



## AMINO ACIDS AND SOLUBLE PROTEIN PROFILE OF RADISH SEEDLINGS UNDER SALT STRESS AS AFFECTED BY GA<sub>3</sub> PRIMING

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### SUMMARY

Radish (*Raphanus sativus* L.) seeds soaked for 24 h in  $10^{-5}$  M of GA<sub>3</sub> were germinated under 3 concentrations of NaCl (50, 100 and 200mM) and seedlings use harvested after 4, 7 and 9 d. Total soluble proteins decreased significantly with the increase of NaCl during germination except at 50mM, and the values were always higher with GA<sub>3</sub> priming. Proline decreased with NaCl with and without GA<sub>3</sub> priming. Arginine decreased with 50mM NaCl in the unprimed seedlings and increased in 50 and 200mM NaCl in the primed ones. Glutamic acid and histidine increased in 200mM salt stressed seedlings and histidine in 50 and 200mM in the primed ones. Glycine was absent in both controls and began to appear with 50 and 200mM in the primed and only in 200mM NaCl in the unprimed seedlings. Salinity increased the total essential amino acids and the increase was higher with GA<sub>3</sub> while the non-essential amino acids decreased slightly with 50mM NaCl and the decrease was greater with GA<sub>3</sub>. The total amino acids accumulated with 200mM NaCl in the unprimed seedlings and the opposite was the case with the primed ones due to the sharp decrease in the non-essential amino acids. GOT activity decreased with the increase of NaCl concentration but began to increase again at 200mM NaCl in the primed and unprimed seedlings; its values were however, always, lower in the primed seedlings than in their unprimed counterparts. GPT activity decreased consistently with salinity and its values were lower on priming with GA<sub>3</sub>. The activity of both enzymes increased with germination age. A new protein with MW of >97kD was detected in the primed seedlings treated with 200mM NaCl. A protein with MW of about 21.5 kD existed at all NaCl concentrations, increased with salinity, and its accumulation was greater with GA<sub>3</sub> priming.

**Key words:** Amino acids, gibberellic acid, proteins, radish, salinity, transaminases

### INTRODUCTION

Salinity affects germinating seeds and growing seedlings of plants in different ways. Thus, it is not uncommon to find that the same salinization treatment causes the accumulation of certain metabolites in some plants and their reduction in others. For instance, Shaddad *et al.* (1990) found that in *Lupinus albus* carbohydrate loss caused by high salinity levels was accompanied by increase in soluble protein, whereas, in *Vicia faba* the

opposite effect was observed. Similarly, high salinity levels caused a decrease in carbohydrates accompanied by an increase in soluble proteins in seedlings of *Cicer arietinum* and *lens culinaris*, whereas the opposite occurred in Seedlings of *Trigonalla foenum-graecum* (El-Tayeb *et al.* 1999). A similar discrepancy concerns the proline content in salt stressed plants. For instance, while some authors (Zidan 1999, Le Dily *et al.* 1993, Delauney and Verma 1993, Hajar *et al.* 1996, Abdel-Samad and Shaddad 1997, Soussi *et al.* 1998) reported

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increased accumulation of proline in seedlings growing under saline conditions, others (Naik and Joshi 1983, Siddiqi and Krishnamoorthy 1987) recorded significant reductions in the proline content of seedlings of other species and cultivars, with several instances in which no significant changes in proline were observed (Stewart and Larher 1980, Rai *et al.* 1983, Ranieri *et al.* 1989, Lutts *et al.* 1996, Gilbert *et al.* 1998). Consequently, such discrepancies have led to a great deal of controversy over the role of proline and other metabolites in the tolerance of different plants to salinity stress.

Attempts have been made to counteract the adverse effects of salinity on plants especially at the different stages of seed germination and seedling growth by pretreatment of seeds with various growth regulating substances. Examples of such attempts include the application of GA<sub>3</sub> or IAA on maize and safflower (Radi *et al.* 1988 and 1989), GA<sub>3</sub> or triadimefon on radish (Muthukumarasamy and Panneerselvam 1997), GA<sub>3</sub> or kinetin on barley (Kabar 1987), GA<sub>3</sub> on wheat and radish (Aldesuquy 1998, and Kasim and Dowidar 2000, respectively), thiamine or ascorbic acid on *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* (El-Tayeb *et al.* 1999), ascorbic acid or pyridoxine or *Lupinus albus* and *Vicia faba* (Shaddad *et al.* 1990), and bitertanol (a triazole fungicide with growth regulating properties) on *Vigna radiata* (Rajan *et al.* 2000). Two amino acids (proline and phenylalanine) were used to alleviate the adverse effects of salinity on *Phaseolus vulgaris* (Heikal *et al.* 1992). Studies on ameliorating such effects with mineral salts include pre-soaking the seeds of *Arachis hypogea* in CaCl<sub>2</sub> (Girija *et al.* 2002), of *Vicia faba* and *Pisum sativum* in CaCl<sub>2</sub> (El-Tayeb and Ahmed 2002), of maize in CaCl<sub>2</sub> or CaSO<sub>4</sub> (Hamada 1994), of *Phaseolus vulgaris* in KNO<sub>3</sub> (Ismail 1996) and of *Sorghum bicolor* in NaCl (Amzallag *et al.* 1990), met with varying degrees of success.

