Journal of Stress Physiology & Biochemistry, Vol. 8 No. 3 2012, pp. 5-12 ISSN 1997-0838

ORIGINAL ARTICLE

Influence of Salinity Stress on proteomic profiles of *Cicer arietinum* L.

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Received February 19 2012

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Salinity induced reaction in growth is the consequence of modifications of several physiological process like, ion balance, water status, mineral nutrition, stomatal behaviour, photosynthesis, carbon allocation and utilization (Munns Termatt, 1986; Flowers *et al.,* 1991). Salt accumulation in soils from natural processes and irrigation adversely affects seed germination, seedling growth as well as related metabolic processes of plants. The response of plants to salinity varied with species, organs and stage of development (Munns, 2002). Plant can act in response and adapt to salt stress by altering their cellular metabolism and invoking various defence mechanisms (Bohnert 2007). However, plants subjected to salt stress showed increased levels of total free amino acids (Dubey and Pessarakli, 1995). In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses (Hare and Cress, 1997; Johnson *et al.,* 2011; Dooslin Mary *et al.,* 2009; Dooslin Mary *et al.,* 2011; Johnson *et al.,* 2011). Carceller *et al.,* (1999) found that the accumulating proline in maize cultivar showed a higher osmotic adjustment capacity than the non accumulating one. Sodium chloride stress negatively influenced

nitrate reductase activity of plants. It increased protease capacity (Muthukumarasamy *et al*., 2000. Harinasut *et al*., (1996) found that exposure of 28-day-old rice seedlings to 150 mM NaCl for 6 days induced drastic decreases in relative water contents, chlorophyll, and protein in leaves. The molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (Sanders, 2000; Zhu, 2000; Hasegawa *et al.,* 2000). With this knowledge the present study was intended to find out the salt influential protein expression of *Cicer arietinum*.

MATERIALS AND METHODS

Seeds of *Cicer arietinum* were surface sterilized with 0.1% $HgCl_2$ and 0.1% sodium lauryl sulfate solution for 3-5 min and rinsed thrice with sterile distilled water. The sterilized seeds were inoculated on to the MS medium supplemented with and without NaCl to study the influence of NaCl (0, 4%, 6%, 8% and 10%) on seedlings of *Cicer arietinum*. The seedlings were grown in the culture room for a period of 15 days. On 15th Day, the randomly collected whole plants were used as a source for protein isolation. 500 to 1000 mg of freshly harvested tissues were taken and homogenized with 3.5 ml of ice-cold 0.1M phosphate buffer (pH 7.0) in a pre-chilled pestle and mortar and centrifuged at 10,000 rpm for 10 min and the supernatant was collected and used for protein separation. The Poly acrylamide gel electrophoresis was performed by Anbalagan (1999) method. SDS – Poly Acrylamide Gel Electrophoresis was carried out at 25°C in the air conditioned room. Separation of protein was carried out at 50v till the tracking dye reaches the separating gel and at100 v thereafter for 3-5 hours or until the tracking dye had migrated to the bottom of the gel. After running the electrophoresis, the gels were carefully removed from the mold and subjected to activity staining (Anbalagan, 1999).

RESULTS

Proteomic analyses have resulted in the identification of many proteins that are inducible by salt stress including the metabolism related genes. The relative positions of the protein bands as revealed by SDS- PAGE of the *Cicer arietinum* seedlings under different stress conditions are shown in Fig 1A. Multiple regions of actively stained system were obtained for SDS-PAGE. On 15th day, a total of 79 bands were observed and their RM values ranged from 0.011 to 0.988. The seedlings treated with 4% of NaCl showed fifteen bands in fifteen different positions. MW-Rf 0.329, 0.517 and 0.764 showed their unique presence in the seedlings treated with 4% of NaCl (Table - 1). The seedlings treated with 6% of NaCl showed their uniqueness by the presence of proteins with MW-Rf 0.094 and 0.552 (Table - 1). The seedlings treated with 8% NaCl demonstrated the expression of 18 different proteins in the protein gel system, with six different specific proteins viz., 0.058, 0.235, 0.741, 0.788, 0.905 and 0.964 .When the concentration of NaCl increases more than 8% the number of bands was decreased and they showed instability in their appearance. The bands were very light and fragile. The seedlings treated with 10 % NaCl showed only thirteen proteins, of which four proteins viz., 0.282, 0.835, 0.882 and 0.988 showed their restricted occurrence. Based on the occurrence and non-occurrence of the proteins in the gel system, the protein profiles were classified in to three categories viz., salt tolerant proteins, salt inducible proteins and salt sensitive proteins. Due to the salt stress, the seedlings of *C. arietinum* elicited the following salt inducible

