%) decreased the germination percentage of bean seeds. This reduction could be induced by the decrease in the relative water contents (Fig. 2), which affected the fresh weight (Table 1), in addition to the toxic effect of accumulated sodium and chloride ions (Table 2). Also sea water treatments re-

duced -amylase activity (Table 3). Several authors reached to similar conclusions, such as El-Saht (1995), and Maramber and Ando (1995), respectively. In the meantime the different concentrations of GA counteracted the inhibitory effects of sea water, salinity where an increase in germination percentage occurred when compared to the corresponding sea water levels. GA at 100 ppm showed to be more effective than 25 and 50 ppm GA. This promotory effect of GA on germination could be induced through the alleviation of the inhibitory effect of sea water on germination by increasing K^+ and decreasing Na^+ and Cl^- accumulation in the germinated seeds (Begum *et al.* 1994). Also GA could increase germination via increasing the hydrolytic enzyme activities (-amylase and protease) (Table 3).

Choudhury and Gupta (1998) confirmed this results in *Catharanthus roseus* cv. *Alba* seeds in response to GA treatment. Moreover, Tipirdamaz *et al.* (1995) found that treated barley seeds with GA increased germination percentage significantly and also significantly increased -amylase activity.

Table 1

Effect of seeds priming in (GA) on osmotic potential (expressed as m osmol/kg^{-1}) and fresh and dry weights (g) in *Vicia faba* seeds under stress imposed by sea water

Treatment		Osmotic potential		Fresh weight (g)		Dry weight (g)	
GA ppm	Sea water%	Time after treatment (hour)		Time after treatment (hour)		Time after treatment (hour)	
		24	36	24	36	24	36
0.0	0.0	87	120	1.813	1.938	0.796	0.745
	5	223	389	1.728	1.841	0.811	0.796
	25	475	490	1.517	1.738	0.886	0.875
	50	685	730	1.475	1.540	0.895	0.886
25	0.0	79	89.0	1.988	2.000	0.754	0.724
	5	210	290	1.790	1.850	0.779	0.743
	25	361	378	1.592	1.776	0.864	0.849
	50	670	69	1.438	1.641	0.889	0.878
50	0.0	67	85	2.110	2.050	0.724	0.701
	5	195	220	1.902	2.000	0.751	0.734
	25	354	365	1.713	1.828	0.846	0.838
	50	605	620	1.639	1.691	0.876	0.851
100	0.0	65	76	2.150	2.166	0.706	0.686
	5	186	210	2.000	2.030	0.746	0.712
	25	341	359	1.772	1.912	0.827	0.810
	50	537	578	1.734	1.822	0.873	0.849
L.S.D. at	5%	5.683	5.032	0.091	0.075	0.024	0.030
L.S.D. at	1%	7.558	6.692	0.122	0.100	0.032	0.041

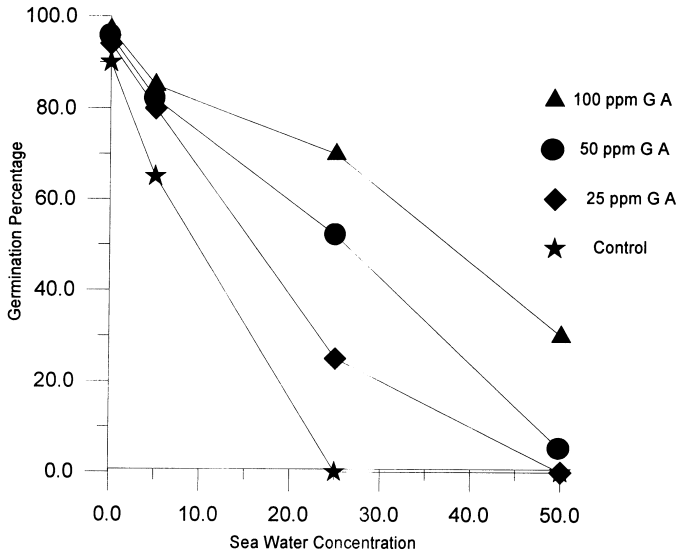

Fig. 1. Effect of seed priming in gibberellic acid (GA) on germination percentage in *Vicia faba* seeds under stress imposed by sea water

