Plant Knowledge Journal
ISSN: 2200-5390
EISSN: 2200-5404
3(2)47-56(2014)

Southern Cross Publishing Group
AUSTRALIA

Influence of salinity and adaptive compounds on growth parameters, carbohydrates, amino acids and nucleic acids in two cultivars of *Vicia faba* contrasting in salt tolerance

Mahmoud Elbaz Younis * and Shaimaa Mohamed Nagy Tourky

Botany Department, Faculty of Science, University of Mansoura, Mansoura, Egypt

***Corresponding author: me_younis@mans.edu.eg**

Abstract

Salinization of two cultivars of broad beans with 50 and 300 mM NaCl, in general, induced significant reductions in all growth parameters, as compared with control values determined in seedlings after 14 days from being set to germinate. The magnitude of reduction was more pronounced in the salt-sensitive than in the salt-tolerant cultivar, especially with 300 mM NaCl. Thus, the calculated relative growth rate (RGR) in response to treatment with the high level of NaCl, in the salt-sensitive cultivar, was lower than that in the salt-tolerant one. Furthermore, significant increases in the contents of glucose, proline (Pro) and glycine associated with significant decreases in the contents of sucrose, polysaccharides, total saccharides, DNA and RNA, as compared with control levels, were obtained. The use of the optimum concentration of each of the adaptive compounds, either alone or in combination with each of the salinity levels, induced significant varied increases (in glucose, Pro, glycine, DNA and RNA) or decreases (in sucrose, polysaccharide and total saccharides), in relation to the contents of untreated- or salinized-controls; thus the magnitude of counteraction of the salinity adverse effects in broad bean seedlings being most pronounced with SA.

Keywords: Amino acids; AsA ; Carbohydrates; DNA; Growth; GSH; NaCl; Pro; RNA; SA; *Vicia faba*.
Abbreviations: AsA- Ascorbic acid; GSH- Glutathine reduced; Pro- Proline; RGR- Relative growth rate; ROS- Reactive oxygen species; SA- Salicylic acid.

Introduction

Adverse plant responses to salinity stress conditions depend on the osmotic and toxic effects of the stressful factors and on the level and duration of the stress (Shalata and Neuman, 2001). Responses range from germination and growth inhibition and accelerated leaf senescence under moderate stress to permanent wilting of shoots with subsequent plant death under severe stress (Munns, 1993).The response of plants to a salinity stress may vary with genotype; nevertheless some general reactions occur in all genotypes. An increase in salinity stress induces not only a reduction in the percentage of germinating seeds, but also can cause complete inhibition of the germination process at salinities beyond the tolerance limits of the species (Ungar, 1995). Salinity can affect plant physiological processes resulting in reduced growth and yield.Thus, in various plants, all growth, development and yield parameters appeared to remain unaltered, accelerated or, in most cases, suppressed with particular significance; depending on the level of the salinity used (Younis et al., 1999; Younis et al. 2008; Mao et al., 2008). In many plants, salt stress has been shown to affect carbohydrate partitioning and metabolism, leading to the synthesis of new compounds (Fougére et al., 1991; Parida et al., 2003). Furthermore, the decrease in total soluble carbohydrates due to salinity could be related to limited carbohydrate availability, as a consequence of a decline in photosynthesis (Younis et al., 1993). Several investigators have demonstrated that amino acid metabolism is strongly influenced by changes in the salinity of the medium. In particular, different amino acids have been reported to accumulate in higher plants under salinity stress (Fougére et al., 1991; Younis et al., 2009a). Pro as well as glycine accumulation can help plants to withstand osmotic stress (Waditee et al., 2005; Younis et al., 2009b). They can also protect plants by maintaining protein structure or increasing scavenging of reactive oxygen species (ROS) (Tester and Davenport, 2003). Bor et al. (2003) reported that stress conditions, as induced by salinity or mannitol, adversely affect the cellular contents of plant cells of different species, such as soluble carbohydrates, nucleic acids and proteins. Furthermore, Younis et al. (2009b) found that increasing NaCl or mannitol concentrations in the culture media, in general, induced a significant decrease in both RNA and DNA contents of *Vicia faba* seeds during germination. Differences in salt tolerance exist not only among different genera and species, but also within the different organs of the same species (Flowers and Hajibagheri, 2001; Ismail, 2003). The problems of soil salinity and improving the salt tolerance of cultivated plants are of particular urgency in semiarid zones where soils have already either been partially salinized or become saline because of irrigation. As an approach to the study of salt tolerance, most of plant physiologists have been directed towards the study of the role of many adaptive (protective) compounds, including compatible osmolytes, antioxidants and hormone-like endogenous regulators. For instance, treatment with Pro counteracted the growth inhibition induced by NaCl in different crop plants e.g., wheat (Raza et al., 2006) and maize (Ali et al., 2007). Also the application of SA has been reported to induce tolerance in plants to many biotic and abiotic stresses (Senaratna et al., 2000). Furthermore, exogenous AsA and GSH have been reported to protect plants against various stresses (Shaddad et al., 1990; Khattab, 2007; Younis et al., 2009b and 2010). The main objective of the present work was thus (i) to investigate and establish, as far as possible, the best available and cheap adaptive compounds and (ii) to study the possible effects on growth and metabolic changes in germinating susceptible and

resistant varieties of *Vicia faba* which is an important crop plant used in developing and industrialized countries. Comparative response studies could provide insights on the salt tolerance mechanism in germinating broad beans; a legume plant that is often grown on saline soils in Egypt.

