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Changes in Free Amino Acids and Stress Protein Synthesis in Two Genotypes of Green Gram under Salt Stress*

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Abstract: Changes in the level of total and Free Amino Acid (FAA) and analysis of protein profiles on SDS-PAGE were investigated and compared in root and shoot tissues of two green gram (*Phaseolus aureus*) cultivars (T-44 and SML-32) differing in salt tolerance during plant growth in the absence and presence of NaCl salinity. Total as well as individual FAA were found to increase progressively with growth in both the tissue and in both the cultivars under absence and the presence of salinity. The level of total and individual FAA was more in shoot than in root during plant growth from day 1 to 5. Salt tolerant cultivar T-44 had significant higher ($p<0.05$) level of total as well as individual FAA than that of salt sensitive cultivar SML-32 in both the tissues in absence as well as in the presence of salt. In salt tolerant cultivar the increased accumulation of amino acid (AA) under salt stress was more pronounced in case of alanine, arginine, aspartic acid, glutamic acid, glutamine, phenylalanine and serine. Analysis of the protein profiles of root and shoot tissues of both the cultivars in absence and presence of salinity on SDS-PAGE revealed that in both tissues of salt tolerant cultivar T-44, 3 to 4 polypeptides (PPs) were specially synthesized while synthesis of some PPs increased under salt stress. Salt induced changes in protein profile in root and shoot of salt tolerant cultivar T-44 were mostly tissue specific. On the other hand no such salt specific proteins were synthesized under salt stress in salt sensitive cultivar SML-32. Correlation between the degree of salt tolerance of the cultivar and levels of AAs and those of stress protein synthesis has been discussed.

Key words: Amino acid, *Phaseolus aureus*, salinity, stress protein

Introduction

Amino acids, being the primary products of inorganic assimilation and precursors of proteins, play essential role in plant metabolism. Accumulation of high concentrations of nitrogenous organic solutes in the cytoplasm has been reported to occur in various plants subjected to salinity stress (Hasegawa *et al*., 2000; Meloni *et al*., 2001, 2003; Greenway and Munns, 1980). Solutes, which accumulate during salinity stress in many species, are glycinebetaine (Ghoulam *et al*., 2002), proline (Ghoulam *et al*., 2002; Girija *et al*., 2002; Balibera *et al*., 1997; Bajji *et al*., 1998; Alian *et al*., 2000)

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and various other AAs like aspartic acid, glycine, asparagine, glutamine and serine, etc. These soluble nitrogenous compounds accumulating under salt stress have been regarded as components of salt tolerance mechanism (Stewart and Larher, 1980) as they contribute for osmotic balance in the cytoplasm when electrolytes are higher in vacuoles and also impart a protective role on enzymes in the presence of high electrolytes in the cytoplasm (Hasegawa *et al*., 2000; Meloni *et al*., 2001, 2003; Greenway and Munns, 1980). Many salt tolerant plants have been reported to possess in built higher levels of certain AAs. In general, plants when grown without NaCl have low levels of such solutes but show increased accumulation under salinization (Stewart and Larher, 1980).

Stress induced changes in protein synthesis have been reported in response to a variety of environmental stresses such as cold (Kawata and Yoshida, 1988), dehydration (Jung and Shin, 1992; Ingram and Bartels, 1996; Ramanjulu and Sudhakar, 1997; Loderio *et al*., 2000), salinity (Ben-Hayyim *et al*., 1993; Lopez *et al*., 1994; Bajji *et al*., 1998), anaerobiosis (Sachs and Ho, 1986) and heat shock (Vierling , 1991; Wu *et al*., 1993). In case of anaerobic stress certain induced proteins have been assigned specific functions in known metabolic pathways (Sachs and Freeling, 1978).