In our previous article (Kasim and Dowidar 2000), we studied the effects of various levels of NaCl salinity on growth criteria, the content and activity of photosynthetic pigments, carbohydrates,  $\alpha$ - and  $\beta$ -amylases of radish (*Raphanus sativus*) seedlings, and the amelioration of these effects by pre-soaking the seeds in gibberellic acid. The present study deals with the use

of gibberellic acid in the alleviation of the effects of the same salinity treatments on the total protein content, protein pattern, the two transaminases GOT and GPT and amino acids of radish seedlings.

## MATERIALS AND METHODS

Radish seeds (*Raphanus sativus*) obtained from the Ministry of Agriculture (Cairo) were selected for uniformity of shape and size and subdivided into two sets. Each set was soaked in 100 ml of either aerated distilled water or 10<sup>-5</sup> M aerated aqueous solution of gibberellic acid (GA<sub>3</sub>) for 24 hrs. Seeds were then washed with distilled water and germinated in 20-cm diameter Petri dishes, each containing 20 seeds and supplemented either with water or with 50, 100 and 200mM NaCl at room temperature (25°C±1) under normal day/night regime. Samples of seedlings were collected when they were 4, 7 and 9 days old and used for the determination of enzyme activities (glutamic-oxaloacetic acid transaminase (GOT) and glutamic-pyruvic acid transaminase (GPT), protein patterns by SDS-PAGE, total soluble protein (TSP) and free amino acids.

Total soluble protein (TSP) was estimated spectrophotometrically using the folin phenol reagent method described by Lowry *et al.* (1951). GOT and GPT were extracted by grinding seedlings in ice cold water, and their activities were determined spectrophotometrically as pyruvate hydrazone for GPT, and as oxalo-acetate hydrazone for GOT according to the method described by Bergmeyer (1974). Amino acid analyses were carried out according to the method of Anderson *et al.* (1977) using the HPLC (Beckman, System Gold, Programmable Solvent Module 126). Protein patterns in the fresh seedlings were qualitatively analyzed by SDS-PAGE according to Laemmli (1970). The degree of significance was calculated for the activity of the two transaminases and the total protein content. Analysis of variance was determined according to the method described by Bishop (1983).

## RESULTS AND DISCUSSION

The increase in the NaCl concentration resulted in a significant decrease in total soluble protein (TSP)

content in unprimed radish seedlings at all the stages of germination examined, except at 50mM NaCl where the TSP increased and the seedlings seemed tolerant to this concentration. These results are in agreement with those of Khan and Singh (1998) for *Curcuma longa*. The priming of seeds by pre-soaking in 10<sup>-5</sup> GA<sub>3</sub> resulted in the alleviation and that the values of TSP in the GA<sub>3</sub>-primed seedlings were always higher than their corresponding values in the unprimed salt-stressed seedlings of the same age. This is in agreement with Radi *et al.* (1989), for maize seeds. A highly significant gradual decline in TSP content of primed and unprimed seedlings with age was observed in all NaCl treatments (Table 1). The highest values of TSP were recorded after 4 days of germination at 50mM NaCl, either with or without GA<sub>3</sub>.

In salt-stressed plants, the decrease in protein level was attributed to: (a) a decrease in protein synthesis, (b) decreased availability of amino acids, and/or (c) denaturation of the enzymes involved in the synthesis of amino acids and proteins (Dubey and Rani 1990, Aldesuquy 1998). It was reported that GA<sub>3</sub> can increase the formation of rough endoplasmic reticulum (RER) which provides the appropriate medium for the increase of polyribosomes (Evins and Varner 1972, Kabar 1987). This notion seems plausible in view of our previous findings (Kasim and Dowidar 2000), where the increase in  $\alpha$ - and  $\beta$ -amylase activities in radish seedlings due to salt stress was more pronounced on priming with GA<sub>3</sub>.