Results and Discussion

Changes in germination and growth parameters

For germination and growth parameters, the changes obtained in 14-day-old variously treated salt-sensitive and salt-tolerant bean seedlings, are indicated in table 1. As compared with control values, salinization of both cultivars with 50 and 300 mM NaCl, induced varied significant decreases in all growth parameters determined, except for the slight significant increase maintained in the salt-tolerant cultivar salinized with 50 mM NaCl. The magnitude of reduction in all growth parameters was more pronounced in the salt-sensitive than in the salt-tolerant cultivar, especially with 300 mM NaCl (Table 1). Furthermore, the RGR calculated in response to treatment with the high level of NaCl in the salt-sensitive cultivar was markedly lower than that in the salt-tolerant one. As indicated in table 1, treatment of either the sensitive or the tolerant cultivar of beans with the optimum concentration of Pro, AsA, GSH or SA, either alone or in combination with the low or high concentration of salinity, induced growth parameter values higher than those obtained for untreated- and salinized-controls, after 14 days of germination; the magnitude of increase being most pronounced with SA as being apparent from the following sequence of treatments: SA > AsA > Pro > GSH. As shown up in table 1, the absolute values of all growth components in the controls and in the variously treated beans were consistently higher for the salt-tolerant seedlings than for the salt-sensitive ones. It is also apparent that the two cultivars of beans used in the present investigation responded differently to different salt stresses and different adaptive compounds, when used either alone or in combination. To substantiate these conclusions and taking into consideration the RGR values after 14 days germination of the salt-sensitive beans; the least RGR value (0.011 mg g^{-1} d^{-1}) was maintained in seedlings treated with 300 mM NaCl. In contrast, the highest RGR (0.027 mg g^{-1} d^{-1}) was maintained in salt-sensitive seedlings treated with 0.09 mM SA. As for the salt-tolerant seedlings, RGR values of 0.018 and 0.047 mg g^{-1} d^{-1} were maintained in response to treatments with 300 mM NaCl and with 0.09 mM SA, respectively (Table 1). One of the most widespread methods of determining plant tolerance to salts is the germination percentage in salt solutions. As well as germination and vigor tests, it is useful to evaluate the seeds physiological quality during salt stress. In general, authors have concluded that salinity is inhibitory to the germination in two ways: either causing a complete inhibition of the germination process at salinities beyond the tolerance limits of a species, or delaying the germination of seeds, at salinities that cause some stress to seeds but do not prevent germination (Ungar, 1995). As being evident from the present work, the percentage of germination appeared to decrease with increased salinity levels used; the magnitude of reduction being higher in the sensitive than in the tolerant cultivar. The decrease in the percent germination, as maintained in the present study, may be due to the combined effect of osmotic pressure and toxicity of salts or due to the effect of added Cl^- ions as pointed out by Gill et al. (2002) who examined the effect of sodium chloride in the cell under conditions that give rise to osmotic stress without exposing seeds to toxic concentrations of salt. In support of the present results, Jamil et al. (2006) observed that root and shoot fresh and dry weights of 4 vegetable species were increased at low salinity levels and decreased at high levels of salinity. Savvas and Lenz (2000) working on eggplant have reported decreases in fresh and dry weights due to salt stress. Furthermore, Kaymakanova and Stoeva (2008) studied the physiological responses of three different bean cultivars to salt stress by Na_2SO_4. They showed that salinity stress had adverse effects on the biomass yield and leaf area of the three cultivars. Differences in shoot dry weight were highly significant depending on the salinity type and of cultivars. RGR in the three cultivars were almost similar under non-saline conditions but declined considerably after salt treatment (Kaymakanova and Stoeva, 2008). To reduce the harmful effects of salinity and to enhance the germination and seedling vigor, this was achieved through treating broad bean seeds and seedlings with four adaptive compounds; the magnitude of response being most pronounced with SA as being apparent from the following sequence of treatments: SA > AsA > Pro > GSH. Also, as indicative in table 1, the magnitude of counteraction of the adverse salinity effects was more operative in the salt-tolerant than in the salt-sensitive broad bean cultivar. The present observations are in accord with those recorded by Sakhabutdinova *et al.* (2003), Farooq et al. (2006) and Khattab (2007) as well as others herein mentioned and elsewhere.

Changes in carbohydrate content

As compared with control, the glucose content of the salinized salt-sensitive and salt-tolerant broad bean seedlings showed a significant increase that appeared to be more pronounced with 300 mM NaCl (Table 2). Treatment of both cultivars of beans with each of Pro, AsA, GSH or SA induced a significant increase of the glucose content above the control value. Additional increments in glucose contents were induced by combination of both levels of NaCl with each of the adaptive compounds; the magnitude of these increments being more pronounced with the high level of NaCl. Furthermore, the contents of glucose in the variously treated salt-tolerant broad beans, were markedly higher than those comparable values maintained in samples of the salt-sensitive beans. For sucrose content of the variously treated salt-sensitive and salt-tolerant broad beans, in general, an opposite situation was observed. Furthermore, the following sequence of treatments: SA > AsA > Pro > GSH was observed with respect to the maintained increase and decrease in the glucose and sucrose contents of the treated samples, respectively. Polysaccharides appear to be the dominant component of the carbohydrate pool in broad beans; the magnitude of their content was consistently higher in the salt-tolerant than those in the salt-sensitive cultivar (Table 2). Consequently, the content of the polysaccharide component as well as that of the total saccharides appear to decrease significantly in response to treatment with NaCl; the magnitude of response being greater with the high NaCl concentration. In response to treatment with the adaptive compounds, there appeared a marked increase in the polysaccharide component and in the total saccharides above those control values. When adaptive compounds were used in

Table 1. Effects of NaCl either alone or in combination with the optimum concentration of Pro, AsA, GSH or SA on germination and growth parameters of salt-sensitive and salt-tolerant broad beans after 14 days of germination.