Increased synthesis of some of the existing proteins as well as synthesis of entirely new proteins under salt stress has been reported. Thus salt induced ten fold increases in a 26 KD PP is reported in tobacco cells (Singh *et al*., 1985), maize callus (Ramgopal, 1986) and in barley root meristems (Ramgopal, 1988). A variety of salt induced proteins have been reported from a number of other sources. Thus, 23, 25 and 27 KD protein from citrus (Ben-Hayyim *et al*., 1989; 1993), 26 and 27 KD from rice (Shirata and Takagishi, 1990) and barley roots (Hurkman *et al*., 1989; Robinson *et al*., 1990), 18, 27 and 49 KD PPs from *Jojoba* calli (Chretein *et al*., 1992), 22 KD PP from leaves of *Raphanus sativus* (Lopez *et al*., 1994). Many salt induced changes in proteins are species specific and that no obvious similarity has existed (Ben-Hayyim *et al*., 1989). Studies have also been carried out in a number of tissues for the identification of the genes responsible for salt tolerance (King *et al*., 1988; Singh *et al*., 1989; Winicov *et al*., 1989). Among these, the 26 KD proteins of salt adapted cells of tobacco (called osmotin) and tomato has been examined in detail and a gene sequence has been cloned (Singh *et al*., 1989; Lopez *et al*., 1994).

We have been pursuing the biochemical studies on the two green gram cultivars (namely T-44 and SML-32, differing in their relative salt tolerance) for identification of biochemical markers for salinity tolerance (Misra and Dwivedi, 1990, 1995). In the present paper we report the metabolic levels of total FAAs and those of individual FAAs and also analysis of the soluble protein profile of root and shoot on SDS-PAGE in the absence as well as presence of NaCl salinity during seed germination.

Materials and Methods

Plant Material and Stress Treatment

Green gram (*Phaseolus aureus* L. Roxb Family: *Leguminosae*) seeds were screened for their salinity during germination in our laboratory and thus cultivar T-44 was found to be fairy tolerant while cultivar SML-32 was found to be sensitive to saline stress (Misra and Dwivedi, 1990). Seeds were surface sterilized and germinated as described (Misra and Dwivedi, 1995). Two concentrations of NaCl viz. 200 mM and 50 mM were used for cultivar T-44 and SML-32, respectively. Starting with the 4 hrs soaked seeds (zero hrs. of germination), the germinated seeds were taken out at 24 h intervals up to 5 days, roots and shoots (along with cotyledons) were separated from the seeds and proceeded further.

Estimation of Total FAAs

Total FAAs were measured by ninhydrin assay (Yemm and Cocking, 1955) with some modifications. Five hundred mg tissue (root/shoot) were extracted in 5 mL of 80% ethanol and centrifuged at 18,000-x g for 30 min. The test extract was taken (0.1 mL) and total FAA was estimated using (7.6 mL) ninhydrin reagents containing 1% ninhydrin in 0.5 M citrate buffer, pH 5.5, glycerol (87%) and 0.5 M citrate buffer pH 5.5 in ratio of 5:12:2. After vigorous shaking contents were heated in boiling water bath for 10 min and after cooling, absorbance was measured at 570 nm in a Gilford Spectrophotometer. 0.1 mL 80% of ethanol served as blank in place of test extract. Absorbance readings were converted to mg amino acid g^{-1} fresh weight tissue using a glycine standard curve.

Estimation of Individual FAAs

For separation and quantitative estimation of individual FAAs, a known volume of test extract of shoot and root tissues were applied on reversed phase HPLC using the method of Brierley *et al*. (1996).

