Indian J. Plant Physiol., Vol. 9, No. 1, (N.S.) pp. 21-28 (Jan.-March, 2004)

VARIABILITY IN RESPONSE OF CHICKPEA (*CICER ARIETINUM* L.) CULTIVARS TO SALT STRESS IN GERMINATION AND EARLY GROWTH OF THE SEEDLINGS

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Received on 31 Aug., 2002, Revised on 20 Jan., 2004

SUMMARY

Eleven cultivars of chickpea (*Cicer arietinum* L.) were tested in order to screen their relative salt tolerance (at EC 2, 4, 6 and 8 dSm^{-1}) at germination and early seedling growth. Significant variations were observed amongst the various cultivars even at the germination stage and the cultivars could be distinctly classified as salt tolerant (Pusa 256, Pusa 329, Pusa 362 and BGD 87), salt sensitive (BG 1006, BG 1008, BG 1009 and BGD 86) and moderately salt tolerant (BG 372, BG 1044 and BGD 94). Germination relative index (GRI) was significantly reduced in the sensitive cultivars, while the tolerant cultivars did not show significant differences in their germinability under different levels of salt stress (2, 4, 6 and 8 dSm^{-1} EC). Similar effects were observed with regard to the early seedling growth measured as root/shoot length as well as their dry weights. The seedlings also showed deleterious effects in the quantity of nitrogen, proteins as well as total chlorophyll at 12, 19 and 26 days after sowing. The effects of salt stress became more pronounced at 26 DAS. On the other hand, a gradual and progressive accumulation of total soluble sugars was observed in all the cutivars under study. However, the quantum of accumulation was maximum in the tolerant cultivars and minimum in the sensitive ones.

***Key words*: Chickpea, cultivars, germination, salt, stress.**

INTRODUCTION

Environmental stresses limit crop yields to about 25 per cent of their potential yield (Boyer 1982). Most common stress in arid and semi-arid zones is soil salinization. With increased irrigation facilitites and poor water management practices, the problem of salinity is intensifying day by day. Global estimates indicate that about 7 percent of world's total land area has become salt affected and has lower yield due to excessive salts in the milieu (Szabolcs 1994).

Legumes generally are sensitive towards saline conditions (Mass and Hoffman 1977). Salinity exercises depressive effects on germination as well as almost all the physiological functions and energy-generating biochemical processes in legumes (Ferri *et al.* 2000, Garg 2002). Soussi *et al.* (1999) and Rao *et. al.* (2002) have screened the salt tolerant lines amongst the arable species of family fabaceae and attempted to correlate their resistance with different components of metabolism. However, no consistent and specific correlations were identifiable. The present investigation was carried out on

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the effects of varying levels of salt stress on the relative response of eleven genotypes of chickpea (*Cicer arietinum* L.) in order to identify the genetic variability in terms of germination, seedling growth and key metabolites responsible for salt sensitivity or tolerance.

MATERIALS AND METHODS

An investigation was carried out with eleven cultivars of chickpea (*Cicer arietinum* L.) namely Pusa 256, Pusa 329, Pusa 362, BG 372, BG 1006, BG 1008, BG 1009, BG 1044, BGD 86, BGD 87 and BGD 94 belonging to family fabaceae. Circular earthen pots (30 x 25 x 25 cms) were lined with polythene bags and each pot was filled with a mixture of thoroughly powdered soil, sand and farm-yard manure in the proportion of 2 : 2: 1 by volume. The seeds of eleven most recommended and released varieties of chickpea (obtained from Pulse Laboratory, Division of Genetics, I.A.R.I., New Delhi) were inoculated with specific strain of Rhizobium F 75 and were sown at the rate of 12 seeds per pot. The pots were divided into various lots and treated with saline solutions (prepared from a mixture of NaCl, $CaCl_2$ and Na_2SO_4) of various EC i.e. 2, 4, 6 and 8 dSm^{-1}. The soils were supplemented with these salt solutions on three consecutive days before sowing in order to get the required salinity level. The desired salinity levels were maintained through out the growing period of the crop by fortification with saline solutions at regular weekly intervals. The control sets of pots were irrigated with tap water only. The data on germination was taken at 6, 8 and 10 days after sowing (DAS), and the germination relative index (GRI) was calculated as per the following equation :

GRI = (S) X_n (k–n) where X_n= number of seeds germinated on n^{th} day, k = number of counts and n = number of days. Three seedlings from each treatment were selected for sampling at weekly intervals (12, 19 and 26 DAS) and analysed for the following biochemical estimations in 3 replicates.