proteins viz., MW- Rf 0.023, 0.058, 0.094, 0.200, 0.235, 0.282, 0.294, 0.329, 0.400, 0.423, 0.435, 0.447, 0.494, 0.517, 0.552, 0.705, 0.717, 0.741, 0.764, 0.776, 0.788, 0.823, 0.835, 0.882, 0.894, 0.964 and 0.988. The following proteins MW–Rf 0.105, 0.164, 0.388, 0.600, 0.635, 0.811, 0.929 and 0.952 were expressed in the control and salt stressed seedlings, and they are called as salt tolerant proteins. The following proteins MW-Rf. 0.011, 0.047, 0.270, 0.470, 0.564 which are absent in the salt treated seedlings, and present only in control (distilled water treated seedlings) are called salt sensitive proteins.

Table 1: SDS – PAGE banding pattern of different concentration of NaCl treated Cicer arietinum seedlings on 15th Day

MW-Rf	Positions	Regions	Concentration of NaCl in %				
			0 (L_1)	4 (L_2)	6 (L_3)	8 (L_4)	10 (L_5)
0.011	PP 1^1	1	+	-	-	-	-
0.023	PP 1^2		-	+	+	+	-
0.047	PP 1^3		+	-	-	-	-
0.058	PP 1^4		-	-	-	+	-
0.094	PP 1^5		-	-	+	-	-
0.105	PP 2^1	2	+	-	-	+	+
0.164	PP 2^2		+	+	+	+	-
0.200	PP 2^3		-	-	-	+	+
0.211	PP 3^1	3	-	+	+	-	-
0.235	PP 3^2		-	-	-	+	-
0.270	PP 3^3		+	-	-	-	-
0.282	PP 3^4		-	-	-	-	+
0.294	PP 3^5		-	+	+	+	-
0.329	PP 4^1	4	-	+	-	-	-
0.341	PP 4^2		-	-	+	+	-
0.388	PP 4^3		+	+	-	-	-
0.400	PP 4^4		-	-	+	+	-
0.423	PP 5^1	5	-	-	-	+	+
0.435	PP 5^2		-	+	+	-	-
0.447	PP 5^3		-	+	+	+	+
0.470	PP 5^4		+	-	-	-	-
0.494	PP 5^5		-	+	+	+	+
0.517	PP 6^1	6	-	+	-	-	-
0.552	PP 6^2		-	-	+	-	-
0.564	PP 6^3		+	-	-	-	-
0.600	PP 6^4		+	-	+	-	-
0.635	PP 7^1	7	+	+	+	+	+
0.705	PP 8^1	8	-	+	-	+	-
0.717	PP 8^2		-	-	+	-	+
0.741	PP 8^3		-	-	-	+	-
0.764	PP 8^4		-	+	-	-	-
0.776	PP 8^5		-	-	+	-	+
0.788	PP 8^6		-	-	-	+	-
0.811	PP 9^1	9	+	+	-	-	-
0.823	PP 9^2		-	-	+	+	-
0.835	PP 9^3		-	-	-	-	+
0.882	PP 9^4		-	-	-	-	+
0.894	PP 9^5		-	+	+	-	-
0.905	PP 10^1	10	-	-	-	+	-
0.929	PP 10^2		+	-	-	-	+
0.952	PP 10^3		+	-	+	-	-
0.964	PP 10^4		-	-	-	+	-
0.988	PP 10^5		-	-	-	-	+

