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Short communication. Nitrate reductase activity in roots and leaves of chickpea cultivars under salt stress

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Abstract

Four chickpea cultivars (CSG 9651, BG 267, CSG 8962 and DCP 92-3) were grown to maturity under salinity conditions of 0, 4, 6 and 8 dS m^{-1}. Nitrate reductase activity decreased progressively with increasing salinity, although variation was seen between the cultivars. Cultivar CSG 9651, a kabuli or Mediterranean type, appeared to be the best adapted to saline stress, maintaining growth and showing efficient nitrate reduction under these conditions.

Additional key words: *Cicer arietinum,* nitrogen assimilation, salinity.

Resumen

Nota corta. Actividad nitrato reductasa en raíces y hojas de cultivares de garbanzo bajo estrés salino

Se cultivaron cuatro cultivares de garbanzo (CSG 9651, BG 267, CSG 8962, DCP 92-3) con diferentes niveles de salinidad (0, 4, 6 y 8 dS m^{-1}) hasta la madurez. Se observó una disminución progresiva de la actividad nitrato reductasa al aumentar los niveles de salinidad, aunque se apreció variabilidad dentro de las variedades. Las plantas del cultivar CSG 9651, de tipo kabuli o mediterráneo, mostraron un mayor crecimiento y una eficiente reducción de nitratos con la salinidad, lo que indica una mejor adaptación al estrés salino.

Palabras clave adicionales: asimilación de nitrógeno, *Cicer arietinum,* salinidad.

Nitrate reductase (NR, E.C. 1.6.6.1) mediates the reduction of nitrate to nitrite, which is regarded as a rate limiting step in plant growth and development (Solmonson and Barber, 1990). Nitrate reductase activity (NRA) provides a good estimate of the nitrogen status of plants and is correlated with growth and plant yield (Srivastava, 1980). Soil salinity is one of the major environmental stresses for cultivation of crops. The continuous deposition of salts in soils affects plant growth, nutrient uptake and metabolism due to osmotic stress, and causes ion toxicity and nutritional imbalances (Dubey, 1994). The inhibitory effect of salinity on NRA is known in many crops (Katiyar and Dubey, 1992; Khan, 1996; Garg *et al*., 1997). Chickpea (*Cicer arietinum* L.) is one of the most important legume crops for human nutrition over large areas of the world. In addition it is widely used as a fodder and green manure. Cultivars grown in India are either native (desi) or Mediterranean (kabuli) types (Van der Maesen, 1987). Like many other legumes, however, chickpea is highly sensitive to salinity (Ashraf and Waheed, 1993). There is very little information on the differences between desi and kabuli cultivars in terms of nitrogen assimilation. The aim of the present study was to determine how salinity affects NRA in leaf and root tissues in chickpea cultivars differing in salt tolerance.

Seeds of chickpea cultivars DCP 92-3, CSG 8962 (desi), BG 267 and CSG 9651 (kabuli) were selected after preliminary investigations into 10 cultivars obtained from the Central Soil Salinity Research Institute (CSSRI), Karnal, India. Seeds were inoculated with the salt tolerant *Mesorhizobium ciceri* strain F: 75 procured from the Indian Agricultural Research Institute (IARI), New Delhi. The seeds were surface sterilized with 30% (w v^{-1}) mercuric chloride for 2 min, washed with sterile water, and then germinated in earthenware pots lined with polythene bags. Plants were subjected to saline stress by adding a salt solution prepared with NaCl, Na_2SO_4 and $CaCl_2$ in the ratio of 4:1:1 respectively. The electrical conductivity (EC) of the different salinity levels (0, 4, 6, 8 dS m^{-1}) was adjusted using an EC meter. Three plants of uniform

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size were maintained in each pot in the greenhouse. Two pots with three plants each were sampled per treatment at 40, 70, 100 days after sowing.