Treatments	% germination	% Change	Fresh Mass	Dry mass	RGR	Water Content	% change	Length of radicle	% change	Length of plumule	% change
			g seedling^{-1}		mg g^{-1} d^{-1}	g seedling^{-1}		cm seedling^{-1}		cm seedling^{-1}	
Salt-sensitive											
Control (1/10 Hoagland soln.)	100	-	1.90	0.41	0.016	1.49	-	3.92	-	3.56	-
50 mM NaCl	98*	-2.00	1.88*	0.37*	0.014*	1.51*	1.34	3.70*	-5.61	3.48*	-2.25
300 mM NaCl	0.0*	-100.00	1.62*	0.32*	0.011*	1.30*	-12.75	0.0*	-100.00	0.0*	-100.00
0.40 mM Pro	100	0.00	2.22*	0.50*	0.024*	1.72*	15.44	4.76*	21.43	4.05*	13.76
50 mM NaCl + 0.40 mM Pro	98.86	-1.14	2.07*	0.53*	0.026*	1.54*	3.36	3.85*	-1.79	3.48*	-2.25
300 mM NaCl + 0.40 mM Pro	5.25*	-94.75	1.80*	0.39*	0.016	1.41*	-5.37	3.37*	-14.03	3.09*	-13.20
4.0 mM AsA	100	0.00	2.30*	0.52*	0.026*	1.78*	19.46	4.78*	21.94	4.28*	20.22
50 mM NaCl + 4.0 mM AsA	99.24	-0.76	2.10*	0.55*	0.026*	1.55*	4.03	3.88*	-1.02	3.50*	-1.69
300 mM NaCl + 4.0 mM AsA	8.05*	-91.95	1.86*	0.40	0.016	1.46*	-2.01	3.47*	-11.48	3.12*	-12.36
1.0 mM GSH	100	0.00	2.18*	0.49*	0.024*	1.69*	13.42	4.74*	21.19	4.00*	12.36
50 mM NaCl + 1.0 mM GSH	98.15	-1.85	2.05*	0.52*	0.027*	1.53*	2.68	3.82*	-2.55	3.46*	-2.81
300 mM NaCl + 1.0 mM GSH	4.88*	-95.12	1.76*	0.37*	0.014*	1.39*	-6.71	3.35*	-14.54	3.03*	-14.89
0.09 mM SA	100	0.00	2.41*	0.55*	0.027*	1.86*	24.83	4.80*	22.45	4.30*	20.79
50 mM NaCl + 0.09 mM SA	99.64	-0.36	2.13*	0.56*	0.024*	1.57*	5.37	3.90*	-0.51	3.54*	-0.56
300 mM NaCl + 0.09 mM SA	8.15*	-91.85	1.91*	0.42	0.017	1.49	0.00	3.48*	-11.22	3.15*	-11.51
Salt-tolerant											
	% germination	% Change	Fresh mass	Dry mass	RGR	Water content	% change	Length of radicle	% change	Length of plumule	% change
			g seedling^{-1}		mg g^{-1} d^{-1}	g seedling^{-1}		cm seedling^{-1}		cm seedling^{-1}	
Control (1/10 Hoagland soln.)	100	-	2.00	0.48	0.021	1.52	-	4.30	-	4.19	-
50 mM NaCl	100.00	0.00	2.11*	0.46*	0.020	1.65*	8.55	4.50*	4.65	4.33*	3.34
300 mM NaCl	38.00	-62.00	1.88*	0.40*	0.018*	1.48*	-2.63	3.16*	-26.51	4.00*	-4.53
0.40 mM Pro	100.00	0.00	2.38*	0.60*	0.030*	1.78*	17.11	5.90*	37.21	5.46*	30.31
50 mM NaCl + 0.40 mM Pro	100.00	0.00	2.58*	0.63*	0.033*	1.95*	28.29	5.05*	17.44	5.00*	19.33
300 mM NaCl + 0.40 mM Pro	39.16*	-60.84	1.94*	0.46*	0.021	1.48*	-2.63	4.11*	-4.42	4.12*	-1.67
4.0 mM AsA	100.00	0.00	2.51*	0.64*	0.036*	1.87*	23.03	5.97*	38.84	5.49*	31.03
50 mM NaCl + 4.0 mM AsA	100.00	0.00	2.76*	0.65*	0.034*	2.11*	38.82	5.15*	19.77	5.11*	21.96
300 mM NaCl + 4.0 mM AsA	40.12*	-59.88	1.97*	0.47	0.021	1.50*	-1.32	4.15*	-3.49	4.16*	-0.72
1.0 mM GSH	100.00	0.00	2.33*	0.58*	0.030*	1.75*	15.13	5.88*	36.74	5.44*	29.83
50 mM NaCl + 1.0 mM GSH	100.00	0.00	2.49*	0.61*	0.030*	1.88*	23.68	4.96*	15.35	4.89*	16.71
300 mM NaCl + 1.0 mM GSH	38.41*	-61.59	1.89*	0.44*	0.020	1.45*	-4.61	4.08*	-5.12	4.10*	-2.15
0.09 mM SA	100.00	0.00	2.63*	0.73*	0.047*	1.90*	25.00	6.00*	39.53	5.51*	31.50
50 mM NaCl + 0.09 mM SA	100.00	0.00	2.77*	0.67*	0.034*	2.10*	38.16	5.29*	23.02	5.22*	24.58
300 mM NaCl + 0.09 mM SA	40.34*	-59.66	2.02*	0.49	0.023*	1.53	0.65	4.18*	-2.79	4.17*	-0.48

*The mean values are significantly different from the control at $P \leq 0.05$

combination with NaCl, a marked decrease in polysaccharides and consequently in the total saccharides was obtained below those values observed with each of the adaptive compounds, when used alone; the magnitude of decrease being more pronounced with the high NaCl level. Again, the following sequence of treatments: SA > AsA > Pro > GSH was displayed with respect to the maintained changes in the polysaccharide content and consequently in the total saccharides content (Table 2). Carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation, and respiration. As respiratory substrates, monosaccharides promote respiration and mitochondrial electron transport which would seem to oppose the onset of quiescence and favour metabolism, energy production, and the formation of ROS (Leprince et al., 1993). In this connection, we should refer to the fact that the rates of respiration of many plant tissues were stimulated by various salinity treatments and the highest rates of respiration were recorded at the highest levels of salt concentration (Divate and Pandy, 1981; Younis et al., 1987; 1989).

The suggested increase in respiration appears to have taken place at the expense of oxidation of metabolites and the decline in dry matter. In support of these results, plants resort to many adaptive strategies in response to different abiotic stresses. Adaptation to all these stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and Pro (Greenway and Munns 1980; Yancey et al., 1982). According to Cram (1976), of the various organic osmotica, sugars contribute up to 50 % of the total osmotic potential in glycophytes subject to saline conditions. In fact, seed carbohydrate metabolism under stress conditions can be considered as a dynamic process involving concomitantly occurring process of polysaccharide degradation and induction of soluble sugar accumulation. Application of the adaptive compounds, either alone or in combination with low and high NaCl levels, might activate the metabolic consumption of the glucose component to form new cell constituents as a mechanism to stimulate the maintained growth rates. Thus, sugars appear to alleviate the salinity stress induced by NaCl in broad bean seedlings and it is apparent that SA, AsA, Pro and GSH play essential roles in plant metabolism and consequently in salt tolerance.

Table 2. Effects of NaCl either alone or in combination with the optimum concentration of Pro, AsA, GSH or SA on the carbohydrate content of salt-sensitive and salt-tolerant broad beans after 14 days of germination.