Protein Analysis

Proteins were extracted from root and shoot (along with cotyledons) at 48 h of seed germination. Two grams of tissues were homogenized in 5 mL Tris-HCl buffer (0.1 M, pH = 7.5) containing 1 mM phenyl methyl Sulfonyl Fluoride (PMSF), at 4°C. The homogenate was centrifuged at 18,000-x g for 30 min. Protein in the supernatant was measured by the some modification of the Bradford (1976) using crystalline bovine albumin as a reference. This protein extract was concentrated before loading over the gel. For concentration, the extract was kept in a dialysis bag and the ends were tied with thread tightly and the dialysis bag containing extract was kept in powdered sucrose for the appropriate time (3-5 h) at 4°C during which period the extract was concentrated more than 3 times. Thereafter, the contents were taken out from dialysis bag, the volume of the extract was measured and protein was again determined in a suitable aliquot Bradford (1976).

Sample preparation and SDS-PAGE at pH 8.3 was done as described (Laemmili, 1970). The gels were photographed and scanned using 2202 ULTROSCAN Laser Densitometer (LKB).

Statistical Analysis

Each treatment was analyzed with at least three replicates and Standard Deviation (SD) was calculated. Statistical analysis was performed using the students t-test; $p<0.05$ was considered statistically significant.

Results

Effect of Salinity on Free Amino Acid

With the progress of germination the FAA level increased throughout in root and shoot of both the cultivars, in the absence as well as in the presence of salinity (Table 1). However, in the presence of salinity the AA content of both root and shoot increased significantly ($p<0.05$) in the cultivar T-44 while those of SML-32 exhibited only a marginal increase. Moreover, shoot maintained higher level of AA as compared to root in both the cultivars. Thus, on day 5 of plant growth in cultivar T-44, root exhibited approximately 3.9 fold ($p<0.05$) increased in the presence of 200 mM NaCl as compared to non saline control while only 1.0 fold increase was observed in root of cultivar SML-32 in the presence of 50 mM NaCl as compared to respective non saline control (Table 1). Similarly, in shoot of cultivar T-44 the AA content was observed aprox 5.9 fold

Table 1: Effect of salinity on total free amino acid (mg/g FW) in root and shoot of green gram cultivar T-44 (a and b) and SML-32 (c & d) during plant growth. Each value represents mean of three independent observations and SD determined. Data are statistically significant at $p<0.05$

Cultivars		Plant growth (in days)	1	2	3	4	5
T-44 (Salt-tolerant)	Root	Non-saline control	0.8±0.02	1.2±0.03	1.68±0.1	1.88±0.2	2.51±0.3
		200 mM NaCl	2.5±0.1	4.5±0.1	6.2±0.3	8.2±0.4	9.5±0.5
	Shoot	Non-saline control	0.95±0.1	1.0±0.02	1.2±0.02	1.5±0.04	1.9±0.06
		200 mM NaCl	1.0±0.03	1.1±0.02	1.3±0.01	1.6±0.02	1.98±0.1
SML-32 (Salt sensitive)	Root	Non-saline control	0.8±0.03	2.1±0.3	2.3±0.3	2.6±0.3	3.92±0.2
		50 mM NaCl	11.3±0.7	14.2±0.8	15.2±0.6	18.76±	22.7±0.3
	Shoot	Non-saline control	1.3±0.4	1.34±0.1	1.4±0.03	1.8±0.1	2.26±0.4
		50 mM NaCl	1.38±0.5	1.4±0.02	1.44±0.2	1.88±0.2	2.32±0.3

Table 2: Effect of salt stress on individual amino acid (mg/g FW) in root and shoot on day 5 plant growth of green gram cultivars T-44 and SML-32. Each value represents mean of three independent observations and SD determined. Data are statistically significant at $p<0.05$