Shoot and root dry mass was taken after drying the samples in an electric oven at 100°C for 10 min, and then at 70°C until a constant weight was reached.

NRA was assayed in leaf and root tissue according to Nair and Abrol (1977). The absorbance of the developed colour was read at 540 nm and the enzyme activity expressed as μmol NO_2 per gram of fresh weight per hour.

Irrespective of varietal tolerance to salinity, dry matter accumulation in the shoots and roots of all the four cultivars decreased in the saline treatments (Tables 1 and 2). The greater dry mass reduction seen in DCP 92-3 and BG 267 showed these cultivars to be more sensitive to salinity than CSG 9651 and CSG 8962. Root growth was more suppressed than shoot growth. This decline in dry mass under salinity conditions might be due to the inhibition of food hydrolysis or of translocation to the growing axis.

The NRA in the leaves and roots of all the chickpea cultivars decreased in response to salt stress at all stages of growth (Tables 3 and 4). However, peak NRA was observed during the second growth

Table 1. Effect of different levels of salinity on the dry weights of shoots (g per plant) in the desi and kabuli cultivars of chickpea. Figures in parenthesis represent percent decrease (–) over control

Days after sowing	Electrical conductivity (dSm^{-1})[a]			
	Control	4	6	8
CSG 9651				
40	0.350 ± 0.002	0.331 ± 0.004 (5.4)	0.295 ± 0.005 (15.7)	0.275 ± 0.005 (21.4)
70	0.477 ± 0.003	0.446 ± 0.005 (6.9)	0.395 ± 0.004 (17.1)	0.365 ± 0.004 (23.4)
100	0.515 ± 0.005	0.473 ± 0.003 (8.1)	0.411 ± 0.002 (20.1)	0.378 ± 0.005 (26.6)
BG 267				
40	0.178 ± 0.001	0.148 ± 0.005 (16.8)	0.141 ± 0.002 (20.7)	0.108 ± 0.004 (39.3)
70	0.374 ± 0.004	0.251 ± 0.001 (32.8)	0.239 ± 0.003 (36.0)	0.165 ± 0.005 (55.8)
100	0.421 ± 0.001	0.279 ± 0.004 (33.7)	0.255 ± 0.004 (39.4)	0.175 ± 0.004 (58.4)
CSG 8962				
40	0.168 ± 0.005	0.146 ± 0.006 (13.0)	0.136 ± 0.008 (19.0)	0.125 ± 0.002 (25.5)
70	0.316 ± 0.006	0.248 ± 0.002 (21.5)	0.228 ± 0.001 (27.8)	0.209 ± 0.002 (33.8)
100	0.340 ± 0.004	0.263 ± 0.003 (25.5)	0.237 ± 0.005 (30.2)	0.216 ± 0.005 (36.4)
DCP 92-3				
40	0.175 ± 0.002	0.111 ± 0.001 (36.5)	0.095 ± 0.005 (45.7)	0.073 ± 0.003 (58.3)
70	0.289 ± 0.003	0.175 ± 0.005 (39.4)	0.135 ± 0.003 (53.2)	0.110 ± 0.004 (61.9)
100	0.337 ± 0.002	0.200 ± 0.002 (40.6)	0.150 ± 0.002 (55.4)	0.115 ± 0.005 (65.8)

[a] Values are means ± SE of six replicates.

Table 2. Effect of different levels of salinity on the dry weight of roots (g per plant) in the desi and kabuli chickpea cultivars. Figures in parentheses represent percent decrease (–) over control

