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In Vitro Assessment of Salinity Tolerance in Chickpea (*Cicer Arietinum* L.)

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Abstract: The calli raised from cell culture of chickpea genotypes viz., CSG 8962 Karnal Chana-1 (Salt tolerant), CSG 8890 (salt sensitive) and Bio 104 on MS medium supplemented with BAP (0.5 mg/1)+ NAA (0.5 mg/l) and 40 g/l sucrose were subjected to different salt and salt mixtures. With increasing concentration of salt and salt mixtures, there was a continuous decline in the Relative Growth Rate of the calli. Among three genotypes, most reduction in RGR was recorded in the calli of Bio 104 genotype. There was a significant difference with Na^+ and K^+ uptake.

Key Words : RGR, Callus, NAA, BAP

INTRODUCTION

Soil salinity is one of the most important problems of crop production in arid and semi arid areas. In India, there are approximately 7 million hectare area is salt affected (Anonymous, 1976). Salts generally after a wide array of metabolic process finally culminate in reduced plant growth and ultimately affect yield. The recent development of *in vitro* technology offers a meaningful tool for determining the tolerance and help in screening the salt tolerant lines (Gosal and Bajaj, 1984). The isolation of NaCl tolerant cultures may offer potential for the retrieval of salt tolerant lines. Such cultures also represent an ideal system to asses the physiological effects of salt at the cellular level. Therefore, the present study is conducted to compare callus growth (fresh and dry weight) Relative Growth Rate (RGR) and Na^+ and K^+ ion uptake.

MATERIALS AND METHODS

Calli were maintained from cell culture of three genotypes viz., CSG 8962 Karnal Chana-1, CSG 8890 and Bio 104 on MS medium supplemented with 0.5 mg/l, BAP+ 0.5 mg/l, NAA+ 40 g/l, sucrose. The 20 days old calli were subcultured to the same medium supplied with control, 0.25%, 0.5% and 1.0% salt and salt mixtures. A mixtures of salts were used for creating chloride salinity so as to have Na^{+}, Ca^{++} and Mg^{++} in 1:1, 1:2:1 and 5:3:1 ratio. The cultures were incubated under 16h photoperiod of cool white fluorescent light at 25 ± 2^{0}C. Growth of the calli were recorded in terms of fresh and dry weights at 20, 40 and 60 days after treatment and finally expressed as Relative Growth Rate (RGR).

RGR = Change in dry weight over initial inoculums after time (t) / Dry weight of initial inoculum

t = 20, 40 and 60 days after treatment

For the estimation of Na^{+} and K^{+}, 500 mg dry weight of the tissue was digested at 80^{0}C in 5 ml of tri acid mixture (10 HNO_3 : 4 $HCIO_4$ (60%) : 1 H_2SO_4) in a corning long necked flask for 24h. A 20-30 ml of deionised water was added to this digested solution in the digestion block at 180^{0}C for 30 minutes resulted in decolouration of the solution. It was filtered by Whatman 42 filter paper and Na^{+} and K^{+} estimated by the Flame photometer from the stock solution using NaCl and KCI as standard.

RESULTS AND DISCUSSION

The growth of the callus as represented by fresh and dry weight in all the treatments was highly affected by the concentration of added NaCl. With the increase in NaCl the growth sharply decreased (Table 1). However, an increase in RGR was observed from 20 to 60 days after treatment. At higher concentration of salts, callus necrosis was observed as was evident from the browning of the calli. Decrease in RGR was more prominent in genotypes Bio 104 when placed on 1.0% NaCl:$CaCl_2$:$MgCl_2$ (5:3:1) than CSG 8890 and CSG 8962 which might be due to salt tolerance among the later genotypes. Inhibition of the calli with increasing salt stress has also been reported in *Cicer arietinum* (Pandey and Ganapathy, 1984) and *Lycopersicon* (Guerrier and Bourgeais-chaillou, 1994). Among various doses of salt and salt mixtures used, 1.0% (highest dose) was found most lethal which suppressed callus induction and drastically reduced RGR irrespective of salt and salt mixtures. Besides, 0.25% concentration of different salt and salt mixtures was found to be very mild in reducing callus induction. The standardization of doses of these salt and salt mixtures was necessary to carryout the *in vitro* selection at different level. There are several reports indicating that various concentration of salt and salt mixtures producing

differential effect on callus induction and its magnitude (Chandler and Vasil, 1984; Sanagwan *et al.,* 1997).

The ionic content of the selected callus line were determined at two consecutive phages. At 1.0% level of NaCl:$CaCl_2$:$MgCl_2$ (5:3:1) concentration the Na^+ ion content were significantly higher in selected callus line (Table 2). The K^+ ion content also increased with the increasing of NaCl concentration. During the third phage of growth i.e. 60 days of culture the Na^+ content reached near the stationary phage. Among genotypes, CSG 8962 showed high tolerance level against 1.0% NaCl:$CaCl_2$:$MgCl_2$ (5:3:1).

The present study thus justifies that salinity has an adverse effect on the growth as reflected in decreased Relative Growth Rate of the calli. The salt selected cells were characterized by ion regulation process which maintained higher intercellular levels of Na^+ and K^+.

Table 1 : Relative growth rate of calli of different genotypes derived from cell cultures of chickpea at different levels of salt and salt mixtures

Genotypes	Salt Concentration	Ratio	RGR	
			20-40 d	20-60 d
CSG 8962	1.0% NaCl	-	0.79	0.80
	1.0% NaCl: $CaCl_2$	1:1	0.40	0.41
	1.0% NaCl: $CaCl_2$:$MgCl_2$	1:2:1	0.41	0.45
	1.0% NaCl: $CaCl_2$:$MgCl_2$	5:3:1	0.21	0.31
CSG 8890	1.0% NaCl	-	0.47	0.51
	1.0% NaCl: $CaCl_2$	1:1	0.37	0.38
	1.0% NaCl: $CaCl_2$:$MgCl_2$	1:2:1	0.24	0.28
	1.0% NaCl: $CaCl_2$:$MgCl_2$	5:3:1	0.15	0.18
Bio 104	1.0% NaCl	-	0.45	0.51
	1.0% NaCl: $CaCl_2$	1:1	0.32	0.34
	1.0% NaCl: $CaCl_2$:$MgCl_2$	1:2:1	0.17	0.27
	1.0% NaCl: $CaCl_2$:$MgCl_2$	5:3:1	0.04	0.06

Table 2 : Na+/K+ uptake in callus grown in growth with different salt and salt mixture

Genotype	Salt concentration	Uptake of Na^+ (ppm)		Uptake of K^+ (ppm)		K^+/Na^+ ratio	
		20-40d	20-60d	20-40d	20-60d	20-40d	20-60d
CSG 8890	1.0% NaCl	170	180	070	080	412	444
	1.0% NaCl: $CaCl_2$ (1:1)	410	460	100	230	144	500
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (1:2:1)	620	690	250	300	403	435
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (5:3:1)	710	790	360	380	503	481

Bio 104	1.0% NaCl	510	610	290	310	508	569
	1.0% NaCl: $CaCl_2$ (1:1)	540	630	280	310	369	519
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (1:2:1)	540	560	280	310	369	519
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (5:3:1)	520	580	320	300	517	615
CSG 8962	1.0% NaCl	660	680	390	280	591	412
	1.0% NaCl: $CaCl_2$ (1:1)	840	540	310	280	369	519
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (1:2:1)	620	690	250	300	403	485
	1.0% NaCl: $CaCl_2$:$MgCl_2$ (5:3:1)	980	990	520	540	525	551

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