	Cultivar T-44				Cultivar SML-32			
	Root		Shoot		Root		Shoot	
Amino Acids	Control	200 mM NaCl	Control	200 mM NaCl	Control	50 mM NaCl	Control	50 mM NaCl
Alanine	0.10±0.01	1.19±0.23	0.12±0.01	2.09±0.78	0.03±0.01	0.04±0.01	0.12±0.02	0.13±0.01
Arginine	0.02±0.01	0.65±0.09	0.06±0.01	1.86±0.32	0.09±0.02	0.09±0.02	0.13±0.05	0.14±0.04
Asparagine	0.23±0.07	1.02±0.13	0.60±0.11	2.16±0.56	0.06±0.01	0.07±0.01	0.12±0.02	0.13±0.03
Aspartic acid	0.02±0.01	0.05±0.01	0.03±0.01	0.09±0.01	0.02±0.01	0.04±0.01	0.03±0.01	0.05±0.01
Cysteine	0.12±0.05	0.46±0.10	0.26±0.07	1.28±0.11	0.08±0.02	0.10±0.03	0.09±0.02	0.12±0.03
Glutamic acid	0.09±0.02	0.45±0.10	0.19±0.05	1.23±0.15	0.02±0.01	0.04±0.01	0.05±0.01	0.08±0.01
Glutamine	0.19±0.06	0.86±0.11	0.29±0.08	2.76±0.67	0.11±0.03	0.12±0.04	0.13±0.05	0.15±0.02
Glycine	0.02±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.09±0.02	0.10±0.01	0.09±0.01	0.14±0.03
Histadine	0.04±0.01	0.25±0.07	0.11±0.06	0.49±0.11	0.02±0.01	0.04±0.01	0.06±0.01	0.09±0.02
Isoluecine	0.10±0.02	0.64±0.08	0.26±0.06	1.58±0.17	0.09± 0.01	0.11±0.04	0.13±0.04	0.16±0.06
Luecine	0.03±0.01	0.10±0.02	0.05±0.01	0.15±0.06	0.02±0.01	0.03±0.01	0.03±0.01	0.06±0.01
Lysine	0.08±0.01	0.16±0.04	0.10±0.05	0.23±0.09	0.06±0.02	0.09±0.02	0.08±0.02	0.13±0.01
Methionine	0.03±0.01	0.02±0.01	0.04±0.01	0.04±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01
Phenylalanine	0.03±0.01	0.11±0.03	0.04±0.01	0.19±0.07	0.05±0.01	0.06±0.02	0.09±0.02	0.15±0.03
Proline	1.07±0.12	2.31±0.34	1.21±0.18	6.21±1.4	0.26±0.06	0.27±0.08	1.10±0.23	1.10±0.21
Serine	0.13±0.03	0.46±0.06	0.16±0.05	1.02±0.23	0.06±0.01	0.07±0.01	0.12±0.03	0.13±0.06
Threonine	0.20±0.04	0.52±0.05	0.25±0.09	0.93±0.15	0.09±0.02	0.13±0.06	0.11±0.08	0.21±0.03
Tryptophane	0.04±0.01	0.09±0.02	0.05±0.01	0.12±0.04	0.02±0.01	0.04±0.01	0.06±0.01	0.09±0.01
Tyrosine	0.03±0.01	0.04±0.01	0.04±0.01	0.06±0.01	0.08±0.02	0.09±0.01	0.12±0.02	0.13±0.02
Valine	0.04±0.00	0.14±0.04	0.09±0.01	0.19±0.08	0.06±0.01	0.07±0.01	0.13±0.03	0.15±0.02

($p<0.05$) increase in the presence of 200 mM NaCl as compared to non saline control while only 1.0 fold increase was observed in root of cultivar SML-32 in the presence of 50 mM NaCl as compared to respective non saline control.

Effect of Salinity on Individual Free Amino Acid

Levels of all 20 AAs measured were found to be increased in presence of salinity in both root and shoot, with shoot maintaining a high level of all these AAs as compared to that of root under the conditions of both absence and presence of salinity in both the cultivars (Table 2). However, the increase in the levels of individual AA under salinity stress was significant in T-44 whereas it was only marginal in SML-32 in both roots as well as in shoot. Thus, in cultivar T-44, AAs whose concentration increased more in both root and in shoot under salinity stress were alanine, arginine, glutamic acid, glutamine, proline, cystein, isoleucine, asparagine and serine (a total of nine Aas).