Nitrogen content was estimated by colorimetric method of Lindner (1944) using the digestion mixture of concentrated sulphuric acid and perchloric acid and nesselerisation. Protein content was determined by protein - dye binding with Coomasie Brilliant Blue (Bradford 1976) and was compared with graded concentration of bovine serum albumin as standard. Chlorophyll content of the first fully-grown leaf was estimated by the method of Hiscox and Israelstam (1979). Total soluble sugars were estimated by the method of Yemm and Willis (1954) using anthrone reagent. Glucose was used as standard.

RESULTS AND DISCUSSION

The results categorized the cultivars into three main groups namely salt tolerant (Pusa 256, Pusa 329, Pusa 362, BGD 87), moderately sensitive (BG 372, BG 1044, BGD 94) and salt sensitive (BG 1006, BG 1008, BG 1009 and BGD 86). However, results for only two genotypes from each category are being presented and discussed in detail. As apparent from the data (Fig. 1) increasing salinity levels adversely affected the germination relative index (GRI), in all the cultivars. The GRI of various cultivars showed little variation in the control series. Salinity level of 2 dSm^{-1} was almost non-significant in all the cultivars. Salinity levels 4, 6 and 8 dSm^{-1} were increasingly deleterious in cultivar BG 1008 and BGD 86, 10 DAS. However, cultivars BG 372, BG 1044 showed moderate negative effects, while in cultivars Pusa 256, Pusa 329 no significant decrease was observed even under higher salinity levels of 6 and 8 dSm^{-1} respectively.

The data on the root and shoot length of different cultivars are presented in Table 1a and 1b. Although, the effect of the treatments was statistically significant at 12 DAS, it became more prounounced with time. Root and shoot length showed maximum reduction in cultivars BG 1008 and BGD 86 under all the salinity levels. However, Pusa 256 and Pusa 329, showed relatively less reduction in their lengths under all the salinity levels. Similar trend was observed with regard to the dry weights of root and

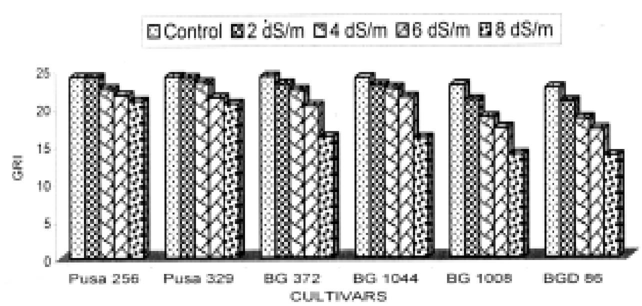

Fig. 1. Effect of different levels of salinity on the germination relative index (GRI) in different cultivars of chickpea 10 DAS. LSD (P=0.05) due to age 1.98, treatment 2.71, interaction 4.43

Table 1a. Effect of different levels of salinity on the root length (cm) in different cultivars of chickpea. Figures in parenthesis represent per cent decrease (-) over control.

Soil salinity (dSm^{-1})	Days after sowing	Pusa 256	Pusa 329	BG 372	BG 1044	BG 1008	BGD 86
Control (0.6)	12	9.5	10.0	10.3	8.5	5.5	5.3
	19	10.5	10.5	11.2	10.5	7.5	6.4
	26	12.0	11.5	12.4	11.6	8.4	7.5
2	12	9.4 (0.2)	9.9 (0.5)	10.1 (1.9)	8.32 (2.1)	5.19 (5.5)	4.99 (5.70)
	19	10.3 (1.3)	10.3 (1.4)	10.8 (2.9)	10.1 (3.4)	6.9 (7.9)	5.90 (7.8)
	26	11.7 (1.7)	11.27 (2.0)	11.8 (4.1)	11.1 (4.3)	7.67 (8.6)	6.84 (8.7)
4	12	9.2 (2.9)	9.7 (3.1)	9.7 (5.3)	8.0 (5.5)	4.91 (10.6)	4.72 (10.9)
	19	9.9 (5.1)	9.9 (4.9)	10.3 (7.2)	9.75 (7.1)	6.6 (11.7)	5.6 (11.8)
	26	11.0 (7.7)	10.5 (8.1)	11.2 (9.5)	10.4 (9.8)	7.1 (14.8)	6.3 (15.0)
6	12	8.6 (8.9)	9.0 (9.1)	9.2 (10.5)	7.5 (10.8)	4.4 (19.7)	4.2 (19.1)
	19	9.2 (11.9)	9.3 (11.4)	9.4 (15.4)	8.8 (15.6)	5.8 (22.3)	4.9 (23.0)
	26	10.3 (14.0)	9.7 (14.9)	9.9 (19.5)	9.3 (19.8)	5.6 (33.0)	4.9 (34.0)
8	12	7.8 (17.0)	8.2 (17.9)	7.8 (24.0)	6.5 (23.0)	3.0 (44.0)	2.9 (43.9)
	19	1.5 (21.0)	8.3 (20.3)	7.9 (29.1)	7.3 (30.0)	4.0 (45.9)	3.3 (47.0)
	26	8.2 (31.7)	7.8 (32.7)	7.9 (35.7)	7.3 (36.7)	4.1 (50.2)	3.6 (51.3)

