PROLINE EFFECT ON SHOOT ORGANOGENESIS AND PROTEIN SYNTHESIS IN SALINITY-STRESSED TOMATO CULTURES

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SUMMARY: Efficient de novo shoot organogenesis from hypocotyls and cotyledons of tomato (Lycopersicon esculentum Mill.) was affected by sodium chloride and proline. Sodium chloride at 100 and 150 mM inhibited the shoot regeneration. The fresh and dry weights were also reduced. Addition of proline (100 mg/L) to the medium containing NaCI counteracted the inhibitory effect of NaCI and enhanced shoot regeneration, especially at high NaCI levels.

SDS-PAGE analyses of extracted proteins, revealed that in cultures grown in medium with proline, extra polypeptides of Mr. (Molecular weight) 190, 58, 45 and 26 kDaa (Kilodaltons) accumulated. These polypeptides were not present in control cultures, but also accumulated at 25 mM NaCI. As NaCI was increased in the medium a new protein of Mr. 67 kDaa also accumulated. Proteins of Mr. 67, 52-45 and 62 kDaa were also accumulated when proline was added to the saline medium. Proline directly or indirectly play an important role in protein accumulation and in cell adaptation to salinity stress.

Key Words: Organogenesis, proline, protein accumulation, salinity.

INTRODUCTION

Salinity impairs normal growth and limits the realization of yield potential of modern cultivars (13). One approach to the improvement of the salt tolerance of tomato utilize the tissue culture technique to derive cell lines tolerant to NaCI stress (2,11,23).

Sodium chloride beyond 80 mM affected fresh and dry weights and differentiation of shoots and roots in tomato cell cultures (29,39). Proline accumulation in plants is a striking consequence of water, salt and temperature stresses (4,36). Exogenously added proline was very effective in counteracting the effect of salt (3,28,32).

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Several new proteins which are synthesized in response to an altered environment have been reported as stress proteins or shock proteins in plants. Sodium chloride induces distinct protein changes in cultured cells (12,15,30,34). In the present study, the shoot organogenesis capacity of tomato (Lycoperiscon esculentum) explants (hypocotyls and cotyledons) at different NaCI levels with and without proline, was investigated. Since protein synthesis is an essential biochemical process during cell division and growth, specific differences in protein patterns between salinity and salinity with proline were examined.

MATERIALS AND METHODS

Tomato seeds (Lycopersicon esculentum Mill Peto 86 3) were floated on distilled water for 10-minutes and then surface-sterilized by immersion 5% chlorox for 5 minutes. The seeds were washed with distilled water three times. Seeds were germinated on Murashige and Skoog (25) medium without hormones (MS-1).

The hypocotyls and cotyledons were excised from 10-12 day-old seedlings in vitro. The excised explants were transferred in aseptic conditions (Clean bench VET-850 G) to 50 ml, conical flaks containing 20 ml, of MS-medium supplemented with (mg dm^{-3}):6 IAA, 5 Kinetin, 40 adenine sulfate, 170 $NaH^2PO^4H^2O$, 100 inositol, 0.1 thiamine-HCI, 0.5 pyridoxine, 0.5 nicotinic acid, sucrose (30 g^{-1} dm^{-3}) and agar (7g^{-1} dm^{-3}) MS-II.

Sodium chloride was incorporated into the MS-II medium at 0.25, 50, 100 and 150 mM. Proline (100 $mg^{-1}dm^{-3}$) was added in combination with NaCI. In controls MS-II was supplemented with only proline. For all combinations of media, pH was adjusted to 5.5 ± 0.2 before autoclaving. Each flask contained three cotyledons or five hypocotyl explants, in three replicates. Cultures were kept in an illuminated incubator at 27°C and under continuous fluorescent white light as recommended by Lercari *et. al.* (22). After 3 weeks shoot formation, fresh and dry weights were recorded. Proline content was determined according to Bates *et. al.* (5).

Protein extraction: Lypholized cultures [0.2 g^{-1} (f.m)] was ground in 0.1 cm^{-3} extraction buffer (50 mM Tris-HCI pH 7.5, 2 mM EDTA, 1% mercaptoethanol, 2% sodium dodecyl sulfate (SDS) and 5% glycerol), boiled for 5 minutes and centrifuged in an Eppendorf micro centrifuge for 15 minutes 0.05 cm^{-3} of extract contain about 4 mg protein was applied.