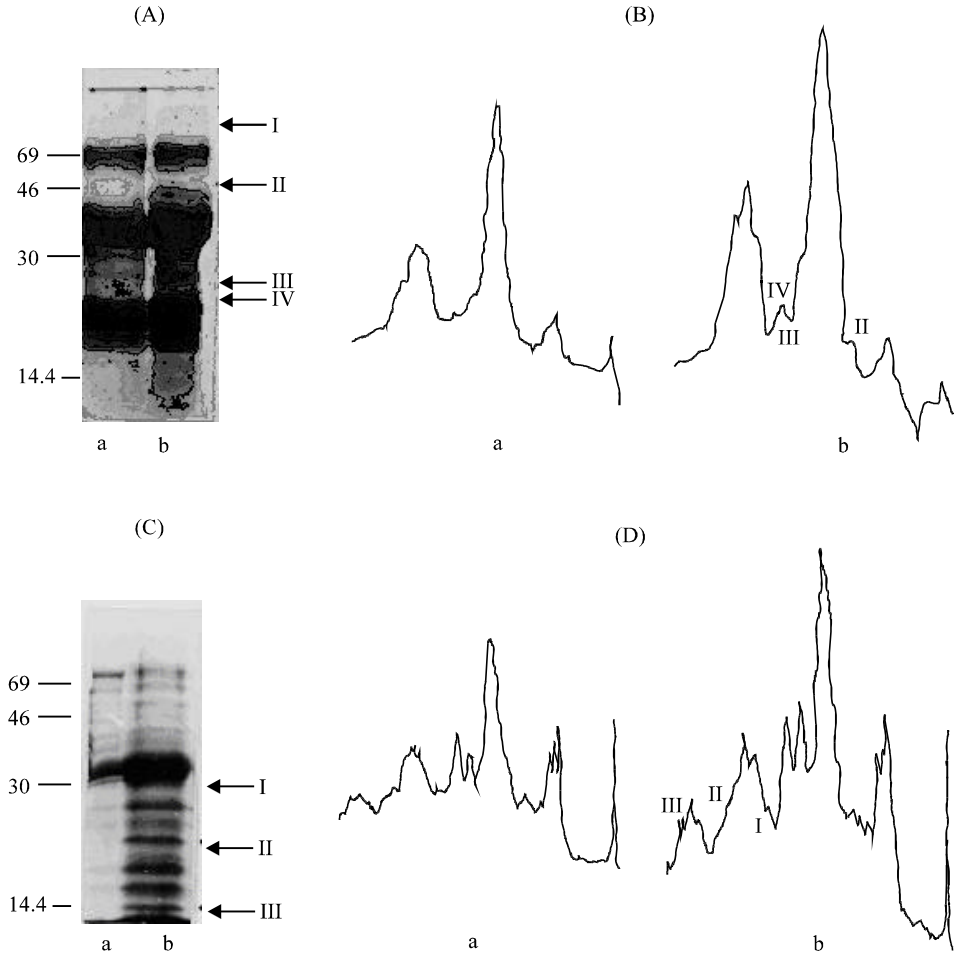

Fig. 1: SDS- PAGE of soluble proteins from shoot (A and B) and root (C and D) of salt tolerant T-44 cultivar in absence (a) and in the presence (b) of 200 mM NaCl. The proteins were extracted, concentrated and approximately 200 μg protein was loaded in each case on a 12% polyacrylamide gel containing SDS. Numbers in KD indicated the position of standard marker. Arrows indicated the position of specific proteins synthesized only during salt stress. B and D show scan of the gels A and C, respectively. Arrows indicate the specific proteins synthesized during salt stress

The corresponding increase in the levels of these AAs under salinity stress in root and shoot of cultivar SML-32 was only between 2-7%.