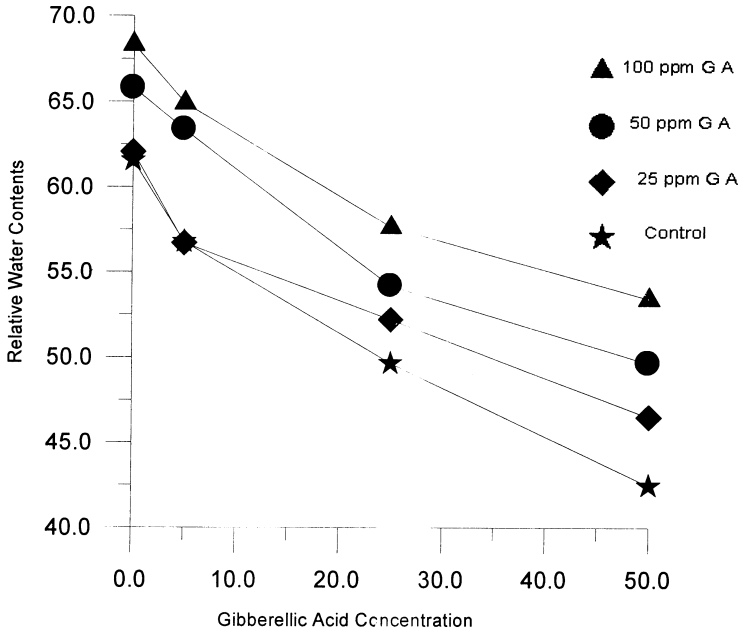

Fig. 2. Effect of seed priming in gibberellic acid (GA) on relative water contents in *Vicia faba* seeds under stress imposed by sea water

The presented data in Table 1 show the changes of fresh and dry weights of the seeds during the early stages of germination (24 and 36 hours) as influenced by the different concentrations of sea water. The fresh weight of the seeds showed a gradual reduction with the increasing sea water concentrations, while the dry weight showed the reverse. These patterns of effects were reported by several authors (Gary and Gupta 1997, Delgado and Sanchez-Raya 1997). This inhibitory effect of salinity on fresh weight of seeds may be due to a decrease in water absorption (Fig. 2), increased the osmotic potential of bean seeds, and decreased the metabolic process. In this respect Khadr *et al.* (1994) reported similar results in case of corn. Moreover, the reduction in germination and consequently in fresh weights also could be due to the disturbance in the balance of the endogenous bioregulator in favour to inhibitors synthesis (El-Nabarawy 1994).

In the meantime seeds priming soaking in different concentrations of GA showed a gradual increase and decrease in their fresh and dry weights, respectively, comparing with the control (Table 1). Meanwhile GA counteracted the

Table 2

Effect of seed priming soaking in GA on ionic contents (expressed as mM g^{-1} dry weight) in *Vicia faba* seeds under stress imposed by seawater (SW)

Treatment		K^+		Na^+		K^+/Na^+		Cl^-		Ca^{2+}	
GA ppm	SW %	Time after treatment (hour)									
		24	36	24	36	24	36	24	36	24	36
0.0	0.0	0.253	0.260	0.041	0.045	6.17	5.78	0.446	0.567	0.022	0.024
	5	0.234	0.250	0.044	0.055	5.32	4.55	0.673	0.767	0.018	0.019
	25	0.215	0.225	0.057	0.076	3.77	2.96	0.731	0.812	0.014	0.015
	50	0.185	0.200	0.065	0.089	2.85	2.25	0.929	1.141	0.011	0.013
25	0.0	0.279	0.321	0.028	0.032	9.96	10.03	0.418	0.531	0.023	0.025
	5	0.264	0.285	0.031	0.036	8.52	7.92	0.617	0.780	0.019	0.020
	25	0.255	0.268	0.053	0.065	4.81	4.12	0.725	0.800	0.015	0.016
	50	0.244	0.257	0.058	0.085	4.21	3.02	0.895	1.127	0.013	0.015
50	0.0	0.350	0.400	0.021	0.030	16.66	13.33	0.375	0.451	0.023	0.026
	5	0.310	0.373	0.026	0.029	11.92	12.86	0.567	0.619	0.020	0.029
	25	0.294	0.344	0.047	0.052	6.26	6.62	0.693	0.715	0.017	0.018
	50	0.288	0.315	0.046	0.065	6.26	4.85	0.739	0.964	0.015	0.016
100	0.0	0.390	0.480	0.018	0.019	21.67	25.26	0.270	0.439	0.024	0.025
	5	0.355	0.398	0.024	0.030	14.79	13.27	0.348	0.502	0.021	0.023
	25	0.337	0.377	0.028	0.050	12.04	7.57	0.531	0.801	0.019	0.020
	50	0.300	0.325	0.039	0.061	7.69	5.33	0.602	0.929	0.015	0.018
L.S.D. at 5%		0.0055	0.0060	0.0035	0.0055	0.165	0.252	0.017	0.019	0.0016	0.0020
L.S.D. at 1%		0.0071	0.0077	0.0045	0.0071	0.213	0.325	0.022	0.025	0.0021	0.0026

