J Indian bot Soc Vol 75 (1996) 69-71

NITROGEN METABOLISM IN BLACK GRAM UNDER NaCl STRESS

S. ANIL KUMAR, M. MUTHUKUMARASAMY AND R. PANNEERSELVAM*

Division of Plant Physiology, Department of Botany, Annamalai University, Annamalai Nagar-608 002 (T.N.), India.

(Accepted February 1996)

The effect of increasing levels of NaCl stress on germination, protein, amino acids and proline content in black gram (*Vigna mungo* L. Hepper cv. Co-5) was studied. Salt stress caused a marked reduction in germination percentage and protein content with concomitant increase in the amino acids and proline content in the root, stem and leaf. The 100, 150 mM NaCl treatment reduced the protein synthesis and increased the amino acid content to a higher extent.

Key Words : *Vigna mungo,* NaCl stress, protein, amino acids, proline.

Soil salinity is well known to affect all the metabolic processes resulting in reduced crop growth and yield (Singh *et al.,* 1994). Crop production in saline areas depends upon germination, seedling emergence and subsequent growth. Blackgram is an important legume crop generally cultivated in marginal low fertile soil with or without fertilizers. Study on the metabolism of black gram during NaCl stress is scanty. Hence an attempt is made in the present investigation to find out the effect of NaCl salinity at 50, 100 and 150 mM level on germination, protein, amino acid and proline content in black gram during growth.

MATERIALS AND METHODS

Seeds of *Vigna mungo* was obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu and were surface sterilized with 0.1 per cent $HgCl_2$ for 2 minutes and thoroughly washed with deionized water to remove the $HgCl_2$. One thousand seeds were soaked in 25 x 20 cm trays lined with distilled water washed filter paper. The filter paper was moistened continuously with respective solution. This setup was maintained up to 72 hours, later no germination takes place. The germination per cents were calculated on three Days After Sowing (DAS).

Surface sterilized seeds were also sown in plastic pots (25 cm dia) filled with 2 Kg of soil mixture containing clay, sand and Farm Yard Manure (FYM) at 1:1:1 ratio. Two seeds were sown in each pot and allowed to germinate in the glass house condition. One liter of each of 60, 100 and 150 mM NaCl solution was used for treatment and the distilled water was used for control. The treatment was divided into 4 instalments and given in alternate days starting from 7th day after sowing (DAS), to minimize the salt shock. Later the plants were irrigated with equal amount of distilled water at every alternate day and 60 pots for each treatment were maintained. Samples were collected randomly on 15th, 25th, 35th and 45th day. The plants were analysed for the contents of protein, amino acid and proline in the root, stem and leaf.

Protein content was estimated following the method of Lowry *et al.* (1951). The total free amino acid was extracted and estimated following the method of Moore and Stein (1948). Free proline estimation was done according to the method of Bates *et al.* (1973). Each experiment was repeated thrice and SD was calculated.

RESULTS AND DISCUSSION

The germination percentage of black gram decreased with the increase in salinity up to 72 hours of germination. The germination per cent was 84 in control and 77, 54 and 41 per cent in 50, 100 and 150 mM NaCl treatments respectively. The 100 and 150 mM NaCl treatments lowered the germination and also delayed the germination. When seeds sown in saline environment, there was a significant decrease in the rate of germination and also delay in completion of germination which inturn fluctuated the germination percentage (Ungar, 1978). With the increase in salinity level there was a concomitant decrease in mung bean germination (Singh and Gangawar, 1984).