We also investigated the developmental profile of all the nine AAs that showed marked increase under salt stress (data not shown). Out of all 20 AAs measured, the most remarkable was the proline, which was increased significantly in the presence of 200 mM NaCl in root as well as in shoot of T-44 cultivar. However in the presence of salinity stress (200 mM NaCl) proline was increased ≈5.0 fold ($p<0.05$) in shoot and 2.1 fold in root of T-44 cultivar. Developmental profiles of these AAs were

characterized by a gradual increase in their content both in absence as well as in the presence of salinity in both root and shoot of both the cultivars. However, the increase in the amount of these AAs in presence of salinity stress was more pronounced in case of cultivar T-44 while it was only marginal in case of cultivar SML-32. Shoot always maintained a higher level as compared to root in both the cultivars in the absence as well as in the presence of salinity (data not shown).

Influence of Salinity on the Soluble Protein Profiles of Shoot

Soluble protein profile of shoot tissue from germinating green gram seeds of salt tolerant cultivar T-44 was analyzed by SDS-PAGE in absence and in the presence of salinity (Fig. 1A). In presence of salinity few PPs were found to be newly synthesized during salt stress while synthesis of some existing proteins increased. Thus, three PPs with approximate mw of 25 KD (IV), 27 KD (III) and 49 KD (II) were specifically synthesized in shoot under salt stress. In addition, nearly four PPs were specifically synthesized in the range of mw 150-170 KD as indicated by zone I on Fig. 3A. The gels were scanned using a laser densitometer as shown in Fig 1B. The 25, 27 and 49 KD PPs in 150-170 KD synthesized specifically in presence of salt are also evident from the scan. Furthermore, the existing PPs whose synthesis increased during salt stress were those in the mw range of 36 and 20 KD as evident from scan by an increase in the peak(s) height as well as total area under the peak Fig 1B. Soluble protein profile from the shoot of germinating green gram seeds of cultivar SML-32 (sensitive) was also analysed by SDS-PAGE. It was observed that no salt stress PPs similar to those of salt tolerant cultivar was specifically synthesized in responce to salt stress. However, few PPs showed increased synthesis under salt stress in comparison to nonsaline control, in the range of 36 and 20 KD similar to those of tolerant cultivar (Data not shown).

Influence of Salinity on the Soluble Protein Profile of Root

SDS-PAGE profile of soluble proteins in root of germinating seed of salt tolerant green gram seed cultivar T-44 was investigated and compared in the absence and in the presence of salinity (Fig . 1C). In presence of salinity few PPs were specifically synthesized in root also. They had mw in the range of 27(I), 20(II) and 13(III) KD. In addition, synthesis of few existing PPs increased under salt stress. The more predominant PPs belonging to this group has mw in the range of 36, 23, 18 and 16 KD. It is noteworthy that in root most of the PP whose synthesis increased under salinity was small mw PPs in the range 23-16 KD. In contrast some high mw PPs in the range of 100 KD showed decreased synthesis under salt stress. No such decrease was found in case of shoot tissue on the contrary, they exhibited specific synthesis of some high mw PPs in this range (150-170 KD). The conclusions drawn from the gel profile were consistent with the data of scan (Fig. 1D). We also investigated the SDS-PAGE profile of soluble proteins from root of germinating seed of cultivar SML-32 (salt sensitive). It was observed that no PPs in the mw range of 27, 20 and 13 KD were specifically synthesized under salt stress, as observed in the case of salt tolerant cultivar T-44 though the PPs which exhibited increased synthesis in T-44 were also observed in SML-32 (36, 23, 18 and 16 KD) (data not shown).