Days after sowing	Electrical conductivity (dSm^{-1})[a]			
	Control	4	6	8
CSG 9651				
40	1.000 ± 0.003	0.965 ± 0.005 (3.5)	0.932 ± 0.002 (6.8)	0.898 ± 0.004 (10.2)
70	1.944 ± 0.004	1.866 ± 0.006 (4.0)	1.773 ± 0.003 (8.7)	1.688 ± 0.005 (13.1)
100	2.561 ± 0.001	2.361 ± 0.001 (6.7)	2.221 ± 0.001 (13.2)	2.112 ± 0.002 (17.5)
BG 267				
40	0.885 ± 0.005	0.763 ± 0.003 (13.7)	0.732 ± 0.002 (16.2)	0.672 ± 0.002 (24.3)
70	1.685 ± 0.004	1.334 ± 0.004 (20.8)	1.272 ± 0.002 (24.5)	1.145 ± 0.004 (33.0)
100	2.300 ± 0.002	1.784 ± 0.002 (22.2)	1.685 ± 0.005 (26.5)	1.476 ± 0.005 (35.8)
CSG 8962				
40	0.571 ± 0.001	0.538 ± 0.005 (5.7)	0.521 ± 0.001 (8.7)	0.475 ± 0.005 (16.8)
70	1.572 ± 0.004	1.348 ± 0.006 (14.6)	1.274 ± 0.004 (19.3)	1.215 ± 0.006 (23.0)
100	2.182 ± 0.002	1.852 ± 0.002 (15.1)	1.678 ± 0.005 (23.0)	1.587 ± 0.005 (27.2)
DCP 92-3				
40	0.517 ± 0.003	0.411 ± 0.001 (20.5)	0.382 ± 0.002 (26.1)	0.348 ± 0.004 (32.6)
70	1.402 ± 0.002	1.041 ± 0.001 (25.7)	0.962 ± 0.003 (31.3)	0.854 ± 0.004 (39.0)
100	1.922 ± 0.002	1.389 ± 0.004 (27.7)	1.232 ± 0.002 (35.9)	1.047 ± 0.006 (45.5)

[a] Values are means ± SE of six replicates.

stage, i.e., at 70 days after sowing. The decreasing trend in NRA was observed in both plant parts, though to a lesser extent in the roots. CSG 9651 and CSG 8962 showed higher NRA compared to BG 267 and DCP 92-3. These observations are in agreement with the findings of other authors who indicate a similar inhibition of NRA due to salinity in other crops (Goula *et al*., 1994; Khan *et al*., 1995). This decline in NRA under conditions of salinity may be due to enhanced degradation of nitrogen reductase, the inhibition of NRA due to salt ions, or a reduced rate of enzyme synthesis due to the salinity. The reduction of NRA in both leaves and roots was proportional to the reduction in root and shoot dry mass accumulation. CSG 9651 (kabuli) was better adapted to saline stress, showing less reduction in growth and efficient nitrate reduction under these conditions. The other kabuli cultivar, BG 267, also maintained higher NRA despite a significant decline in dry mass in conditions of salinity. DCP 92-3 proved to be the most salt-sensitive in terms of nitrate reductase function. These results might be useful in attempts to screen and select chickpea germplasm tolerant to salt stress.

Table 3. Effect of different levels of salinity on nitrate reductase (NR) activity (μmol NO_2/g FW/h) in the leaves of desi and kabuli chickpea cultivars. Figures in parentheses represent percentage decrease (–) over control