LSD (P = 0.05) due to age 1.04, treatment 2.61, interaction 4.68

shoot (Table 2a and 2b). Cultivars BG 1008 and BGD 86 showed marked negative effects on dry weight with increasing salinity levels. Varieties Pusa 256 and Pusa 329 showed relatively less decrease in dry weight and showed higher salt tolerance.

The data on the nitrogen content (Fig. 2) revealed a sequential decrease under each salt treatment, in all the cultivars, and the decline became more with increasing salts in the medium as well as with time. While the lowest salinity level of 2 dSm^{-1} did not significantly alter the nitrogen content in all the cultivars, the higher levels of 4, 6 and 8 dSm^{-1} caused a 7.1, 14.3, 30.1 per cent decrease over control respectively in cultivar Pusa 256. The same treatments led to significnatly higher decline of 14.4, 32.3, 52.4 percent under 4, 6 and 8 dSm^{-1} respectively in cultivar BG 1008.

Salt treatments also reduced the total chlorophyll content in all the cultivars and the effects became more pronounced with time (Fig. 3). BG 1008 and BGD 86 showed more decrease in total chlorophyll under all the

Table 1b. Effect of different levels of salinity on the shoot length (cm) in different cultivars of chickpea. Figures in parenthesis represent per cent decrease (-) over control.

Soil salinity (dSm^{-1})	Days after sowing	Pusa 256	Pusa 329	BG 372	BG 1044	BG 1008	BGD 86
Control (0.6)	12	10.5	12	12.5	9.0	9.0	9.0
	19	16.5	16.0	14.0	13.5	12.5	13.0
	26	19.5	18.0	16.0	15.5	15.4	17.4
2	12	10.4 (0.3)	11.9 (0.6)	12.3 (1.3)	8.8 (1.9)	8.5 (5.4)	8.5 (5.2)
	19	16.2 (1.4)	15.8 (1.2)	13.6 (2.7)	13.0 (3.1)	11.5 (7.7)	11.9 (7.9)
	26	18.9 (2.6)	17.4 (2.9)	15.2 (4.5)	14.7 (4.7)	14.02 (8.9)	15.8 (8.8)
4	12	10.1 (3.4)	11.5 (3.6)	11.8 (5.6)	8.4 (5.7)	8.0 (10.9)	8.0 (10.5)
	19	15.5 (5.7)	15.1 (5.4)	12.9 (7.2)	12.5 (7.1)	10.9 (12.3)	11.4 (11.9)
	26	17.9 (7.9)	16.5 (7.8)	14.4 (9.8)	14.0 (9.6)	13.1 (14.8)	14.8 (14.9)
6	12	9.5 (8.6)	10.9 (9.1)	10.91 (12.7)	7.8 (12.8)	7.2 (19.0)	7.24 (19.5)
	19	14.5 (12.0)	13.9 (12.6)	11.8 (15.7)	11.3 (15.6)	9.6 (23.0)	9.9 (23.5)
	26	16.5 (15.0)	15.2 (15.1)	12.8 (19.8)	12.4 (19.7)	10.4 (32.0)	11.7 (32.4)
8	12	8.70 (17.1)	9.7 (19.1)	9.42 (24.6)	6.79 (24.5)	5.2 (42.2)	5.0 (43.5)
	19	12.8 (22.1)	12.3 (23.0)	9.9 (29.1)	9.4 (29.9)	6.6 (46.5)	7.04 (45.8)
	26	13.3 (31.7)	11.9 (33.6)	9.7 (39.1)	9.6 (37.8)	7.7 (49.8)	8.6 (50.4)