Treatments	Glucose	% change	Sucrose	% change	Polysaccharides	% change	Total saccharides	% Change
Salt-sensitive								
Control (1/10 Hoagland soln.)	40.66	-	100.14	-	138.44	-	279.24	-
50 mM NaCl	52.42*	28.92	94.24*	-5.89	134.51*	-2.84	281.17*	0.69
300 mM NaCl	56.16*	38.12	87.52*	-12.60	120.91*	-12.66	264.59*	-5.25
0.40 mM Pro	50.72*	24.74	124.72*	24.60	163.21*	17.89	338.65*	21.28
50 mM NaCl + 0.40 mM Pro	57.15*	40.56	96.24*	-3.89	135.72*	-1.96	289.11*	3.53
300 mM NaCl + 0.40 mM Pro	67.22*	65.32	88.66*	-11.46	124.55*	-10.03	280.43*	0.43
4.0 mM AsA	51.32*	26.22	126.44*	26.26	168.26*	21.54	346.02*	23.91
50 mM NaCl + 4.0 mM AsA	58.12*	42.94	97.61*	-2.53	136.51*	-1.39	292.24*	4.66
300 mM NaCl + 4.0 mM AsA	69.72*	71.47	90.71*	-9.42	127.81*	-7.68	288.24*	3.22
1.0 mM GSH	49.91*	22.78	120.66*	20.49	161.35*	16.55	331.92*	18.87
50 mM NaCl + 1.0 mM GSH	56.44*	38.81	96.00*	-4.13	134.46*	-2.87	286.90*	2.74
300 mM NaCl + 1.0 mM GSH	65.85*	61.95	87.91*	-12.21	122.11*	-11.80	275.87*	-1.21
0.09 mM SA	51.55*	26.78	130.92*	30.47	168.91*	22.01	351.38*	25.83
50 mM NaCl + 0.09 mM SA	59.63*	46.66	103.00*	2.86	137.25*	-0.86	299.88*	7.39
300 mM NaCl + 0.09 mM SA	70.48*	73.34	90.92*	-9.21	129.92*	-6.15	291.32*	4.33
Salt-tolerant								
	Glucose	% change	Sucrose	% change	Polysaccharides	% change	Total saccharides	% Change
Control (1/10 Hoagland soln.)	46.51	-	123.22	-	142.83	-	312.56	-
50 mM NaCl	62.77*	34.96	119.67*	-2.88	139.92*	-2.04	322.36*	3.14
300 mM NaCl	68.77*	47.86	114.00*	-7.48	126.70*	-11.79	309.47*	-0.99
0.40 mM Pro	59.63*	28.21	156.71*	27.18	174.98*	22.50	391.32*	25.20
50 mM NaCl + 0.40 mM Pro	70.63*	51.86	133.60*	8.42	159.20*	11.46	363.43*	16.28
300 mM NaCl + 0.40 mM Pro	76.56*	64.61	110.50*	-10.32	133.53*	-6.51	320.59*	2.57
4.0 mM AsA	60.00*	29.00	157.15*	27.54	176.80*	23.78	393.95*	26.04
50 mM NaCl + 4.0 mM AsA	76.84*	65.21	135.86*	10.26	160.27*	12.21	372.97*	19.33
300 mM NaCl + 4.0 mM AsA	78.96*	69.77	116.33*	-5.59	134.00*	-6.18	329.29*	5.35
1.0 mM GSH	57.73*	24.12	150.82*	22.40	172.56*	20.81	381.11*	21.93
50 mM NaCl + 1.0 mM GSH	70.34*	51.24	131.40*	6.64	157.65*	10.38	359.39*	14.98
300 mM NaCl + 1.0 mM GSH	75.15*	61.58	108.03*	-12.33	132.35*	-7.34	315.53*	0.95
0.09 mM SA	60.24*	29.52	161.92*	31.41	177.51*	24.28	399.67*	27.87
50 mM NaCl + 0.09 mM SA	78.93*	69.71	141.80*	15.08	160.66*	12.48	381.39*	22.02
300 mM NaCl + 0.09 mM SA	80.93*	74.01	118.60*	-3.75	134.14*	-6.08	333.67*	6.75

*The mean values are significantly different from the control at $P \leq 0.05$.

**Values of saccharide components are given as mg glucose equivalent/100 g dry mass.

Changes in amino acids content

The contents of Pro and glycine appeared to increase with an increase in NaCl concentration, after 14 days of germination, in both bean cultivars (Table 3). Exogenous application of Pro, AsA, GSH or SA, appeared to induce more or less comparable significant increases in the Pro and the glycine contents of both cultivars above control values. The observed increase was most pronounced with SA in both bean cultivars as compared with those increases induced by the other adaptive compounds (Table 3). When these compounds were used singly or in combination with the low concentration of NaCl, an apparent significant increase in both Pro and glycine contents, of both salt-sensitive and salt-tolerant broad beans, was observed above those levels maintained for the control- and for the adaptive compound-treated samples. With high NaCl, further additive significant increases were obtained for both cultivars. Again, all values of Pro (mg g^{-1} fresh mass) and glycine (mg g^{-1} dry mass) contents, were markedly higher in the salt-tolerant beans than those values maintained in the salt-sensitive ones. Furthermore, the following sequence of treatments: SA > AsA > Pro > GSH, was displayed with respect to the detected levels of both Pro and glycine. In connection with the above mentioned changes in amino acid contents of *Vicia faba* seedlings as influenced by low and high levels of NaCl, either alone or in combination with Pro, AsA, GSH or SA, Saunier et al. (1968), Stewart and Lee (1974), Agarwal and Pandey (2004) and Younis et al. (2009a and 2009b) reported that under moisture and saline conditions, the increase in total amino acids in halophytes and glycophytes was predominantly due to increase in the levels of Pro, phenylalanine, glutamic acid, taurine, glycine and alanine. Significant increases were also observed in alanine, arginine, histidine, isoleucine and valine. Increases and decreases in other amino acids were not significant (Saunier et al., 1968). Waditee et al. (2005) working on *Synechococus* and *Arabidopsis*, and Younis et al. (2009a), working on lettuce plants, both reported that glycine seems to meet the requirements of a compound that can have a role in stress tolerance. Of interest in this context, Waditee et al. (2005) obtained results indicating that glycine limits maximal salt-stress tolerance in plants. It is thus possible that in halophytes and glycophytes similar amino acids can attribute for or work along with Pro in osmotic adjustment. To substantiate our present observations, Sohn et al. (2005) investigated the physiological and biochemical bases for salt tolerance in two rice cultivars: relatively salt-tolerant (Dongin) and salt-sensitive (Kumnam) under NaCl stress

Table 3. Effects of NaCl either alone or in combination with the optimum concentration of Pro, AsA, GSH or SA on amino acids content of salt-sensitive and salt-tolerant broad beans after 14 days of germination.

Treatments	Salt-sensitive				Salt-tolerant			
	Proline		Glycine		Proline		Glycine	
	mg / 100 g fresh mass	% change	mg / 100 g dry mass	% change	mg / 100g fresh mass	% change	mg / 100 g dry mass	% change
Control (1/10 Hoagland soln.)	50.46	-	34.29	-	56.36	-	40.04	-
50 mM NaCl	61.22*	21.32	45.22*	31.88	68.73*	21.95	53.60*	33.87
300 mM NaCl	66.14*	31.07	47.00*	37.07	75.43*	33.84	55.50*	38.61
0.40 mM Pro	53.81*	6.64	42.22*	23.13	62.46*	10.82	49.83*	24.45
50 mM NaCl + 0.40 mM Pro	64.72*	28.26	50.46*	47.16	76.46*	35.66	60.31*	50.62
300 mM NaCl + 0.40 mM Pro	71.63*	41.95	54.73*	59.61	85.20*	51.17	72.84*	81.92
4.0 mM AsA	54.96*	8.92	42.55*	24.09	64.26*	14.02	50.21*	25.40
50 mM NaCl + 4.0 mM AsA	67.61*	33.99	51.79*	51.04	77.81*	38.06	63.70*	59.09
300 mM NaCl + 4.0 mM AsA	73.26*	45.18	57.55*	67.83	85.50*	51.70	74.63*	86.39
1.0 mM GSH	52.95*	4.93	42.11*	22.81	61.93*	9.88	49.70*	24.13
50 mM NaCl + 1.0 mM GSH	63.42*	25.68	50.35*	46.84	75.81*	34.51	59.30*	48.10
300 mM NaCl + 1.0 mM GSH	69.91*	38.55	54.66*	59.41	84.73*	50.34	71.90*	79.57
0.09 mM SA	55.96*	10.90	43.76*	27.62	64.73*	14.96	51.92*	29.67
50 mM NaCl + 0.09 mM SA	69.83*	38.39	52.92*	54.33	78.73*	39.69	66.76*	66.73
300 mM NaCl + 0.09 mM SA	76.41*	51.43	58.49*	70.57	85.66*	51.99	74.92*	87.11

*The mean values are significantly different from the control at $P \leq 0.05$.