inhibitory of sea water salinity on germination. Interestingly this inhibitory effect was decreased with the increase of GA concentrations. The positive effect of seed priming in GA hastened the emergence of seedling stage than the control as reported by Kumar and Singh (1996). In most cases an increase in water uptake of the GA treated plants was recorded presumably as a result rather than a cause of cell expansion which was induced by plant bioregulator treatments. It was also suggested, that under the influence of salt stress, the level of naturally synthesised hormones may be suppressed (Roy *et al.* 1995), and that the exogenous application of plant bioregulators supplies more or less sufficient quantities, which are involved in growth promotion (Durusoy *et al.* 1995), and enough to adjust the balance in favour of growth promoters and normal germination and seedling growth.

Ions uptake

Vicia faba seeds showed a linear increase and decrease in sodium and potassium uptake, respectively, in response to the gradual increase of sea water concentrations (Table 2). A confirmation to these results was reported by Rasul *et al.* (1994), and Chen and Zhao (1996) stated that the irrigation with saline water increased Na^+ contents and decreased K^+/Na^+ ratio in *Panicum antidotale* and maize seeds, respectively.

Priming soaking of seeds to each concentration of GA (Table 2) shows that there was a significant increase and decrease in K^+ and Na^+, respectively comparing with the control (Begun *et al.* 1994). So, the treatment of seeds by sea water at different concentrations with priming seeds by GA were significantly increased and decreased in K^+ and Na^+ contents, respectively, and consequently K^+/Na^+ ratio was increased as compared to the corresponding salinity level and the control, except at 25 ppm GA, in the presence of 25% and 50% sea water. In this connection, Kumar and Singh (1996) stated, that wheat soaking in 100, 200 or 300 ppm GA before salinity will decrease Na^+ and increase K^+ and the increase in K^+/Na^+ selectivity ratio was in agreement with the mechanism of salinity tolerance hypothesised for salt-tolerant cultivars of *Olea europaea* (Tattini *et al.* 1994).

Chloride uptake was linearly increased parallel to external solution (Table 2), where there was a drastic increase in Cl^- concentrations occurred at the two periods (24 and 36 hours). On the other hand, the seeds priming soaking in different GA concentrations of showed that there was a highly significant decrease in Cl^- uptake as compared to the control (Table 2).

Meanwhile the seeds priming soaking in GA and germinated in different levels of sea water showed a significant decrease as compared with the corresponding salinity levels. Similar results were confirmed by Begum *et al.* (1994).

Moreover, GA treatments to the seeds adjusted the inorganic solutes (Table 2) and osmotic potentials (Table 1). This suggests that plants from GA primed seeds reduce seed osmotic potential more strongly. Where this is considered a less expensive process from an energetic point of view regarding a healthy seedling growth (Yeo 1983).

Calcium concentration in the seeds was significantly reduced by salinity throughout the experimental period (Table 2). Ca^{2+} concentration was inversely correlated with increased level of salinity. In accordance with those obtained by Garg and Gupta (1996), Chen and Zhao (1996), Kumar and Singh (1996). Ca^{2+} is widely recognized to play an important part in regulating the passive entry of Na^+ and K^+/Na^+ selectivity (He and Cramer 1992).