LSD (P = 0.05) due to age 2.01, treatment 2.75, interaction 4.35

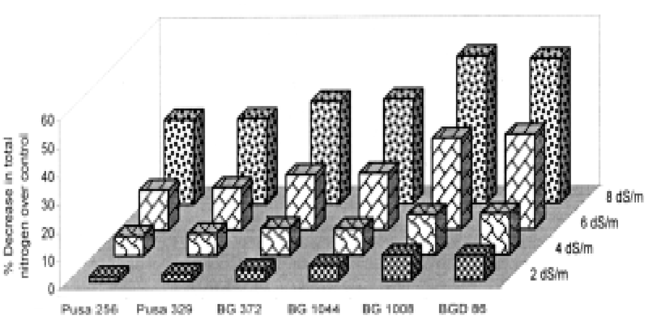

Fig. 2. Effect of different levels of salinity on the per cent decrease in total nitrogen content in different cultivars of chickpea 26 DAS. LSD (P=0.05) due to age 2.46, treatment 3.89, interaction 5.54

salinity levels, thereby showing variations almost parallel to nitrogen content. Cultivars Pusa 256 and Pusa 329, which showed less decline in nitrogen content also showed a less decrease in total chlorophyll content under 4, 6 and 8 dSm^{-1}. Salinity level of 8 dSm^{-1} was consistently more detrimental to total chlorophyll.

Protein content (Fig. 4) varied with the type of cultivar. Protein degradation was higher in BG 1008 and BGD 86 and lower in Pusa 256 and Pusa 329. The extent of decrease in total protein was significantly higher in the

Table 2a. Effect of different levels of salinity on the root dry weight (mg) in different cultivars of chickpea. Figures in parenthesis represent per cent decrease (-) over control.

Soil salinity (dSm^{-1})	Days after sowing	Pusa 256	Pusa 329	BG 372	BG 1044	BG 1008	BGD 86
Control (0.6)	12	26.3	26.4	26.1	25.8	21.6	20.7
	19	49.1	48.4	42.4	41.3	45.6	41.1
	26	85.0	84.2	75.5	74.3	74.8	67.3
2	12	26.11 (0.7)	25.9 (1.6)	25.2 (3.4)	24.9 (3.2)	19.5 (9.7)	18.6 (9.9)
	19	48.4 (1.3)	46.8 (3.3)	39.3 (7.1)	38.1 (7.7)	40.3 (11.6)	36.3 (11.5)
	26	83.1 (3.7)	79.4 (4.8)	69.2 (8.3)	67.9 (8.5)	66.1 (14.3)	57.4 (14.7)
4	12	24.4 (7.2)	24.4 (7.5)	22.4 (13.8)	21.6 (16.2)	16.9 (21.7)	15.8 (23.2)
	19	45.0 (8.3)	44.3 (8.4)	34.6 (18.3)	33.5 (18.8)	33.8 (25.7)	30.4 (25.9)
	26	76.5 (9.9)	74.3 (9.6)	61.6 (21.2)	57.7 (22.3)	53.3 (28.7)	47.4 (29.5)
6	12	23.5 (10.4)	23.4 (11.1)	19.1 (26.8)	19.7 (26.2)	14.1 (34.7)	12.4 (39.9)
	19	41.6 (14.7)	40.8 (15.5)	28.7 (32.1)	27.3 (33.8)	25.1 (44.8)	22.5 (45.2)
	26	69.5 (18.2)	65.6 (20.1)	43.7 (42.1)	42.8 (42.3)	33.4 (55.3)	30.4 (52.8)
8	12	20.1 (23.5)	19.7 (25.1)	12.7 (51.2)	12.9 (50.0)	8.2 (62.0)	8.1 (60.5)
	19	35.1 (28.4)	34.8 (27.9)	17.5 (58.6)	16.8 (59.1)	13.4 (70.4)	11.5 (71.8)
	26	58.2 (31.5)	55.9 (33.6)	29.2 (61.3)	26.5 (64.2)	16.0 (78.5)	13.6 (79.7)