*Amino acids:* Histidine and arginine constituted about 57% of the total essential amino acid content, whereas proline and glutamic acid formed nearly 84% of the non-

essential amino acids in unprimed 7 day-old radish seedlings. These amino acids constituted nearly 70% of the total amino acid pool of the unprimed seedlings at this age. This is in apparent agreement with the results reported by Laroche *et al.* (1984) with regard to arginine and glutamic acid. Therefore, these 4 amino acids seem to be the main degradation products of the seed storage proteins.

Proline content of the seedlings decreased under NaCl treatment, whether the seeds were primed in GA<sub>3</sub> or not. Furthermore, proline quantities were lower in the case of GA<sub>3</sub> primed seedlings than in their unprimed counterparts. These findings are similar to the results of Gilbert *et al.* (1988), who reported that proline was not found to accumulate in either source or sink tissues of *Coleus blumei* during the exposure to salinity. Arginine content in unprimed seeds decreased with the increase in NaCl but in primed seeds an increase was observed under both concentrations of NaCl. Glutamic acid increased only at 200mM NaCl, as was also observed by Aldesuquy (1998), while on priming with GA<sub>3</sub>, glutamic acid increased at 50mM and then decreased with 200mM NaCl. Histidine increased only under 200mM NaCl in unprimed seeds but in primed seed it increased under both NaCl concentrations. The other amino acids showed different responses to salinity with and without priming of the seeds in GA<sub>3</sub>. However, glycine deserves a special mention as it was the only amino acid not detectable in both control samples, but began to appear with 50mM in the primed seedlings and with 200mM NaCl in both primed and un-primed seedlings; a 9-fold increase of glycine occurred in the primed seedlings treated with 200mM NaCl.

**Table 1.** Effect of pre-soaking of radish seeds in GA<sub>3</sub> (10<sup>-5</sup>M) on total soluble protein (TSP) content (mg/g dw) in seedlings germinated under different concentrations of NaCl

Age of seedlings (days)	Unprimed seedlings				GA <sub>3</sub> -primed seedlings			
	NaCl concentrations (mM)							
	0	50	100	200	0	50	100	200
4	116±1.15	13.2±1.35	9.5±0.8	9.2±1.1	12.5±1.45	15.8±1.1	12.3±1.5	10.8±1.25
7	6.8±0.5	7.7±0.83	5.9±0.91	4.9±0.6	8.1±0.7	11.2±1.1	7.9±0.93	6.2±0.45
9	3.7±0.6	4.6±0.5	3.3±0.28	2.8±0.26	5.2±0.7	6.8±0.32	5.1±0.21	2.8±0.18

**Table 2.** Effect of pre-soaking of radish seeds in GA<sub>3</sub> (10<sup>-5</sup>M) on amino acid content (mg/100 g dw) in 7 days old seedlings germinated under two NaCl concentrations

GA <sub>3</sub> Treatment	Unprimed			primed		
	NaCl concentrations (mM)					
	0	50	200	0	50	200
<b>Essential amino acids</b>						
Histidine	3.24	3.20	4.74	3.92	4.76	4.94
Arginine	2.41	2.32	1.49	1.33	1.50	1.90
Threonine	1.10	1.80	1.15	0.82	1.20	0.77
Valine	0.13	0.10	0.08	0.07	0.10	0.09
Methionine	0.06	0.07	0.04	0.03	0.06	0.03
Leucine	0.87	0.81	1.01	0.88	1.01	0.96
Isoleucine	0.40	0.42	0.47	0.37	0.50	0.46
Phenylalanine	1.17	1.12	1.95	2.56	1.98	3.08
Lysine	0.56	0.51	1.21	0.21	1.25	0.26
<b>Non-essential amino acids</b>						
Aspartic acid	0.87	0.81	1.11	1.12	1.14	1.25
Glutamic acid	5.08	5.00	9.89	6.26	9.89	1.56
Serine	0.50	0.45	0.60	0.67	0.62	0.20
Glycine	0.00	0.00	0.04	0.00	0.04	0.37
Alanine	0.29	0.20	0.34	0.38	0.30	0.54
Proline	8.68	8.62	6.19	11.95	6.20	2.57
Tyrosine	0.93	0.90	1.27	2.18	1.30	2.67
Cysteine	0.09	0.11	0.12	0.00	0.13	4.61
Total essential amino acids	9.94	10.35	12.14	10.19	12.36	12.49
Total non-essential amino acids	16.44	16.09	19.56	22.56	19.62	13.77
Total amino acids	26.38	26.44	31.70	32.75	31.98	26.26

The total essential amino acids increased with increasing salinity, whether the seeds were primed in GA<sub>3</sub> or not, although these values were always higher in the primed seedlings than their non-primed counterparts. On the other hand, the total non-essential amino acids slightly decreased in 50mM NaCl and increased in 200mM NaCl. In the case of priming in GA<sub>3</sub>, non-essential amino acid content decreased in both NaCl concentrations.