Table 4. Effects of NaCl either alone or in combination with the optimum concentration of Pro, AsA, GSH or SA on nucleic acid contents of salt-sensitive and salt-tolerant broad beans after 14 days of germination.

Treatments	Salt-sensitive				Salt-tolerant			
	DNA		RNA		DNA		RNA	
	mg / 100 g fresh mass	% change	mg / 100 g fresh mass	% change	mg / 100 g fresh mass	% change	mg / 100 g fresh mass	% change
Control (1/10 Hoagland soln.)	39.24	-	55.14	-	49.16	-	65.41	-
50 mM NaCl	38.55*	-1.76	52.14*	-5.44	48.53*	-1.28	67.49*	3.18
300 mM NaCl	32.54*	-17.07	46.65*	-15.40	41.35*	-15.89	60.09*	-8.13
0.40 mM Pro	45.55*	16.08	56.76*	2.94	61.20*	24.49	83.66*	27.91
50 mM NaCl + 0.40 mM Pro	46.94*	19.62	53.92*	-2.21	61.61*	25.33	77.91*	19.11
300 mM NaCl + 0.40 mM Pro	36.73*	-6.40	48.62*	-11.82	47.23*	-3.93	64.22*	-1.81
4.0 mM AsA	47.90*	22.07	58.60*	6.27	61.66*	25.43	84.80*	29.64
50 mM NaCl + 4.0 mM AsA	48.00*	22.32	55.30*	0.29	61.83*	25.77	78.67*	20.27
300 mM NaCl + 4.0 mM AsA	37.90*	-3.41	49.62*	-10.01	50.32*	2.36	64.86*	-0.84
1.0 mM GSH	44.25*	12.77	55.90*	1.38	60.52*	23.11	83.12*	27.08
50 mM NaCl + 1.0 mM GSH	44.77*	14.09	51.55*	-6.51	59.11*	20.24	75.73*	15.78
300 mM NaCl + 1.0 mM GSH	35.62*	-9.23	47.51*	-13.84	47.21*	-3.97	64.11*	-1.99
0.09 mM SA	48.96*	24.77	58.83*	6.69	62.97*	28.09	85.55*	30.79
50 mM NaCl + 0.09 mM SA	49.22*	25.43	56.00*	1.59	62.53*	27.20	79.51*	21.56
300 mM NaCl + 0.09 mM SA	38.59*	-1.66	50.11*	-9.12	51.50*	4.76	64.99*	-0.64

*The mean values are significantly different from the control at $P \leq 0.05$.

conditions. They showed that Pro accumulation in both cultivars were closely associated with the NaCl concentration; being significantly higher in (Kumnam) than in (Dongin). This experimental evidence showed that their capacity to accumulate Pro under NaCl stress was not an indicator of salt tolerance nor a protective value, but merely a consequence or symptom of the stress (Sohn et al., 2005). Furthermore, under increasing levels of salinity, germinating seeds of salt- tolerant rice cultivars contain higher levels of Pro and other free amino acids than do salt-sensitive cultivars (Dubey and Rani, 1989). This observation lend a strong support to our observation indicating higher content of Pro in the salt-tolerant broad bean seedlings than that observed in the salt-sensitive seedlings under the present set of experimental conditions. Furthermore, soaking rice seeds in AsA or SA improved seed germination, seed and seedlings vigor under salinity levels compared with other antioxidants (α-tochopherol, citric acid and humic acid) and distilled water

(Yousof et al., 2010). Also as indicated by Khattab (2007), working on two different cultivars of canola, exogenous application of GSH and polyadenylic acid significantly enhanced the stimulatory role of salt stress on the production of free amino acids; the highest level of amino acids being found in plants pretreated with GSH and polyadenylic acid and exposed to salt stress. In addition, as indicated by Okuma et al. (2004) and Hoque et al. (2007), the improvement of salt tolerance by exogenous Pro or betaine was accompanied by varied increases of intracellular accumulation of Pro or betaine. Apart from protection of macromolecules and enzyme activities from denaturation and carbon and nitrogen reserve for stress relief, Pro has several other functions during stress: e.g. osmotic adjustment (Solomon et al., 1994), osmoprotection (Kishor et al., 2005), free radical scavenger and antioxidant activity (Sharma and Dietz, 2006). However, the role of other similar amino acids, e.g. glycine (Waditee et al., 2005; Hasaneen et al., 2008; Younis et al., 2009a) cannot be eliminated.

Changes in nucleic acid contents

Both nucleotide levels detected appeared to show a significant decrease, below the control levels, with an increase in salinity; the magnitude of decrease being most pronounced with 300 mM NaCl. Also, the magnitude of decrease in both nucleotide contents was, in general, more pronounced in the salt-sensitive than in the salt-tolerant one (Table 4). Treatment of both cultivars of broad beans with Pro, AsA, GSH or SA induced a significant increase in DNA and RNA contents as compared with control levels after 14 days of germination. The magnitudes of increase in both DNA and RAN contents were most pronounced with SA. When the adaptive compounds were used in combination with either 50 or 300 mM NaCl, an apparent significant increase in DNA and RNA contents was observed above those contents maintained in response to each of the salinity levels used. This indicated marked partial nullification of the reduction effects induced by salinity. Again the magnitude of response was most pronounced with SA followed by AsA. Pro appeared to have an intermediate effect followed by GSH which appeared to be the least in action. Nevertheless, the absolute values of DNA and RNA contents as well as the magnitude of effects in response to the various treatments were higher in the salt-tolerant cultivar than that observed for the salt-sensitive one (Table 4). In support of the present changes, in DNA and RNA contents, in broad bean seedlings under varied experimental conditions, Mukhtar and Hasnain (1994) found that callus cultures of *Brassica oleeracea* L. grown as control gave the highest value of DNA content , where at 100 mM NaCl there was a significant decrease in DNA content. This decrease in DNA content was probably a result of impaired synthesis. Also Mukhtar and Hasnain (1994) showed that there was about 45 % increase in RNA content of calli grown on 100 mM salt over that of control. Increase in RNA content at 100 mM NaCl as compared to control suggests that perhaps more transcription was required to cope with the adverse conditions. El-khallal et al. (2009) found that salt-stress significantly decreased DNA content in maize plants. SA markedly increased DNA in shoots of 3 weeks old plants, as compared with stressed control. However, the level of RNA increased in maize shoots in response to salt stress and/or SA. Furthermore, Younis et al. (2009b) found that *Vicia faba* seeds are able to germinate and grow in the presence of different levels of either NaCl or mannitol but, these levels induced reduction in DNA and RNA contents, especially when the former were present at high concentrations. AsA is an important primary metabolite that functions as an antioxidant, an enzyme cofactor and a cell signaling modulator in a wide array of physiological processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress resistance, photoprotection, cell division and growth (Wolucka et al., 2005). The addition of AsA as an antioxidant compound increased tissue levels in *Vicia faba* seedlings and confirmed earlier reports that plants will take up exogenous AsA (Shalata and Neumann, 2001). Thus, as indicated by Younis et al. (2009b), the exogenous AsA increased the percentage of *Vicia faba* seedlings that become able to survive the toxic effects of exposure to salinity or moisture stresses. The presence of AsA in the stressing media also appeared to partially alleviate the inhibitory effects of NaCl or mannitol on RNA and DNA contents. Furthermore, it is apparent that AsA can improve the salt tolerance of broad beans by protecting the protein turnover machinery against stress damage and by up-regulating stress protective proteins (Younis et al., 2009b). In this connection, Shalata and Neumann (2001) pointed out that the very distinct protective effect of exogenous AsA appeared to be specifically related to its antioxidant activity, rather than its possible utility as an organic substrate for respiratory energy metabolism. Salinity is reported to disturb nucleic acid metabolism and cause growth inhibition and thus Khattab (2007) showed that salinity significantly reduced the level of both DNA and RNA in two cultivars of canola compared to the corresponding unstressed controls. Presoaking of seeds in GSH and polyadenylic acid before sowing promoted the synthesis of DNA and RNA and/or prevented their degradation by nucleases enzymes. Higher levels of DNA and RNA were observed in canola seedlings exposed to either GSH or polyadenylic acid before the exposure to 100 mM NaCl (Khattab, 2007). It was also reported that GSH, by direct or indirect reaction with ROS, contribute to maintain the integrity of cell structures such as proteins, lipids and nucleic acid from damage induced by salt stress (levitt, 1980).