On the other hand the Ca^{2+} level in the primed seeds with GA increased (Table 2). In this connection Gilroy and Jones (1992) showed that GA_3 induced

Table 3

Effect of seed priming in (GA) on enzyme activities (expressed as enzyme activity/g.f.wt/hour), total soluble sugars (expressed as mg glucose g^{-1} D.wt) and proline (expressed as $\mu g/g^{-1}$ D.w) in *Vicia faba* seeds under stress imposed by sea water

Treatment		-amylase		Total soluble sugar		Protease		Proline	
Gibberellic acid ppm	Sea water %	Time after treatment (hour)		Time after treatment (hour)		Time after treatment (hour)		Time after treatment (hour)	
		24	36	24	36	24	36	24	36
0.0	0.0	1.25	0.97	5.84	5.99	408.8	415.5	0.93	2.11
	5	0.86	0.81	3.743	3.98	882.2	912.8	1.35	4.94
	25	0.40	0.31	2.463	2.95	941.3	963.5	2.50	11.52
	50	0.30	0.17	1.645	1.93	1067.8	1071.4	9.88	15.34
25	0.0	2.29	2.16	5.896	6.20	427.2	455.1	0.88	0.91
	5	0.90	0.85	4.407	5.20	595.9	601.8	1.15	3.51
	25	0.44	0.38	3.162	3.85	780.5	810.3	2.35	6.78
	50	0.32	0.26	3.098	3.28	912.3	940.6	6.54	12.53
50	0.0	2.35	2.20	7.307	8.50	463.6	486.9	0.75	0.82
	5	0.99	0.80	6.635	7.10	2193.6	451.0	0.95	1.15
	25	0.64	0.53	4.619	5.11	688.0	708.5	1.95	4.35
	50	0.56	0.40	4.00	4.45	850.9	890.5	4.33	8.14
100	0.0	2.50	2.41	9.136	9.90	429.3	442.5	0.53	0.61
	5	1.05	0.85	7.850	8.40	425.5	448.6	0.83	0.98
	25	0.73	0.68	5.977	6.81	630.8	681.9	1.51	2.45
	50	0.60	0.51	4.635	5.37	705.11	880.31	2.45	5.33
L.S.D. at 5%		0.065	0.055	0.123	0.145	7.238	7.338	0.1101	0.887
L.S.D. at 1%		0.084	0.071	0.159	0.188	9.340	9.468	0.142	1.144

a sustained increase in [Ca^{2+}] from 50 to 150 nM in barley aleurone protoplasts. The increase in [Ca^{2+}] preceded the GA_3. induced increase in -amylase synthesis (Table 3).

Meanwhile the Ca^{2+} level in the primed seeds with GA and germinated in different concentrations of sea water increased, although they were still lower than those of controls.

Organic solutes and enzymes

The results demonstrated that soluble sugars and proline are the major organic solutes which contributing to osmotic adjustment in bean germination under salt stress or different sea water concentrations. Similar conclusions recorded by several authors (Alarcon *et al.* 1993). So, during the germination stages of bean seeds (in the absence of GA treatment) total soluble sugars were significantly reduced. This could be due to the reduction in hydrolytic enzyme activities such as -amylase (Table 3). Similar results were obtained with the following germinating seeds of pea and maize by Kord and Khalil (1995), and Chen and Zhao (1996), respectively.

On the other hand, the priming of seed by GA significantly increased the -amylase activity compared with the control (Durusoy *et al.* 1995, Kord and Khalil 1995) and consequently soluble sugars were increased. In case of seeds priming soaking in different concentrations of GA and germinated in different levels of sea water showed a significant increase in -amylase activities and soluble sugars as compared with the corresponding salinity levels. In this respect Basra *et al.* (1989) indicated that metabolic acceleration in germination embryos of seeds treated with GA revealed an increased in a soluble sugars and protein accumulations under stress conditions. They added that the activities of some enzymes were seemingly related to the alleviation metabolism. In addition GA may have positive regulatory effects in triggering system for stress alleviation.

In respect to proline contents there were gradual increments during germination of bean seeds in the different concentrations of sea water. These results are in harmony with those obtained by Hajar *et al.* (1996) on black cumin. Mattioni *et al.* (1997) stated in their investigation that the accumulation of compatible solutes might help to maintain the relatively high water content necessary for growth and cellular functions. Although, a general increase in all amino acids was observed in *Triticum durum* under salt stress, only the proline content was high enough. So the proline is considered to the principle solute in osmoprotection.