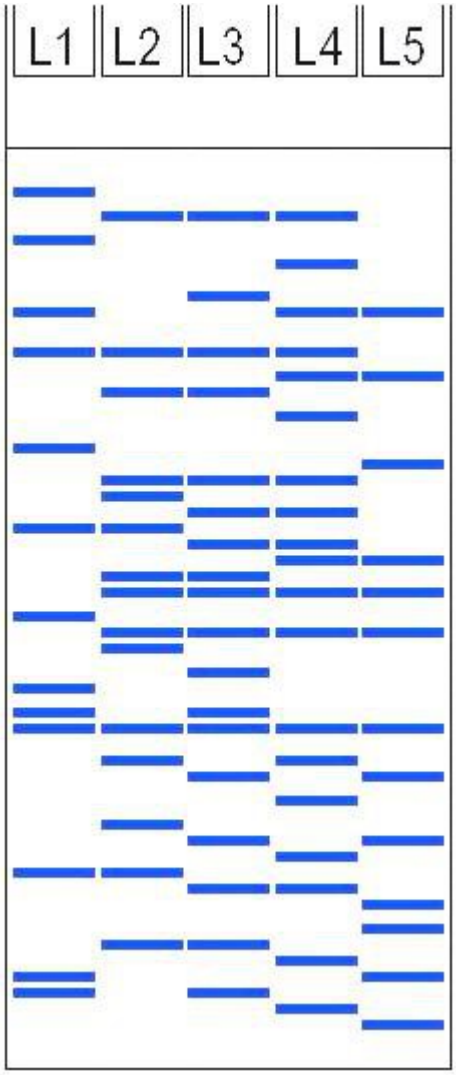

Figure 1. Zymogram of Salt Treated and Control Seedlings of *Cicer arietinum*

L_1 - Control Seedlings; L_2 – 4% of NaCl treated Seedlings C. *Arietinum;* L_3 – 6% of NaCl treated Seedlings; L_4 – 8% NaCl treated Seedlings; *L5* – 10% NaCl treated Seedlings.

DISCUSSION

Seeds of *Cicer arietinum* soaked for 24h in 1% NaCl, showed different percentage of seed germination and seedlings growth. The seeds soaked for more than 24 h showed very less percentage of germination and growth. The germinated seedlings were cultured on MS medium augmented with various percentage viz., 0, 4, 6, 8 and 10 of salt (NaCl). The seedlings showed the maximum tolerance up to 4% of NaCl. Growth of any organ is associated with an additional synthesis of proteins which are building blocks of protoplasm and are again the resultant on inter- mediatory metabolism. Proteome analyses have resulted in the identification of many proteins that are inducible by salt stress including the metabolism related genes.

The results of the present study also revealed the expression of number of proteins which are induced by salt stress. The present observation directly coincides and supplements with the previous observations. When the seedlings were

treated with above 8 % of NaCl the number of protein bands and strength of bands were decreased.

Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Boyer, 1982; Pal *et al.,* 2004). Accumulation of metabolites that act as compatible solutes is one of the probable universal responses of plants to changes in the external osmotic potential. Metabolites with osmolyte function like sugar alcohols, complex sugars, proteins, isoenzymes and charged metabolites are frequently observed in plants under unfavorable conditions (Hasegawa *et al.,* 2000; Satiropoulos, 2007).

Accumulation of organic solutes under saline conditions is an adaptive response of plants against osmotic stress (Tejera *et al*., 2004). This is in part due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu, 2001). One of the most common stress responses in plants over production of different types of compatible organic solutes viz., aminoacids, proteins, PGRs, soluble sugars and inorganic ions (Serraj and Sinclair, 2002). Salinity induces distinct protein changes in root and shoot tissues of barley (Ramagopal *et al.,* 1987). The proteins and isoenzymes profiles of many plants are affected by addition of salts. In the present study also we observed the proteins expression with reference to salt concentrations. The banding positions and regions of activity are variable based on the developments of plants and concentrations of salts supplementations. Similar to the previous observation, the banding positions of C. *arietinum* salt treated seedlings showed the variation in the banding profile and expression in the protein gel system. Some proteins and isoenzymes show their marked presence by the supplementation of salts in the media (Salt Induced Proteins), some are failed to express due to salt supplementations (salt sensitive proteins) and some showed their presence in the normal plants and salt supplemented seedlings also (Salt tolerant proteins). The results of the present study also revealed the expression of salt sensitive, salt induced and salt tolerant proteins in the C. *arietinum* salt treated seedlings. In this respect, Ebad *et al.,* (1987) found a progressive and consistent decrease in concentration of total protein of maize plants with increasing NaCl. Moreover, Dell' Aquila and Spada (1992) detected a novel expression of the 26 kDa protein (osmotin) on germinating wheat embryos under salinity conditions. In addition, Hamada (1994) proved that low concentration of NaCl stimulated soluble protein production, but higher concentrations decreased the content of soluble proteins. Similar to Hamada's observation, in the present study also we observed the decreases in content and number of proteins in the seedlings treated above 8 % NaCl.

In conclusion, the data presented here revealed that salinity induced changes in protein profiles in the seedling of *Cicer arietinum*. This study reported the molecular weights of some salt responsive proteins. It is necessary to study further the structural and functional roles of these salt stress responsive polypeptides to enhance our understanding of the salt stress responses in *Cicer arietinum*.

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