Discussion

Quantitative and qualitative changes in AAs revealed increased accumulation of total FAAs as well as a group of nine individual FAAs in salt tolerant cultivar (T-44) under salt stress during plant growth. The corresponding increase in salt sensitive cultivar was negligible. The individual AAs, alanine, arginine, glutamic acid, glutamine, proline, cystein, isoleucine, asparagine and serine (a total

of nine AAs) that showed significant increase during salt stress in tolerant cultivar. Proline was unique in all that it was significant under salinity stress in salt tolerant cultivar. Similar reports of increased accumulation of total as well as a group of six individual AAs namely alanine, arginine, valine, leucine, glutamine and proline have been reported for rice plants differing in salt sensitivity under salinity stress (Dubey and Rani, 1990). Increased accumulation of proline (Misra and Gupta, 2005; Ghoulam *et al*., 2002), glycine, aspartic acid, serine, asparagine and glutamine (Ahmed *et al*., 1978), glycinebetaine (Girija *et al*., 2002) under salinity stress has been reported. Increased accumulation of asparagine, aspartic acid, glycine, serine, glutamic acid, alanine, proline under salinity stress was also observed in pigeon pea (Rao and Rao, 1981). Similar observations of high in built levels of certain AAs or combination of 3-4 AAs were markedly increased accumulation in salt tolerant cultivars have also been reported under salinity stress (Stewart *et al*., 1978). A combination of asparagine, glutamine, serine and proline is reported to be specific for salt tolerance in many higher plants (Ahmed *et al*., 1978). Increased level of arginine has been correlated with polyamine synthesis, which are involved in osmotic regulation during salinity stress (Bray *et al*., 1991). It has been suggested that some of them AAs functions as compatible cytoplasmic solutes and their increased accumulation serves as means of intracellular osmotic adjustment in order to maintain the osmotic potentials of cytoplasm in adverse conditions of salinity (Bray *et al*., 1991).

Proline accumulation is also related with salt tolerance in many higher plants (Bray *et al*., 1991; Perez-Alfocea and Larher, 1995; Lopez *et al*., 1994; Ghoulam *et al*., 2002; Girija *et al*., 2002). Higher osmolyte accumulation, especially proline seem to be related to salt tolerance in salt tolerant genotype T-44 not to be a consequence of tissue dehydration or tissue reaction to stress damage (Misra and Gupta 2005). Proline accumulation also appeared to be a reaction to salt stress damage and not a plant response associated with salt tolerance (Lacerda *et al*., 2003). Proline has been shown to protect the PEG induced precipitation of some enzymes and protein complex *in vitro* (Paleg *et al*., 1985). Such protection of proteins has been shown to be increased further in presence of a combination of 3-4 AAs along with proline. These observations suggested that the protective effect of some AAs are at least additive and are consistent with the conclusion that the compatible solutes protect protein–containing systems against the unfavorable consequences of dehydration (salt stress also has dehydration component) by increasing the tendency of the system to maintain the status quo. A similar suggestion can be made from our findings.

Analysis of the protein profiles of the shoot of cultivar T-44 on SDS-PAGE revealed that some PPs are specifically synthesized in response to salt stress (25, 27 and 49 and 150-170 KD) while synthesis of few already existing PPs (20 and 36 KD) were increased under salt stress. In root of the cultivar T-44 the PPs specifically synthesized under salt stress were 13, 20 and 27 KD while PPs whose synthesis increased during salt stress were 36, 23, 18 and 16 KD. Similar reports of specific synthesis of proteins under salt stress have been reported. Thus, a 25 KD protein was found to be specially synthesized in salt adapted cells of *Citrus sinensis*, which was found to be associated with salt tolerance (Ben-Hayyim *et al*., 1989, 1993). Increased synthesis of some 10 PPs in cells adapted to salinity has been also reported (Singh *et al*., 1985). Synthesis of a 26 KD protein under salt stress was unique in such tobacco cells which they latter called as osmotin (Singh *et al*., 1987a,b), because it was synthesized and accumulated by cell undergoing gradual osmotic adjustment to either salt or desiccation stress (Chretein *et al*., 1992; Perez-Alfocea and Larher, 1995). Chretein *et al*. (1992) reported that PPs of mw of 18, 27 and 49, synthesized in salt adapted calli of *Jojoba*, which were suggested as marker proteins for salt adaptation. Ericson and Alfinito (1984) reported increased synthesis of a 20 and 32 KD PPs in salt adapted tobacco cells. Ramgopal and Carr (1991) reported that in sugarcane suspension cells 15 proteins were induced or enhanced and other three proteins were