On the other hand, the seeds priming soaking in GA had a significant decrease on the proline contents in bean seeds during germination below the cor-

Table 4

Comparative analysis of molecular weight, and relative concentrations of the different types of protein bands of *Vicia faba* seed treated with different concentrations of sea water and the possible role of gibberellic acid concentrations

Band No.	M.wt K.Da	Band %															
		Treat-ment	Sea water (S)			25 ppm Gibberellic acid				50 ppm Gibberellic acid				100 ppm Gibberellic acid			
		Ctrl	5% S	25% S	50% S	0	5% S	25% S	50% S	0	5% S	25% S	50% S	0	5% S	25% S	50% S
1	119.25	–	–	–	1.52*	–	–	2.03*		–	–	1.43*	–	–	–	1.18*	–
2	102.03	1.52	–	4.91	4.54	1.92	–	4	–	1.6	0.83	4.31	5.28	1.69	–	0.632	4.54
3	91.16	2.00	–	–	–	2.73	1.76	–	5.45	1.82	2.28	1.88	2.04	–	4.03	4.51	3.17
4	80.42	1.28	4.2	–	–	1.35	3.36	1.04	1.85	1.25	0.594	–	–	4.3	–	1.05	0.725
5	72.76	0.379	1.25	–	–	0.633	–	–	–	0.612	1.29	–	–	0.634	1.45	0.869	–
6	66.65	0.666	–	–	–	–	0.888	1.56	1.48	0.736	–	1.44	–	0.88	–	1.4	1.52
7	63.16	1.18	1.12	1.6	1.6	0.851	–	–	–	–	–	–	–	–	–	–	–
8	59.12	–	–	1.35*	1.06*	1.26*	1.11*	1.58*	1.37*	0.909*	1.37*	1.55*	1.89*	1.05*	1.98*	1.34*	1.79*
9	55.54	0.727	1.21	–	–	1.16	1.91	–	–	0.95	–	–	1.56	1.61	1.91	–	2.34
10	51.30	–	–	4.03*	4.35*	–	–	4.5*	–	–	1.862*	5.58*	–	1.03*	4.52*	5.65*	–
11	47.79	–	1.26*	2.42*	1.14*	–	1.43*	1.82*	4.88*	–	1.3*	1.58*	11.1*	–	–	1.68*	6.31*
12	43.98	4.45	–	–	–	4.46	4.5	1.31	–	5.37	5.35	–	–	5.14	1.73	1.69	–
13	40.63	2.6	4.35	2.75	3.12	2.6	0.822	2.79	3.53	2.22	3.78	3.03	3.16	1.81	2.82	3.72	8.15
14	39.37	3.38	–	–	–	3.53	1.47	4.73	2.87	1.06	–	4.86	–	1.44	1.89	3.79	–
15	36.79	–	2.55*	4.67*	3.92*	–	112*	2.68*	5.83*	–	3.78*	0.998*	4.08*	–	3.36*	3.26*	1.80*
16	35.15	4.55	1.46	4.21	5.22	3.2	2.78	2.31	5.89	4.25	2.44	4.44	3.37	2.96	–	3.47	2.49
17	33.73	2.64	–	–	–	–	0.999	–	–	–	–	–	–	1.52	–	–	2.71
18	31.11	1.3	4.73	–	–	2.42	3.29	–	–	3.12	1.83	0.466	–	2.46	2.99	–	–
19	30.67	–	3.08*	0.787*	0.618*	3.53*	–	2.47*	–	4.15*	–	0.466*	–	1.07*	2.11*	–	–
20	29.83	0.971	–	–	–	–	1.7	–	–	–	2.35	–	–	0.994	–	–	–
21	27.67	1.54	–	–	–	–	2.36	–	–	0.585	–	–	–	2.81	–	–	–
22	26.86	–	3.41*	8.13*	12.7*	–	1.3*	10.8*	13.3*	1.37*	–	13*	9.06*	–	2.22*	13.3*	13.9*

Table 4 (continued)