Materials and Methods

Plant material

Two homogeneous lots of broad beans: *Vicia faba* var. Giza 716 (salt-sensitive cultivar) and Giza 843 (salt-tolerant cultivar) were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Estimation of carbohydrates

The method of extraction of carbohydrate fractions was patterned after those adopted by Yemm and Willis (1954) and Van Handel (1968). Thus dried powdered bean seedlings were submerged overnight, in 10 cm^3 80 % ethanol at 25 ^{0}C, with periodic shaking. The ethanolic mixture was filtered and the filtrate made up to volume, ready for analyses of glucose and sucrose. Glucose was determined using the anthrone method as described by Yemm and Willis (1954). Sucrose content was estimated by the procedures adopted by Yemm and Willis (1954) and Van Handel (1968).The method used for extraction and estimation of polysaccharides, being considered mainly as starch, was that of Thayumanavan and Sadasivam (1984).

Estimation of free amino acids

The method of extraction of glycine was essentially that adopted by Yemm and Willis (1956). Glycine was estimated by the method of Muting and Kaiser (1963). The extract was deproteinized with ethanol/acetone mixture and the glycine content was then determined colourimetrically with ninhydrin. The method adopted for extraction and estimation of proline was essentially that described by Bates et al. (1973).

Estimation of nucleic acids

As adopted by Mohammed and El-Sayed (1982), a known fresh weight of seedlings was crushed with acid-washed sand in a glass mortar using 4 cm^3 of Tris-EDTA buffer (0.05 M Tris-EDTA + 0.1 M NaCl + 0.1 M EDTA, pH 8.0), 2 cm^3 SDS and 1 cm^3 of 1 × standard saline citrate solution (0.015 M NaCl + 0.015 M trisodium citrate, pH 7.0). The mixture was transferred into a measuring flask with 7 cm^3 of chloroform: isoamyl alcohol (24:1). The contents were shaken for 10 min, and then centrifuged at 3000 g for 10 min at 4^0C. The clear supernatant contained the nucleic acids.

RNA. The widely used reaction of ribose in RNA with orcinol as described by Sadasivam and Manickam (1996) is herein used. An aliquot of extract was pipetted into a tube, followed by the sequential addition of 6 cm^3 of orcinol acid reagent (2 cm^3 10 % solution (w⁄v)) $FeCl_3.6\ H_2O$ + 400 cm^3 of concentrated HCl) and 0.4 cm^3 6.0 % alcoholic orcinol (6 g orcinol in 100 cm^3 95 % ethanol). The tubes were first shaken, heated in a boiling water bath for 20 min and then allowed to cool. Absorbance was read at 600 nm against a blank prepared by adding all reactants plus water instead of the plant extract.

DNA. The method herein adopted is based on the quantitative reaction of deoxyribose with diphenylamine reagent (Sadasivam and Manickam, 1996). In a test tube, an aliquot of extract was followed by addition of 6 cm^3 of diphenylamine reagent (mixture of 5 g crystallized diphenylamine, 500 cm^3 glacial acetic acid and 13.75 cm^3 concentrated H_2SO_4). The contents were then mixed, heated in a boiling water bath for 10 min, then cooled and the absorbance of the blue colour developed was read at 600 nm against a blank.

Time course experiments

An initial step was to find out the appropriate low and high concentrations of NaCl as well as the optimum concentrations of the used adaptive compounds namely: Pro, AsA, GSH and SA; this term being used throughout this investigation. Thus, using the procedures adopted in the main experimental reaction, several preliminary experiments were carried out and considering the results obtained for all growth parameters, it was possible to find out the appropriate concentrations of NaCl and the optimum concentration of each adaptive compound herein used with the sensitive and tolerant broad beans. In the main experimental section, two uniformly sized lots of salt-sensitive and salt-tolerant broad beans were selected and surface sterilized by immersion in 0.1 % $HgCl_2$ solution for 5 minutes. The sterilized beans were thoroughly washed with distilled water. Thereafter, the seeds of each cultivar were divided into 15 groups. Each group of seeds were then primed separately for 24 h in an aerated appropriate culture solution according to the following treatment scheme:

1-1⁄10 Hoagland solution (control)
2- 50 mM NaCl
3-300 mM NaCl
4- 0.40 mM Pro
5- 50 mM NaCl + 0.40 mM Pro
6- 300 mM NaCl + 0.40 mM Pro
7- 4.0 mM AsA
8- 50 mM NaCl + 4.0 mM AsA
9- 300 mM NaCl + 4.0 mM AsA
10-1.0 mM, GSH
11- 50 Mm NaCl + 1.0 mM GSH
12-300 Mm NaCl + 1.0 mM GSH
13-0.09 mM SA
14-50 mM NaCl + 0.09 mM SA
15-300 mM NaCl + 0.09 mM SA

All appropriate weights of NaCl and adaptive compounds, required for the appropriate culture solutions, being maintained in 1⁄10 Hoagland nutrient solution.