repressed or abolished. The most obvious change concerned a 22 KD, PI 7.5 PP, which accumulated after exposure of the *Raphanus sativus* to NaCl. A cDNA clone corresponding to the radish 22 KD PP was obtained and sequenced (Lopez *et al*., 1994).

From our study it is noteworthy, that the PPs specifically synthesized under salt stress were not common in root and shoot except for 27 KD PPs. Similarly, PPs synthesized at increased rates under salt stress were also not common in root and shoot except for 36 KD PP. In contrast to shoot, where none of the PPs exhibited decreased synthesis, root exhibited a PP in the range of 100 KD whose synthesis decreased under salt stress as reported for some PPs in tobacco cells (Singh *et al*., 1985). Polypeptides synthesized specifically under salt stress in root and shoot tissues of salt tolerant cultivar T-44 were found to be missing from those of salt sensitive cultivar SML-32 under salinity. Similar results have been reported from barley roots (Hurkman *et al*., 1989) in which 26 and 27 KD PPs synthesized in salt tolerant cultivar under salinity stress was missing from the salt sensitive cultivar in presence of salinity. Since the proteins which are specifically synthesized (in shoot as well as in root tissue) under salt stress only in cultivar T-44 and was absent in the cultivar SML-32 under the conditions of absence as well as presence of salinity, these proteins may be considered as markers for salt tolerance in salt tolerant cultivar T-44. These proteins that are synthesized either specifically or at increased rate under salt stress are suggested to have adaptive role in plants in osmotic adjustments (osmotic as well as ionic components of salt stress) (Shirata and Takagishi, 1990; Singh *et al*., 1987a,b) protecting the key cytoplasmic enzymes and protein synthesizing apparatus against adverse effects of high salt concentrations. Salt induced changes in protein in our case are predominantly, tissue specific and that no obvious similarity exist, which is consistent with the differences in the physiological functions played by the two types of tissue (root and shoot) and also with the environment around these tissues. However, the possibility that a variety of environmental stresses may lead to the production of one or more common proteins suggesting a general stress tolerance mechanism and the possibility that there may be a keyset of proteins (Harrington and Alm, 1988) is not ruled out from our findings. A number of proteins synthesized in our case are, at least, in close range, on the basis of mw, to those of proteins specifically synthesized under heat shock in tobacco cell lines, namely 15 to 20 KD (Harrington and Alm, 1988). Thus, conclusions drawn from the studies of Ben-Hayyim *et al*., (1993) that salt induced proteins are species specific and that no obvious similarities exists among them is not consistent with our findings, as a number of proteins synthesized in response to salt stress in our case are common (on the basis of their molecular weight) with those reported from other sources under salt stress cf. PPs 23,25,27, 20 KD, etc. (Ben-Hayyin *et al*., 1989) 49 KD (Chretein *et al*., 1992).

In conclusion, green gram cultivars differing in salt tolerance have in built varying levels of AAs, alanine, arginine, glutamic acid, glutamine, proline, cystein, isoleucine, asparagine and serine (a total of nine AAs) and higher levels of these AAs in germinating seed parts (root and shoot) of tolerant cultivar T-44. Increased accumulation of these AAs in tolerant cultivars T-44 under salinization can be correlated with salt tolerance ability of this green gram cultivar. These all AAs along with proteins synthesized/accumulated under salt stress should be considered together in combination as indices for salt tolerance in green gram as suggested by other workers (Dubey and Rani, 1989).

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