Band No.	M.wt K.Da	Band %															
		Treat-ment	Sea water (S)			25 ppm Gibberellic acid				50 ppm Gibberellic acid				100 ppm Gibberellic acid			
		Ctrl	5% S	25% S	50% S	0	5% S	25% S	50% S	0	5% S	25% S	50% S	0	5% S	25% S	50% S
23	24.93	13.8	–	–	–	16.4	1.69	–	–	1.35	–	–	–	3.33	7.88	–	–
24	23.39	–	2.87*	1.32*	1.98*	–	–	1.58*	2.6*	–	–	2.05*	7.01*	–	–	1.99*	2.31*
25	21.20	–	5.18*	1.95*	1.22*	–	9.25*	1.45*	1.14*	–	7.6*	1.31*	1.76*	–	1.18*	1.02*	–
26	20.62	2.38	1.44	1.38	–	2.59	–	–	–	14.6	11.4	–	–	10.6	2.11	–	–
27	19.31	1.21	1.38	–	–	–	–	–	–	–	–	–	–	–	–	–	–
28	18.73	–	2.63*	3.22*	1.89*	1.27*	2.7*	3.11*	2.66*	3.16*	1.57*	2.84*	–	1.96*	2.06*	3.32*	–
29	18.26	–	–	–	–	–	1.27*	3.61*	5.72*	–	1.21*	1.98*	8.69*	1.48*	–	2.23*	3.48*
30	17.70	3.95	1.29	–	–	2.74	–	–	–	–	–	2.41	–	–	–	2.33	–
31	16.17	4.84	1.15	4.32	4.87	–	1.16	–	–	2.41	–	–	–	2.59	2.96	–	–
32	15.36	–	0.755*	0.78*	–	2.25*	1.57*	–	–	3.98*	2.5*	–	–	1.97*	2.47*	–	–
33	14.26	–	3.22*	4.46*	3.69*	1.83*	1.98*	3.97*	–	1.13*	2.63*	4.95*	–	1.81	2.55*	–	–
34	13.35	–	–	–	–	–	3.18*	–	–	–	1.9*	–	–	–	4.06*	–	–
35	12.44	2.7	1.75	8.81	11.7	5.13	–	9.72	14.7	–	–	9.38	12.8	4.3	5.01	14.9	15.3
36	11.79	–	5.94*	6.22*	6.55*	1.6*	5.1*	5.66*	4.94*	5.02*	–	3.15*	10.02*	12.5*	5.2*	5.92*	5.2*
37	10.50	29.5	–	–	–	–	11	4.03	–	–	23	–	–	–	–	–	2.85
38	8.81	–	11.4*	2.31*	4.47*	3.41*	3.06*	–	4.28*	9.5*	3.84*	3.91*	–	6.39*	7.25*	3.85*	–
39	7.87	–	9.44*	–	–	5.6*	5.08*	–	–	7.46*	1.71*	–	–	5.25*	–	–	–
40	7.46	–	4.56*	–	–	–	5.1*	–	–	–	4.24*	–	–	–	3.54*	–	–
Total number		22	25	20	19	23	29	22	17	24	24	23	14	27	24	23	17
No of new responsive proteins (*)			13	13	13	8	14	13	10	9	13	14	8	10	13	12	7

responding salinity levels at the two periods of germination the magnitude of proline reduction was increased with increasing GA concentrations (Table 3). These results may be due to the ability of GA treatments alleviated the inhibitory effect of high salinity on germination through increasing K^+ contents and decreasing Cl^- and Na^+ accumulation in the germinated seeds (Table 2). These results are in confirm with the statement reported by Begum *et al.* (1994).

Protease activity was increased in bean seeds during germination in response to the increase of sea water concentration (Table 3). This may be attributed to the synthesis of enzymatic hydrolysis of protein leading to an increase in certain polypeptides during salt stress. Where this step could be important in the adaptation of plants to saline conditions (Table 4). In this respect, Salam and Ismaeil (1992) concluded that salinity increased protease activity in sunflower at certain concentrations.

In case of the bean seeds priming soaking in different concentrations of GA showed a significant increase in protease activity as compared to the control (Table 3). In this respect Choudhury and Gupta (1998) found that both respiratory (dehydrogenases) and hydrolytic (amylase and protease) enzymes showed enhanced activity in *Catharanthus roseus* cv. *Alba* seeds germinated in GA.

Salt responsive proteins

The changes in protein electrophoretic pattern of bean seeds germinated in different concentrations of sea water and priming soaking in different concentrations of gibberellins are shown in Figure 3. These results were analysed and recorded in Table 4.

In untreated bean seeds (control) the separation of 22 protein bands was apparent, where their molecular weights ranged between 102.03 KDa and 10.50 KDa. In respect to sea water treatments the following response was occurred. The seeds germinated at 5% sea water where the protein bands were increased to 25 bands and then decreased to 20 and 19 at 25% and 50% sea water, respectively, in comparison to the control. The decrease in protein synthesis in seeds germinated in 25% and 50% sea water could be attributed to the effect of Na^+ ions on impairing the metabolic activities (Greenway and Munns 1980).