LSD (P = 0.05) due to age 2.03, treatment 3.71, interaction 4.60

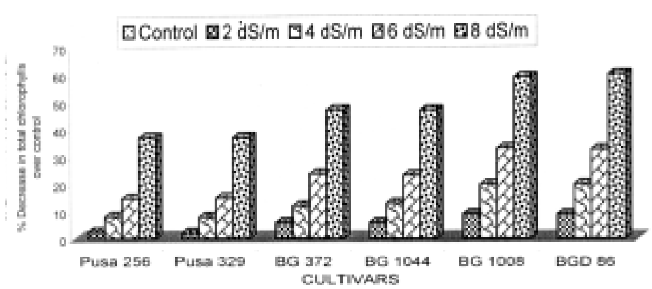

Fig. 3. Effect of different levels of salinity on the per cent decrease in chlorophyll content in different cultivars of chickpea 26 DAS. LSD (P=0.05) due to age 3.69, treatment 4.25, interaction 5.62

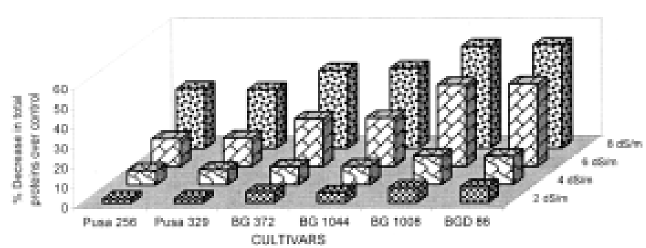

Fig. 4. Effect of different levels of salinity on the per cent decrease in total proteins in different cultivars of chickpea 26 DAS. LSD (P=0.05) due to age 1.99, treatment 3.98, interaction 5.01)

Table 2b. Effect of different levels of salinity on the shoot dry weight (mg) in different cultivars of chickpea. Figures in parenthesis represent per cent decrease (-) over control.

Soil salinity (dSm^{-1})	Days after sowing	Pusa 256	Pusa 329	BG 372	BG 1044	BG 1008	BGD 86
Control (0.6)	12	79.3	71.4	72.4	73.9	73.5	71.7
	19	126.0	122.1	128.1	125.3	128.3	125.2
	26	176.4	177.5	173.2	174.5	175.3	173.2
2	12	78.9 (0.4)	70.54 (1.2)	70.51 (2.6)	71.83 (2.8)	67.69 (7.9)	65.89 (8.1)
	19	125.11 (0.7)	120.3 (1.4)	122.84 (4.1)	119.66 (4.5)	115.59 (9.9)	112.05 (10.5)
	26	172.6 (2.1)	172.7 (2.7)	160.9 (7.1)	160.7 (7.9)	153.5 (12.4)	149.1 (13.9)
4	12	74.4 (6.1)	66.8 (6.4)	64.21 (11.3)	65.1 (11.9)	58.8 (20.0)	55.42 (22.0)
	19	115.9 (8.0)	113.5 (7.0)	103.3 (19.3)	101.99 (18.6)	97.37 (24.1)	94.02 (24.9)
	26	160.5 (9.0)	161.7 (8.9)	139.7 (19.3)	139.94 (19.8)	132.70 (24.3)	128.8 (25.6)
6	12	71.05 (10.4)	63.83 (10.6)	53.35 (26.3)	55.35 (25.1)	47.70 (35.1)	44.88 (37.4)
	19	108.9 (13.5)	105.25 (13.8)	88.97 (32.1)	83.82 (33.1)	74.28 (42.1)	70.8 (43.4)
	26	147.29 (16.5)	146.97 (17.2)	101.49 (41.4)	104.7 (40.0)	87.47 (50.1)	82.44 (52.4)
8	12	65.02 (18.0)	58.26 (18.4)	37.93 (47.6)	37.98 (48.6)	25.87 (64.8)	25.38 (64.6)
	19	99.91 (20.7)	94.38 (22.7)	54.57 (57.4)	51.87 (58.6)	34.38 (73.2)	32.42 (74.1)
	26	131.24 (25.6)	129.39 (27.1)	60.44 (65.1)	58.63 (66.4)	43.12 (75.4)	41.74 (75.9)

LSD (P = 0.05) due to age 2.01, treatment 3.50, interaction 4.71

susceptible varieties, and much less in the relatively resistant ones.

