- Short communication

Plant Tissue Cult. & Biotech. **17**(2): 217-220, 2007 (December)

PTC&B

Accumulation of Proline in NaCl-treated Callus of Six Tomato (*Lycopersicon esculentum* Mill.) Cultivars

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Key words: Lycopersicon escutentum, Callus, Proline

The synthesis, accumulation and degradation of proline are highly regulated by environmental stresses such as salt, drought and metals. This phenomenon has been documented in many plants (Aspinall and Paleg 1981, Kavi-Kishor et al. 2005). In response to stress conditions plants increase osmotic potential within their cells by synthesizing and accumulating compatible osmolytes. Proline is considered one of the most common compatible osmolytes (Claussen 2005) which is directly involved with the osmotic adjustment of the plant cell to external salt stress (Rhodes et al. 1986, Rhodes and Handa 1989). The accumulation of free proline has been studied in a number of taxa subjected to hyperosmotic stress conditions for over 45 years. The accumulation of proline under environmental stress conditions depends on the plant species and the stress level (Kavi-Kishor et al. 2005). Proline seems to have diverse roles under osmotic stress, such as stabilization of protein structures against denaturation; it also stabilizes cell membranes by interacting with phospholipids, and subcellular structures thereby protecting cellular functions (Aspinall and Paleg 1981, Samaras et al. 1995, Kavi-Kishor et al. 2005)

Soil salinity is a major constraint in limiting plant growth. Although proline is known to confer osmotic tolerance during stress conditions, its specific role during plant growth is not completely known (Kavi-Kishor et al. 2005).

In some Solanaceae family like potato, proline plays a vital role in osmotic adjustments (Bussis and Heineke 1998). In tobacco cells this response seems to be related to their salt tolerance since proline content is higher in salt adapted cells (Rhodes and Handa 1989). Preliminary experiments showed that tomato plants subjected to salt stress conditions accumulated relatively high amount of proline (Hernandez et al. 2000). There is a positive correlation in proline accumulationin reported in tomato callus under salt stress (Tal et al. 1978).

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The plant tissue culture approach has proven effective for obtaining salt tolerant plants (Patnaik and Debata 1997b; Purohit et al. 1998) and for quick evaluation of germplasm against salt stress. This technique has also proved useful in the study of salt tolerance mechanisms and the identification of specific cellular pathways involved in proline biosynthesis during stress conditions (Dutta-Gupta et al. 1995, Patnaik and Debata 1997a, Claussen 2005, Kavi-Kishor et al. 2005).

The calli of six tomato *(Lycopersicon esculentum* Mill.) cultivars raised from hypocotyl explants were tested with five levels (0, 25, 50, 75 and 100 mM) of NaCl and their effects on proline accumulation were examined. The cultivars used in the present experiment were Pascal, Imperial, Queen, Tnshet crystal, Tnshet star and Pahuja.

Seeds of the tomato cultivars were surface sterilized by 8% Clorox for 10 minitues and rinsed four times with sterile distilled water. Seed germination was carried out *in vitro* with MS under complete darkness in growth chamber (digitally controlled) with 80% relative humidity at 25°C for one week. The hypocotyl segments were cut (10 mm) from germinated seeds and cultured on MS supplemented with 2.26 µM 2,4-D and 2.32 µM Kn for callus initiation. The media were solidified with agar (Difco-Bacto, 7 g/l) and added 30 g/l sucrose and the pH was adjusted to 5.7. The calli were incubated with 14 h photoperiod and a light intensity of 6.26 µmol $m^{-2}s^{-1}$ (white fluorescent tubes) at 25 ± 1°C for one month. Subsequently the proliferating calli were transferred to same medium with different levels of NaCl (0, 25, 50 , 75 and 100 mM). The NaCl treated calli were grown under the same photoperiod and temperature regime for one month. Fresh calli obtained from NaCl treatments were harvested and proline was measured according to the method of Bates et al. (1973). The 400 mg fresh weight of callus samples were used for extraction and estimation of proline. The samples were homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and the homogenate was filtered through filter paper (Whatman No. 1). In a test tube 2 ml of the filtrate was mixed with 2 ml acid ninhydrin and 2 ml glacial acetic acid and incubated in 100°C water bath for 1h .The reaction mixture was terminated by placing in ice bath, extracted with 4 ml toluene and the chromophore phase was aspirated from the aqueous phase. The absorbance was read at 520 mM using spectrophotometer.

The experiment was designed as completely randomized and with four replications. Data were subjected to analysis of variance and the means were separated using LSD at 5%. To confirm results the experiment was repeated twice.

The callus on medium having NaCI showed cream in colour and no necrotic patches were found in callus during tile culture period. The NaCl treated calli

exhibited higher levels of proline than that of non-treated (NaCl free) counterpart. Highest amount of proline accumulated in callus of Pahuja derived from 100 mM NaCI medium, while the lowest was noted in Imperial in NaCl free medium (Table 1). Callus produced the highest amount of proline with a content of 7.09 mg/g fresh weight in Pahuja; i.e. values two times higher than those of Pascal and Tnshet Crystal treated with 100 mM NaCl added to the medium.

Table 1. Proline accumulation of NaCI treated callus derived from hypocotyl explants of six tomato cultivars.

Cultivars	Proline accumulation (mg/g fresh weight)				
	NaCl Level (mM)				
	0	25	50	75	100
Pascal	0.20 ± 0.05 c	0.40 ± 0.10c	0.36 ± 0.12c	1.29 ± 0.39b	3.13 ± 0.71a
Imperial	0.10 ± 0.01c	0.22 ± 0.06c	0.58 ± 0.15c	0.81 ± 0.37b	4.00 ± 1.26a
Queen	0.19 ± 0.01e	0.43 ± 0.08c	0.90 ± 0.19c	2.37 ± 0.91b	3.59 ± 0.79a
Tnshet Crystal	0.16 ± 0.02c	0.26 ± 0.05c	0.32 ± 0.05c	0.90 ± 0.13b	2.31 ± 0.38a
Tnshet star	0.26 ± 0.05c	0.27 ± 0.07c	0.74 ± 0.13c	1.47 ± 0.52b	4.36 ± 1.29a
Pahuja	0.24 ± 0.04c	0.34 ± 0.09 c	1.58 ± 0.65c	2.95 ± 0.82b	7.09 ± 1.35a

Values shown are means of four replicates ± SE. In the column, values followed by same letters are not significantly different according to DMRT at p = 0.05.

The proline accumulation was not significantly different on medium from 0 to 50 mM NaCI while those on higher range (75 - 100 mM NaCl) it was significantly different (Table 1). Callus obtained from Pahuja was more efficient to proline accumulation than other cultivars at 50 - 100 mM NaCl. In response to NaCl treatments Pahuja, Queen and Tnshet crystal were found significantly different among other one, while Pascal, Imperial and Tnshet star showed moderate response in proline accumulation.

The results indicated that all tomato cultivars tested were able to accumulate proline under *in vitro* salinity stress. The rates of accumulation were different depending on cultivars and NaCl levels. Proline content increased significantly with an increase NaCl concentrations. The results are in agreement with Emilio et al. (1998) for *Lycopersicon esculentum* and *L. pennellii.* Using the same methods Martinez et al. (1996) found a positive relationship between proline accumulation and NaCl tolerance in potato.

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