Several explanations for the accumulation of free amino acids in a number of monocots and dicots under salinity stress have been suggested. These include (i) the stimulated synthesis and/or the inhibited degradation of amino acids, and (ii) the impaired protein synthesis and/or enhanced protein degradation (Ranieri *et al.* 1989, Fougère *et al.* 1991, Good and Zaplachinski 1994, Schubert *et al.* 1995, Gilbert *et al.* 1998). Several authors

**Table 3.** Effect of pre-soaking of radish seeds in GA<sub>3</sub> (10<sup>-5</sup>M) on the activities of glutamic-oxaloacetic acid transaminase (GOT) and glutamic-pyruvic acid transaminase (GTP) (µg/g dry weight/min.) in radish seedlings germinated under different concentrations of NaCl

Age of seedlings (days)	GA <sub>3</sub> -unprimed				GA <sub>3</sub> -primed			
	NaCl concentrations (mM)							
	0	50	100	200	0	50	100	200
Glutamic-oxaloacetic acid transaminase (GOT)								
4	14.3±0.45	12.0±0.06	10.5±0.05	14.0±0.7	12.5±0.3	10.2±0.4	8.4±0.07	12.8±0.07
7	19.8±0.45	16.8±0.15	12.9±0.06	17.4±0.08	16.8±0.45	13.5±0.08	9.0±0.07	13.9±0.06
9	24.4±0.54	20.2±0.08	15.9±0.06	20.3±0.06	19.4±0.6	15.3±0.05	10.5±0.35	15.7±0.09
Glutamic-pyruvic acid transaminase (GPT)								
4	1.4±0.22	1.3±0.06	1.2±0.07	1.1±0.04	1.2±0.21	1.1±0.07	0.9±0.06	0.7±0.05
7	3.1±0.35	2.9±0.08	2.5±0.23	2.2±0.04	2.8±0.29	2.7±0.06	2.1±0.07	1.6±0.06
9	4.6±0.31	4.4±0.05	3.9±0.07	3.5±0.07	4.1±0.12	3.8±0.07	3.2±0.08	2.7±0.07

maintain that this accumulation is mainly the result of the induction of protein hydrolysis by protease (Newton *et al.* 1986, Vernon *et al.* 1993, Olmos and Hellin 1996, Gilbert *et al.* 1998).

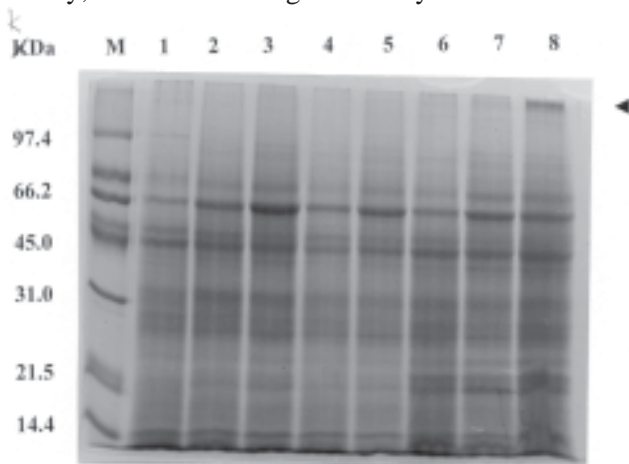
Among the compatible cytoplasmic solutes accumulated by plants in response to salinity stress are amino acids and some carbohydrates (Rabe 1990, Bohnert *et al.* 1995). A number of possible functions of these compatible solutes has been put forward by various authors. These include: (a) osmotic adjustment by lowering the water potential to improve the uptake of water against the external gradient, (b) serving as a readily available energy source, or as a nitrogen source during limited growth and photosynthesis, (c) detoxification of excess ammonia under periods of stress, and (d) stabilization of enzymes and/or membranes (Rabe and Lovatt 1984, Smirnoff and Cumbes 1989, Vernon *et al.* 1993). The decrease in the total amino acids in radish seedlings germinated under salt stress from seeds primed in GA<sub>3</sub> may be due to the role of GA<sub>3</sub> in enhancing the incorporation of free amino acids into conjugated proteins or iso-enzymes in order to increase the salt tolerance of the seedlings as has already been suggested by Ali (1992) and Aldesuquy (1998). This possible explanation seems to be compatible with the previous results of Dubey and Sharma (1990) who found a greater number of iso-

enzymes in the embryo axes of salt-tolerant than in salt-sensitive rice varieties.