Primary soaking in different concentrations of GA 25, 50 and 100 ppm increased the protein bands to 23, 24 and 27, respectively. The increase in protein synthesis in bean seeds by gibberellin treatments could be attributed to the effect of GA on hydrolytic (amylase and protease) enzymes (Table 3) (Tipirdamaz *et al.* 1995, Choudhury and Gupta 1998). Several investigators demonstrated similar results. Mostafa (1978) found that GA treatment altered and in-

creased in the synthesis of proteins in *Sinapis arvensis* seeds. These results were confirmed by the earlier foundation of Higgins *et al.* (1976) where they showed that GA alters the pattern of proteins synthesised by the activity at the gene level, activating some genes and repressing others.

In the same time priming soaking of seeds in GA at (25, 50 and 100 ppm) and germinated in 5% sea water increased the number of bands to 29, 24 and 24, respectively. However, in 25% sea water accompanied with 50 and 100 ppm GA showed a slight increase in the number of bands up to 23 as compared with the number of bands of control (Table 4). On the other hand, the seeds germinated in 50% sea water and priming soaking in 25, 50 and 100 ppm of GA showed a decrease in number of protein bands to 17, 14 and 17, respectively (Table 4).

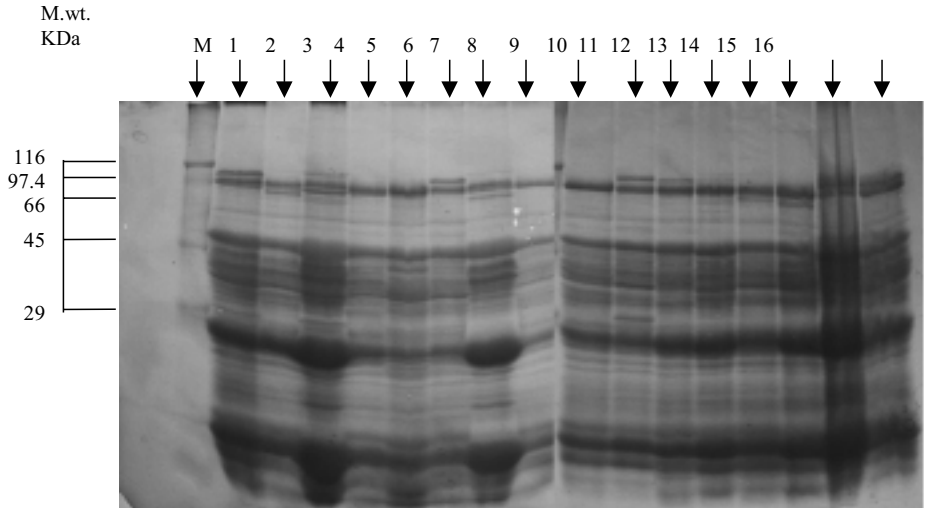

Fig. 3. Electrograph of soluble protein patterns by one-dimensional SDS-PAGE showing the changes of protein bands (marked by arrowheads), in response to sea water and combination with GA. Each lane contains equal amounts of protein extracted from *Vicia faba* seeds. Protein bands in the gel were visualized by coomassie blue stain. Lane M protein markers: Lane 1 Control seeds; Lane 2 seed treated with 25 ppm GA; Lane 3 seed treated with 50 ppm GA; Lane 4 seed treated with 100 ppm GA; Lane 5 seed treated with 5% seawater; Lane 6 seed treated with 5% seawater 25 ppm GA; Lane 7 seed treated with 5% seawater 50 ppm GA; Lane 8 seed treated with 5% seawater 100 ppm GA; Lane 9 seed treated with 25% seawater; Lane 10 seed treated with 25% seawater + 25 ppm GA; Lane 11 seed treated with 25% seawater+ 50 ppm GA; Lane 12 seed treated with 25% seawater + 100 ppm GA; Lane 13 seed treated with 50% seawater; Lane 14 seed treated with 50% seawater + 25 ppm GA; Lane 15 seed treated with 50% seawater + 50 ppm GA; Lane 16 seed treated with 50% seawater +100 ppm GA