Days after sowing	Electrical conductivity (dSm^{-1})[a]			
	Control	**4**	**6**	**8**
CSG 9651				
40	0.372± 0.003	0.350± 0.002 (–5.9)	0.342± 0.003 (–8.0)	0.322 ± 0.004 (–13.4)
70	0.427 ± 0.002	0.395 ± 0.001 (–7.4)	0.378 ± 0.002 (–11.4)	0.367 ± 0.004 (–14.0)
100	0.351 ± 0.005	0.320 ± 0.004 (–8.8)	0.302± 0.003 (–13.9)	0.291 ± 0.005 (–17.0)
BG 267				
40	0.350 ± 0.001	0.300 ± 0.001 (–14.2)	0.284 ± 0.003 (–18.8)	0.270 ± 0.002 (–22.8)
70	0.390 ± 0.003	0.328 ± 0.002 (–15.8)	0.309 ± 0.001 (–20.7)	0.292 ± 0.002 (–25.1)
100	0.319 ± 0.002	0.248 ± 0.004 (–21.5)	0.231 ± 0.004 (–27.8)	0.218 ± 0.003 (–31.0)
CSG 8962				
40	0.364 ± 0.004	0.330 ± 0.003 (–9.4)	0.315 ± 0.005 (–13.4)	0.309 ± 0.005 (–15.1)
70	0.412 ± 0.005	0.361 ± 0.004 (–12.3)	0.347 ± 0.003 (–15.7)	0.332 ± 0.004 (–19.4)
100	0.336 ± 0.003	0.287 ± 0.002 (–14.5)	0.270 ± 0.004 (–19.6)	0.262 ± 0.003 (–22.0)
DCP 92-3				
40	0.333 ± 0.002	0.276 ± 0.003 (–17.6)	0.247 ± 0.003 (–25.8)	0.219 ± 0.004 (–34.2)
70	0.356 ± 0.001	0.281 ± 0.003 (–21.0)	0.252 ± 0.006 (–29.2)	0.225 ± 0.003 (–36.7)
100	0.300 ± 0.003	0.211 ± 0.004 (–29.6)	0.188 ± 0.003 (–37.3)	0.171 ± 0.004 (–43.0)

[a] Values are means ± SE of six replicates.

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Table 4. Effect of different levels of salinity on nitrate reductase (NR) activity (μmol NO_2/g FW/h) in roots of desi and kabuli chickpea cultivars. Figures in parentheses represent percentage decrease (–) over control

Days after sowing	Electrical conductivity (dSm^{-1})[a]			
	Control	**4**	**6**	**8**
CSG 9651				
40	2.85 ± 0.060	2.73 ± 0.080 (–4.2)	2.64 ± 0.030 (–7.3)	2.52 ± 0.020 (–11.5)
70	3.10 ± 0.030	2.89 ± 0.010 (–6.7)	2.76 ± 0.010 (–10.9)	2.69 ± 0.050 (–13.2)
100	2.34 ± 0.040	2.15 ± 0.040 (–8.1)	2.04 ± 0.040 (–12.8)	1.97 ± 0.050 (–15.8)
BG 267				
40	2.75 ± 0.060	2.38 ± 0.010 (–13.4)	2.26 ± 0.060 (–17.8)	2.19 ± 0.060 (–20.3)
70	2.94 ± 0.040	2.49 ± 0.045 (–15.3)	2.36 ± 0.060 (–19.7)	2.23 ± 0.020 (–24.1)
100	2.28 ± 0.070	1.82 ± 0.060 (–20.1)	1.67 ± 0.070 (–26.7)	1.59 ± 0.030 (–30.2)
CSG 8962				
40	2.54 ± 0.050	2.32 ± 0.030 (–8.6)	2.28 ± 0.060 (–10.1)	2.19 ± 0.050 (–13.7)
70	3.06 ± 0.060	2.71 ± 0.060 (–11.4)	2.60 ± 0.030 (–15.0)	2.53 ± 0.030 (–17.3)
100	2.32 ± 0.040	2.01 ± 0.010 (–13.3)	1.90 ± 0.050 (–18.1)	1.83 ± 0.030 (–21.1)
DCP 92–3				
40	2.40 ± 0.020	2.00 ± 0.010 (–16.6)	1.88 ± 0.040 (–21.6)	1.67 ± 0.010 (–30.4)
70	2.84 ± 0.030	2.25 ± 0.020 (–20.7)	2.03 ± 0.040 (–28.5)	1.82 ± 0.010 (–35.9)
100	2.14 ± 0.010	1.52 ± 0.020 (–28.9)	1.37 ± 0.070 (–35.9)	1.28 ± 0.040 (–40.1)

[a] Values are means ± SE of six replicates.

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