Thus, for each cultivar of the broad beans used, a total of fifteen treatments representing all planned possible combinations of salinity levels and adaptive compounds were allotted. The group of seeds allotted for each treatment were subdivided into a number of sets, each of 25 seeds, that were allowed to germinate in plastic boxes (22×17× 9 cm) furnished with Whatman No.1 paper moistened by adding 20 cm^3 of the appropriate culture solution to each box. The germination boxes were incubated in the dark at 20± 0.1 ^{0}C over a period of 14 days under complete sterile conditions. During the experimental period, and when required, each box was supplied with 20 cm^3 of 1⁄10 Hoagland nutrient solution or the specified NaCl or the adaptive compound solution. Seeds were periodically examined and the percentage of germination was calculated after 14 days from the start of the germination period. Also seedlings were examined for determination of length of radicle and plumule to the nearest cm using a ruler. Since seed coat, in general, is not being utilized in germination, the weight of the decoated seed represents the weight of the living portion, i.e. embryo and cotyledons, in which resides the potential for growth. Thus, after 14 days, triplicate samples of decoated seeds were dried on paper towels for determination of fresh weight before being dried at 80 ^{o}C in an aerated oven to constant dry weight; water content was thus obtained by simple calculation. Comparable samples of decoated seeds were subjected to chemical analyses.

The relative growth rate was calculated as follows:

$$GR = \frac{\ln (M_2) - \ln (M_1)}{t_2 - t_1}$$

Where M_1 and M_2 is the plant dry mass at time t_1 and t_2, respectively. For better quantitative comparison among the different treatments, the percentage change (increase or decrease) in response to each treatment, in relation to control level, was calculated throughout this investigation as follows: Percentage change (increase or decrease) in response to each specific treatment: [level maintained in response to each treatment-control level) /control level] ×100. The results obtained from the analyses of duplicate determinations and triplicate samples were remarkably close, thus the data presented in the corresponding tables are the means of triplicate samples. The full data of the different stressed groups of germinating beans were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the Post Hoc L.S.D. with a significant level at * $P \leq 0.05$. All the analyses

were made using the SPSS 13.0 for Windows software package (SPSS Inc., Chicago, IL, USA).

Conclusions

In the present study, we have confirmed the contribution of accumulated Pro, glycine and glucose to stress tolerance in *vicia faba* seedlings. The constitutive accumulation of these metabolites, in addition to protection of cellular integrity; as being evident from an initial fall followed by an increase in nucleic acid contents, may be a promising approach to creating salt tolerant crops.We have also confirmed that the constitutive contents of certain carbohydrates, Pro and glycine were higher in the tolerant seedlings than the comparable contents in the sensitive ones. This can be considered as acquired varietal adaptation to salinity tolerance in broad beans. Our results thus demonstrate that cultivar Giza 843 was more tolerant to NaCl than cultivar 716; by preferentially enhancing the accumulation of glucose, Pro and glycine as well as nucleic acids. Combination of the various adaptive compounds with salinity levels led to different rates of counteraction of the maintained varied inhibitory effects of salinity on the various growth and metabolic components in both cultivars. The magnitude of response appeared higher in the tolerant than in the sensitive cultivar. This can be attributed to the fact that, in addition to the acquired varietal salt tolerance, there is additive salt tolerance conferred by exogenous application of adaptive compounds. Although all the selected adaptive compounds are multifunctional molecules, yet the maintained changes in growth and metabolic components go along the following sequence of adaptive compound treatments: SA > AsA > Pro > GSH. Thus, SA appeared to be the most effective among the 4 selected adaptive compounds mainly because of its being a hormone-like growth regulator of phenolic nature.

Today, when the availability of fresh water is scarce, there is increasing use of saline water in agriculture in arid and semiarid regions. Although faba bean is often grown on saline soils in Egypt, yet few studies have been published on its response to salinity. Taking into consideration the present results, we may suggest cultivation of beans, in particular the tolerant variety, in newly reclaimed areas in which the use of saline water or even diluted sea water are the only available source of irrigation. However, with the aid of SA, further studies on growth and yield of faba bean plants are required.

References

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol Plant. 48: 555-560.

Ali Q, Ashraf M, Athar, HR (2007) Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak J Bot. 39: 1133-1144.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-208.

Bor M, Ozdemir F, Turkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Sci. 164: 77-84.

Cram WJ (1976) Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Luttge U., Pitman M.G. (eds.): Encyclopaedia of Plant Physiology, New Series, Vol. 2. Springer-Verlag, Berlin.

Divate MR, Pandy RM (1981) Salt tolerance in grapes. III. Effect of salinity on chlorophyll, photosynthesis and respiration. Ind J Plant Physiol. 24: 74-79.

Dubey RS, Rani M (1989) Salinity induced accumulation of free amino acids in germinating rice seeds differing in salt tolerance. J Agron Crop Sci. 163: 236-247.

El-Khallal SM, Hathout TA, Ashour AA, Kerrit AA (2009) Brassinolide and salicylic acid induced antioxidant enzymes, hormonal balance and protein profile of maize plants grown under salt stress. Res J Agric Biol Sci. 5: 391-402.

Farooq M, Basra SMA, Walid A, Khan MB (2006) Rice seed invigoration by hormonal and vitamin priming. Seed Sci Technol. 34:775-780.

Flowers TJ, Hajibagheri MA (2001) Salinity tolerance in *Hordeum vulgare:* ion concentrations in root cells of cultivars differing in salt tolerance. Plant and Soil 231: 1-9.

Fougére F, Le Rudulier D, Streeter JG (1991) Effects of salt stress on amino acid, organic acid and carbohydrate composition of roots, bacteriods and cytosol of alfalfa (*Medicago sativa* L.). Plant Physiol. 96: 1228-1236.

Gill P, Sharma A, Singh P, Bhullar S (2002) Osmotic stress induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. Bulg J Plant Physiol. 29:12-25.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Ann Rev Plant Physiol Plant Mol Biol. 31: 149–190.

Hasaneen MNA, Younis ME, El-Bialy DMA (2008) Plant growth metabolism and adaptation in relation to stress conditions. XXII. Further studies supporting nullification of harmful effects of salinity in lettuce plants by urea treatment. Plant Soil Environ. 54: 123-131.

Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007) Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. J Plant Physiol. 164: 553-561.

Ismail AM (2003) Effect of salinity on physiological responses of selected lines / variety of wheat. Acta Agron Hung. 51: 1-9.

Jamil M, Lee D, Jung KY, Ashraf M, Lee SC, Rha ES (2006) Effect of salt stress on germination and early seedling growth of four vegetable species. J Cent Eur Agric. 7: 273-282.

Kaymakanova M, Stoeva N (2008) Physiological reaction of bean plants (*Phaseolus vulgaris* L.) to salt stress. Gen Appl Plant Physiol. 34 : 177-188.

Khattab H (2007) Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline condition. Aust J Basic and Appl Sci. 1: 323-334.

Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KS (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci. 88: 424-438.

Leprince O, Hendry GAF, McKersie BM (1993) The mechanisms of desiccation tolerance in developing seeds. Seed Sci Res. 3: 231-246.