The protein content in the root, stem and leaf increased from 15 DAS to 25 DAS and declined later on in plants raised under control and NaCl stress (Table 1). The protein content in the roots, stem and leaves in

Received November 1995

Table 1. Effect of NaCl on the protein and amino acid contents of blackgram (Values are mean±SD of 3 samples expressed in mg/g fresh weight)

Protein/ Amino acid contents	Days after sowing (DAS)											
	Leaf				Stem				Root			
	15	25	35	45	15	25	35	45	15	25	35	45
	CONTROL											
Protein	10.20	26.08	22.49	9.38	8.82	12.49	5.88	4.66	4.79	7.53	7.09	4.59
	±0.38	±1.09	±0.89	±0.36	±0.44	±0.58	±0.17	±0.23	±0.20	±0.25	±0.23	±0.16
Amino acid	0.622	0.814	2.402	2.769	0.698	0.997	1.340	1.947	0.746	0.987	1.393	1.773
	±0.02	±0.02	±0.09	±0.10	±0.02	±0.03	±0.05	±0.07	±0.02	±0.01	±0.05	±0.07
	50 mM NaCl											
Protein	12.15	29.89	25.12	13.93	10.46	15.12	6.09	5.53	6.22	8.54	7.14	6.85
	±0.44	±1.10	±1.00	±0.48	±0.52	±0.75	±0.23	±0.26	±0.27	±0.33	±0.30	±0.22
Amino acid	2.355	3.638	4.293	4.347	0.798	1.237	1.814	2.293	1.432	1.857	1.975	2.657
	±0.07	±0.10	±0.15	±0.13	±0.03	±0.04	±0.07	±0.08	±0.05	±0.07	±0.08	±0.10
	100 mM NaCl											
Protein	11.17	23.03	19.82	8.82	7.79	10.56	5.38	4.04	3.84	6.85	5.02	3.54
	±0.43	±0.80	±0.86	±0.34	±0.33	±0.45	±0.25	±0.16	±0.14	±0.26	±0.18	±0.12
Amino acid	3.062	3.950	4.447	4.750	0.854	1.774	2.347	2.985	1.843	2.335	2.846	2.934
	±0.11	±0.13	±0.16	±0.14	±0.03	±0.06	±0.08	±0.11	±0.07	±0.08	±0.11	±0.11
	150 mM NaCl											
Protein	8.01	20.84	18.33	6.62	6.32	9.42	4.79	3.55	2.84	5.54	4.32	3.22
	±0.31	±0.87	±0.65	±0.19	±0.20	±0.33	±0.15	±0.13	±0.10	±0.19	±0.16	±0.13
Amino acid	3.774	4.675	4.891	4.947	0.903	1.984	2.631	3.146	1.994	2.684	3.093	3.257
	±0.13	±0.18	±0.17	±0.19	±0.03	±0.06	±0.10	±0.12	±0.07	±0.09	±0.12	±0.13

Table 2. Effect of NaCl on the proline content of blackgram (Values are mean±SD of 3 samples expressed in mg/g fresh weight)

NaCl Concentration in mM	Days after sowing (DAS)											
	Leaf				Stem				Root			
	15	25	35	45	15	25	35	45	15	25	35	45
Control	7.66	15.17	18.25	23.72	6.75	9.87	16.87	21.23	5.55	9.19	14.80	19.48
	±0.24	±0.48	±0.60	±0.75	±0.23	±0.34	±0.59	±0.74	±0.21	±0.34	±0.55	±0.70
50	18.72	21.02	27.36	35.03	15.78	19.87	23.66	29.48	11.68	16.51	19.84	24.08
	±0.59	±0.67	±0.87	±1.15	±0.55	±0.69	±0.82	±0.94	±0.38	±0.54	±0.71	±0.86
100	23.20	25.78	29.40	37.07	18.10	23.80	28.50	32.31	15.90	20.85	26.83	30.17
	±0.74	±0.92	±1.05	±1.29	±0.59	±0.85	±1.02	±1.13	±0.57	±0.72	±0.96	±1.08
150	27.47	29.91	32.40	39.48	23.97	28.74	32.12	38.00	19.76	26.23	31.38	35.40
	±0.96	±0.95	±1.16	±1.42	±0.88	±1.12	±1.12	±1.33	±0.71	±0.86	±1.03	±1.23

50 mM NaCl treatment was higher over control. But the higher dosage of NaCl reduced protein content in all the three sampled organs; the roots being most affected. Levitt (1972) manifested the relation between protein metabolism with respective sodium chloride stress in plants. Salinity adversely affected the protein metabolism. Protein degradation under saline environment have been reported due to decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acids and denaturation of enzymes involved in protein synthesis. The reduction in protein content may be due to decrease in protein synthesis or by accelerated proteolysis after 25th DAS.