*Transaminases:* The accumulation of total amino acids under salt stress and the decrease in their amounts under the effect of pre-soaking in GA<sub>3</sub> prompted us to study the effect of these treatments on two of the transaminases (GOT and GPT) involved in the biosynthesis of amino acids. It was observed that GOT activity decreased significantly with the increase in salt concentration but increased at 200 mM NaCl, whether the seeds were primed in GA<sub>3</sub> or not. Furthermore, each value of GOT activity is noticeably lower in GA<sub>3</sub> treatment than its corresponding value of the untreated seedlings. On the other hand, for every salt concentration GOT activity is enhanced with seedling age. GPT activity decreased gradually with the increase in salt concentration in both the GA<sub>3</sub>-primed and unprimed seedlings. As in the case of GOT, each value of GPT activity is clearly lower in GA<sub>3</sub> priming than the corresponding value of the unprimed seedlings, and for every salt concentration, the activity increased with seedling age. These results indicated that unlike the activity of GPT (which decreased with all salt concentrations), GOT is re-enhanced at salt concentration of 200mM. This is the same salt concentration which caused the largest accumulation of total amino acids in

the unprimed seedlings as well as the largest decrease in total amino acids of the primed ones (see Table 2). Hence, the effect of GA<sub>3</sub> tended to reverse the NaCl stress effects. The decrease in the amino acids in the GA<sub>3</sub> primed seedlings may be explained by their incorporation in new proteins. It is most probable that these proteins included  $\alpha$ - and  $\beta$ -amylases as may be deduced from their increased activity reported previously by Kasim and Dowidar (2000).

*Effect on protein pattern:* SDS-PAGE of protein extracts of 7-days old seedlings, with and without priming with GA<sub>3</sub> is shown in Fig. 1. A new unique protein band appeared at molecular weight >97 kD in the GA<sub>3</sub> primed seedlings treated with 200 mM NaCl (lane 8) as well as higher intensity of all other bands in lane 8 than corresponding bands in lane 4 (unprimed and stressed with 200 mM NaCl). This indicated relatively higher protein concentrations in the primed seedlings than in their unprimed counterparts. In Fig. 1, the protein of about 21.5 kDa molecular weight is faintly recognizable in lane 1 (i.e. without salinity and GA<sub>3</sub>), but begins to accumulate in the salt-stressed seedlings (lanes 2-4). A similar result was reported by Lopez *et al.* (1994) and Ilami *et al.* (1997) in *Raphanus sativus* and *Brassica napus*, respectively. The latter authors were of the view that this protein could be involved in the decrease of protease activity, thus contributing to a delay in the senescence



**Fig. 1.** SDS-PAGE of protein extracts of 7-days-old *Raphanus sativus* seedlings grown under 0.0, 50, 100 and 200 mM NaCl. Molecular weights of marker (M) proteins are indicated in kDa. Lanes (1-4) and (5-8) represent the extract of unprimed and GA<sub>3</sub> primed seedlings, respectively. The arrow indicates a unique protein with molecular weight of more than 97 kDa.

process. The accumulation of the 21.5 kDa protein increased considerably in the GA<sub>3</sub> primed seedlings treated with 50, 100 and 200 mM NaCl (lanes 6-8 in Fig. 1, respectively) than in their unprimed counterparts (lanes 2-4, respectively). The protein which appears in the 31 kDa region (previously called cruciferin A by Laroche *et al.* 1984, Kasim 1990, Hamada *et al.* 2002) seemed to be unaffected by GA<sub>3</sub> treatment.

Taking the present results collectively into consideration, it may be reasonable to assume that the multiple effects of GA<sub>3</sub> on salt-stressed radish seedlings include: (i) the *de novo* synthesis of a new protein (Fig. 1), (ii) the increased accumulation of certain existing proteins (Fig. 1), (iii) the decrease in the non-essential and in the total free amino acids (shown in Table 2) which might have been taken up in the two previous processes, and (iv) the reduction in the rate of degradation of existing (storage) soluble proteins (Table 1). The diminishing activity of the two transaminases GOT and GPT which increased salinity (Table 3) may be another factor contributing to the decreased contents of the non-essential and the total free amino acids.

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