Levitt J (1980) Responses of Plants to Environmental Stress, Volume 2, Water, radiation, salt and other stresses. Academic Press, New York, USA, pp: 365-488.

Mao H, Iwanaga F, Yamanaka N, Yamamoto F (2008) Growth, photosynthesis, and ion distribution in hydroponically cultured *Populus alba* L. cuttings grown under various salinity concentrations. Landscape Ecol Eng. 4:75-82.

Mohammed Y A, El-Sayed MA (1982) The role of cobalt in nucleic acid content of *Azotobacter chlorococcum*. Iraqui J Sci. 23:421-424.

Mukhtar CZ, Hasnain S (1994) Effect of NaCl stress on DNA, RNA and soluble protein content of callus cultures of *Brassica oleracea* L. Var. Capitata. Pak J Agric Res. 15: 233-238.

Munns R (1993) Physiological processes limiting plant growth in saline soils. Some dogmas and hypotheses. Plant Cell Environ. 16: 15-24.

Muting D, Kaiser E (1963) Spectrophotometric method of determining of alfa-amino-N in biological material by means of the ninhydrin reaction. Hoppe-Seyler`s Zschr Physiol Chem. 332: 276-289.

Okuma E, Murakami Y, Shimoishi Y, Tada M, Murata Y (2004) The effects of exogenous proline and betaine on tobacco cultured cells under saline conditions. Soil Sci Plant Nutr. 50: 1301-1305.

Parida AK, Das AB, Mittra B (2003) Effect of NaCl stress on the structure, pigment complex composition and photosynthetic activity of mangrove *Bruguiiera parviflora* chloroplast. Photosynthetica 41: 191-200.

Raza SH, Athar HR, Ashraf M (2006) Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. Pak J Bot. 38: 341-351.

Sadasivam S, Manickam A (eds) (1996) Biochemical metho ds. 2nd edn. New Age Int. Publ., New Delhi.

Sakhabutdinova AA, Fatkhutdinova DR, Bezrukova MV, Shakirova FM (2003) Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg J Plant Physiol. Special Issue, 314-319.

Saunier RE, Hull HM, Ehrenreich JH (1968) Aspects of the drought tolerance in creosote bush (*Larrea divaricata*). Plant Physiol. 43: 401-404.

Savvas D, Lenz F (2000) Effects of NaCl or nutrient-induced salinity on growth, yield, and composition of eggplants grown in rockwool. Sci Hort. 84: 37-47.

Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (asprin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul. 30: 157-161.

Shaddad MA, Radi AF, Abdel-Rahaman AM, Azooz AA (1990) Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine (B_6). Plant and Soil 122:177-183.

Shalata A, Neumann PM (2001) Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J Exp Bot. 52: 2207–2211.

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot. 57: 711-26.

Sohn YG, Lee BH, Kang KY, Lee JJ (2005) Effects of NaCl stress on germination, antioxidant responses and proline content in two rice cultivars. J Plant Biol. 48: 201-208.

Solomon A, Beer S, Waisel Y, Jonesand GP, Paleg LG (1994) Effects of NaCl on the carboxylating activity of rubisco from *Tamarix jordanis* in the presence and absence of proline-related compatible solutes. Physiol Plant. 90: 198-204.

Stewart GG, Lee JA (1974) The role of proline accumulation in halophytes. Planta. 120: 279-289.

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot. 91: 503-507.

Thayumanavan V, Sadasivam S (1984) Physiological basis for the preferential uses of certain rice varieties. Qual Plant Foods Hum Nutr. 34: 253-259.

Ungar IA (1995) Seed germination, seed-bank ecology in halophytes. In: Kigel J., Galili G. (eds.), Seed development, germination: 529-544. Marcel Dekker, New York.

Van Handel E (1968) Direct micro determinations of sucrose. Anal Biochem. 22: 280 - 283.

Waditee R, Bhuiyan MNH, Rai V, Aoki K, Tanaka Y, Hibino T, Suzuki S, Takano J, Jagendorf AT, Takabe T, Takabe T (2005) Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*. Proc Natl Acad Sci. USA, 102: 1318-1323.

Wolucka BA, Goossens A, Inze D (2005) Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. J Exp Bot. 56: 2527-2538.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: Evolution of osmolyte systems. Sci. 217: 1214-1222.

Yemm EW, Willis A J (1954) The estimation of carbohydrates by anthrone. Biochem J. 57: 508-514.

Yemm EW, Willis AJ (1956) The respiration of barley plants. IX. The metabolism of roots during assimilation of nitrogen. New Phytol. 55: 229-252.

Younis ME, Hasaneen MNA, Nemet-Alla MM (1987) Plant growth, metabolism and adaptation in relation to stress conditions. IV. Effects of salinity on certain factors associated with the germiantion of three different seeds high in fats. Ann Bot. 60: 337-344.

Younis ME, Hasaneen MNA, El-Saht HM (1989) Plant growth, metabolism and adaptation in relation to stress conditions. VII. Respiration, nitrogen and proline contents in french bean and maize plants as influenced by salinity. Qatar Univ Sci Bull. 9: 125-137.

Younis ME, Abbas MA, Shukry WM (1993) Plant growth, metabolism and adaptation in relation to stress conditions. XV. Effects of salinity on growth and metabolism of *Phaseolus vulgaris*. Biol Plant. 35: 417-424.

Younis ME, Shukry WM, EL-Nimr MAM (1999) Plant growth, metabolism and adaptation in relation to stress conditions. XX. Comparative effects of salinity on growth, metabolism and yield of three different bean plants. Egypt. J Physiol Sci. 23: 99-122.

Younis ME, Hasaneen MNA, Ahmed AR, El-Bialy DMA (2008) Plant growth metabolism and adaptation in relation to stress conditions. XXI. Reversal of harmful NaCl-effects in lettuce plants by foliar application with urea. Aust J Crop Sci. 2: 83-95.

Younis ME, Hasaneen MNA, Tourky SMN (2009a) Plant growth, metabolism and adaptation in relation to stress conditions. XXIV. Salinity-biofertility interactive effects on proline, glycine and various antioxidants in *Lactuca sativa*. Plant Omics J. 2: 197-205.

Younis ME, Hasaneen MNA, Kazamel AMS (2009b) Plant growth, metabolism and adaptation in relation to stress conditions. XXVII. Can ascorbic acid modify the adverse effects of NaCl and mannitol on amino acids, nucleic acids and protein patterns in *Vicia faba* seedlings? Protoplasma, 235: 33-47.

Younis ME, Hasaneen MNA, and Kazamel AMS (2010) Plant growth metabolism and adaptation in relation to stress conditions. XXVI. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. Protoplasma, 239: 39-48.

Yousof F I, Mersal IF, EL-Emam AAM (2010) Effect of soaking rice (*Oryza sativa* L.) seed in some antioxidant solutions on germination and seedling vigor under different salinity levels. J Plant Prod. 1: 279-290.