The root, stem and leaf of NaCl treated plants possessed higher quantities of free amino acids than control (Table 1); the content being nearly identical in root and leaf but lower in stem. Ramanjulu *et al.* (1993) reported that an increase in amino acid pool in the NaCl treated mulberry plants. The magnitude of increased amino acid was found to be stress intensity and duration dependent. Reddy and Vora (1985) reported that in bajra, amino acid content increased in the leaves of the salt stressed plants. Accumulation of free amino acids were observed both in glycophytes (Chu *et al.*, 1976) and halophytes (Stewart and Lee, 1974) under saline conditions. The increase in the amino acid content in the root, stem and leaf due to NaCl stress in *Vigna mungo* coincides with observation of the above mentioned authors in different plants.

The proline content in the leaf, stem and root was very high immediately after treatment when compared to control and it increased till 45 DAS in all the parts of the treated plants. The leaf showed a higher proline accumulation than the other parts of the salt stressed plant (Table 2). Rapid accumulation of proline in the tissues of many plant species as a response to salt, drought or temperature stress was demonstrated, and the implication of proline in enzyme stabilization and osmoregulation under salt stress conditions was documented (Flowers *et al.*, 1977). The free proline content in the leaves and roots increase significantly with stress intensity and duration (Ramanjulu *et al.*, 1993). Application of salinity stress to *Pisum sativum and Tamarix tetragyna* resulted in an elevation of both free and protein bound proline content (Bar-Nun and Poljakoff-Mayer, 1977).

The above physiological parameters studied the salinity at 50 mM NaCl level does not have an adverse effect on the plant growth and biochemical content of *Vigna mungo*. The 100 and 150 mM NaCl treatments have reduced the germination and protein synthesis to a larger extent. The increase in amino acid and proline may be attributed to the stress alleviating mechanism and they may be taken as stress indicating parameters.

REFERENCES

Bar-Nun N & Poljakoff-Mayber 1977 Salinity stresses and the content of proline in roots of *Pisum sativum and Tamarix tetragyna*. *Ann Bot* **41** 173-179.

Bates L S, R P Waldren & I D Teare 1973 Rapid determination of free proline for water stress studies. *Plant and Soil* **39** 205-207.

Chu T M, D Aspinall & L G Paleg 1976 Stress metabolism. VII. Specific ion effects on proline accumulation in barley. *Aust J Plant Physiol* **3** 503-512.

Flowers T J, P F Troke & A R Yeo 1977 The mechanism of salt tolerance in halophytes. *Ann Rev Plant Physiol* **28** 89-121.

Levitt J 1972 *Response of plants to environmental stress*, Monograph, Academic Press, New York.

Lowry O H, N J Rosebrough, A L Farr & R J Randall 1951 Protein measurement with Folin-Phenol reagent. *J Biol Chem* **193** 265-275.

Moore S & W H Stein 1948 Photometric ninhydrin method for use in the chromatography of amino acids. *J Biol Chem* **176** 367-388.

Ramanjulu S, K Veeranjaneyulu & C Sudhakar 1993 Physiological changes induced by NaCl in mulberry var. Mysore local. *Indian J Plant Physiol* **36** 273-275.

Reddy M P & A B Vora 1985 Effect of salinity of protein metabolism in bajra *(Pennisetum typhoides)* leaves. *Indian J Plant Physiol* **28** 190-195.

Singh B A & P K Gangawar 1984 Relative salt tolerance in some cultivars of mung bean during early seedling growth. *Comp Physiol Ecol* **9** 421-426.

Singh S P, B B Singh & Maharaj Singh 1994 Effect of Kinetin on chlorophyll, nitrogen and proline in mungbean *(Vigna radiata)* under saline conditions. *Indian J Plant Physiol* **37** 37-39.

Stewart G R & J A Lee 1974 The role of proline accumulation in halophytes. *Planta* **120** 279-289.

Ungar I A 1978 Halophyte seed germination. *Bot Rev* **44** 233-264.