In addition, some proteins disappeared in bean seeds subjected to sea water treatments while a new set of proteins was *de novo* synthesised (Table 4). These results indicated that protein bands increments in germinated bean seeds in presence of 5% sea water and GA, where due to GA effect which induced salt acclimation or salt alleviation via the marked increased in the number of salt responsive proteins. In this respect Robinson *et al.* (1990) suggested that the disappearance of polypeptides during stress were compensated by the increased synthesis of others. Moreover, to salt stress, despite the reduction in protein levels (Singla and Grover 1994), the cells preferentially synthesised a few specific proteins that are termed stress proteins (Pureek *et al.* 1995). In this respect it should be stated that one of the most important mechanisms involved in the cell protection against salt stress is the induction of *de novo* synthesis of a specific set of proteins (Kermode 1997). Therefore, in the present investigation, salinity, in general, induced *de novo* synthesis of a new set of proteins and increased the net synthesis of some other proteins in bean seeds (Table 4). Therefore the different concentrations of sea water (5%, 25% and 50%) induced *de novo* synthesis of 13 proteins (M wt: 47.79, 36.79, 30.67, 26.86, 23.39, 21.20, 18.73, 15.36, 14.26, 11.79, 8.81, 7.87 and 7.46 KDa) and (59.12, 51.30, 47.79, 36.79, 30.67, 26.86, 23.39, 21.20, 18.73, 15.36, 14.26, 11.79 and 8.81 KDa) and (119.25, 59.12, 51.30, 47.79, 36.79, 30.67, 26.86, 23.39, 21.20, 18.73, 14.26, 11.79 and 8.81 KDa), respectively (Table 4). In this respect Kuznetsov and Shevyakova (1997) observed that the NaCl, tolerant cells could be related with selective phosphorylation of several polypeptides (23–24, 27–31–32, 47 KDa). Moreover, the seeds germinated in different concentrations of sea water were characterised by the presence of a new protein (M wt: 36.79 KDa). This appeared to be dehydrin. Similar results were obtained by Galvez *et al.* (1993) who found that upon osmotic dehydrin mRNA levels were much higher in roots of *Lophopyrum* and *Triticum*. Also the polypeptides of the seeds of the same treatments were characterised by the appearance of *de novo* synthesis proteins of molecular weight: 26.86 KDa (Table 4). These results indicate that the increase in certain polypeptides during salt stress could be important in the adaptation of plants to saline conditions for example, a 26 KDa polypeptide was increased followed the adaptation of bean seeds treatments to saline water. This protein may play a role in osmoregulation. In this respect Bressan *et al.* (1987) have also shown the induction of 26 KDa polypeptide which was increased with the adaptation of cultured tobacco cell in media contained high levels of NaCl.

The protein (23.39 KDa) which was detected in bean seeds appeared to be osmotin. Accumulation of osmotic is linked to increased growth survival in the presence of high levels of NaCl and it is a major protein, which accumulates in tobacco cells adapted to low water potentials. Quantitation of osmotin

levels by immunoblots indicated that cells adapted to 428 mM NaCl contained 4 to 30 times the level of osmotin found in unadapted cells (La Rosa *et al.* 1989). Osmotin appeared to provide osmotic adjustment to the cells by facilitating the accumulation of solutes and/or providing metabolic alteration in the cells, which may be helpful in osmotic adjustment (Singh *et al.* 1987).

Also, proteins with molecular weights of (59.12, 40.63, 35.15, 30.67, 20.62, 18.73, 14.26 and 8.81 KDa) were appeared in the bean seed of salt acclimated or seeds priming soaking in different concentrations of GA. Similar results were obtained by Yen *et al.* (1997) who showed that the accumulation of five polypeptides with estimated molecules masses of (40, 34, 32, 29, 20, 18 and 14 KDa) were enhanced by the addition of 200 mM NaCl to the culture media in and *Mesembryanthemum crystallinum* callus. Moreover, the protein of molecular weight 8.81 KDa appeared to be ubiquitin is known to be a very small heat stress protein consisting of 76 amino acids, 8.5 KDa (Ozkaynak *et al.* 1984). Ubiquitin appeared to protect protein from degradation by proteases through tagging the protein (Hershko 1988).

According to the obtained results and discussion it may show that there is a positive correlation between the gene expression induced by salt acclimation and GA treatments to cope with salinity stress during germination where certain genes were expressed through *de novo* synthesis of proteins which may be involved in increasing the salt tolerance of bean germinated seeds. In this respect, Garcia *et al.* (1998) showed that gibberellic acid had a synergistic effect on the induction of the salt gene when combined with NaCl. Moreover Fisher *et al.* (1994) concluded that among the mechanism which underlie the adaptation of plants to osmotic adjustment is that responsible for the prevention of cytoplasmic accumulation of toxic Na^+ ions. The low molecular weight proteins (60 KDa) could play a role in ion homeostasis.

*

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