While a reduction in nitrogen, chlorophyll and protein content was observable in all the cultivars, there was a progressive and gradual accumulation of total soluble sugars (Fig. 5) with increasing salinity treatments in all the cultivars. Cultivars Pusa 256 and Pusa 329, which showed less reduction in nitrogen, chlorophyll and protein contents, accumlated higher amount of total soluble sugars. On the ohter hand, cultivars BG 1008 and BGD 86, which showed more reduction in the seedling growth as well as the levels of nitrogen, chlorophyll and protein contents showed less accumulation of total soluble sugars, thereby indicating that accumulation of total soluble sugars served as an index of osmotic adjustment by the plant.

The investigation revealed variations in relation to germination and early seedling growth amongst the different cultivars. The relative salt tolerance of a cultivar seems to be associated with its metabolic profile. Salinity is known to decrease total nitrogen content in both shoots as well as roots in legume species (Cordovilla *et al*. 1999). Sheokand *et al*. (1995) and Soussi *et al*. (1999)

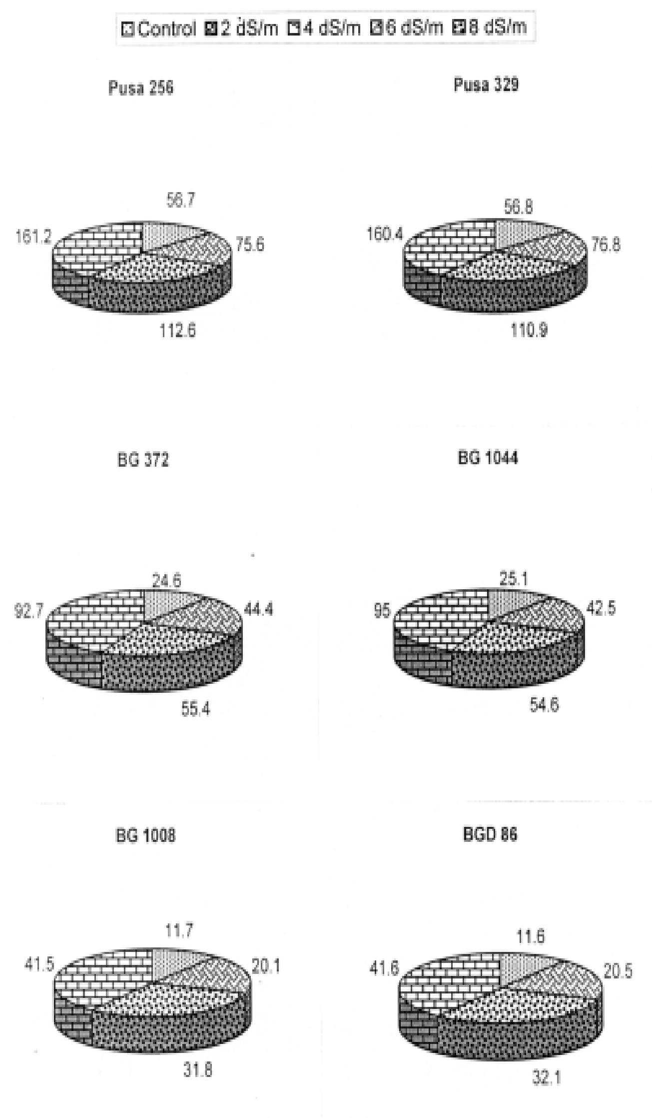

Fig. 5. Effect of different levels of salinity on per cent increase in total soluble sugars in different cultivars of chickpea 26 DAS. LSD (P=0.05) due to age 2.61, treatment 3.91, interaction 4.75

reported a significant decrease in the chlorophyll content under salinity and accumulation of total soluble sugars, probably because of increase in the activity of hydrolytic enzymes involved in sugar breakdown. Tramontano *et al*. (1997) have reported a decrease in the total protein content with a concomitant accumulation in the amino acid content. There is also an increase in proteolytic activity under various abiotic stresses resulting in accumlation of free amino acids and decrease in protein content. The tolerant cultivars showed comparatively lesser decrease in nitrogen, chlorophyll, protein and higher levels of sugars, compared to susceptible cultivars.

ACKNOWLEDGEMENTS

The financial support of CSIR, New Delhi is gratefully acknowledged.

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