SDS-PAGE (Sodium dodecyl sulphate-Polyacrylamide Gel Electroporosis): was performed as described (19) by means of vertical slab gel unit SE 600 (Hoefer scientific instrument). The running gel was 12% acrylamide (0.4% bis) at pH 8.8 and stacking was 2.5% acrylamide 0.625% bis) at pH 6.8. Both solutions of running gel stacking gels were supplemented with SDS 0.4%. The reservoir buffer was 1.5 M Tris-glycine plus SDS 2%. Electrophoresis was performed at room temperature 25°C, 140 volt and 60 mA with phenol red as "Tracking dye". Molecular weight markers ranged from 200-8 kDaa. Gels were stained with coomassie brilliant blue R-250 and destained with a 5% MeOH/acetic acid mixture. Stained bands were scanned at 525 nm with a Seroscan elvi 146 densitometer.

RESULTS AND DISCUSSION

All the cultured hypocotyl and cotyledon explants produced calli at all levels of NaCI treatments, except 150 mM. Addition of proline in combination with NaCI induced callus formation at high level.

The percentage of shoot regeneration of hypocotyl explants was reduced sharply as NaCI increased in the culture medium and completely inhibited at 150 mM NaCI. Exogenously supplied proline (Table 1), increased shoot regeneration, especially at high levels of NaCI (100 and 150 mM).

Table 1: Shoot regeneration of hypocotyl and cotyledonary explants, cultured for 3 weeks on MS-II supplemented with different levels of NaCl, or NaCl with proline (100 mg^{-1} dm^{-3}).

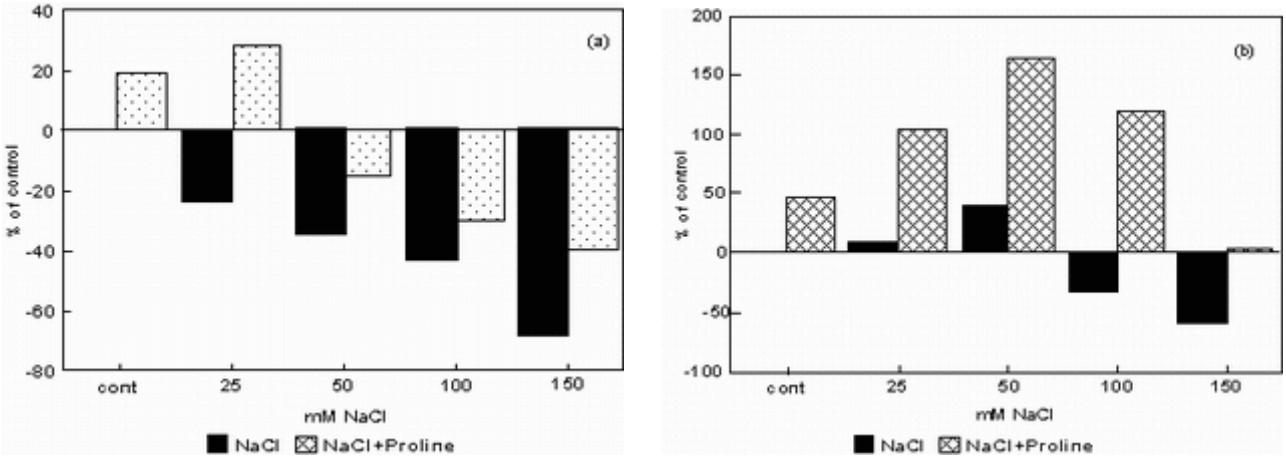
NaCI	Hypocotyl explants						Cotyledonary explants					
	No. of ERS		ERS (% of control)		x of S/E		No. of ERS		ERS (% of control)		x of S/E	
Treat (mM)	NaCI	NaCI+ Prol	NaCI	NaCI+ Prol	NaCI	NaCI+ Prol	NaCI	NaCI+ Prol	NaCI	NaCI+ Prol	NaCI	NaCI+ Prol
0	27	27	100	100	3.77	9.0	9.9	9.0	100	100	6.0	6.45
25	27	27	100	100	1.22	2.35	7.0	9.0	65	100	4.0	6.02
50	15	18	55	66	0.88	3.75	6.0	7.0	66	77	4.0	5.78
100	12	15	44	55	0.55	1.44	-	5.0	-	55	-	4.34
150	-	15	-	55	-	1.00	-	4.0	-	45	-	4.44

- means no response

Ers= explants regenerated shoots

x of S/E = average-number of shoots per explants.

Figure 1: Dry wt. of hypocotyl explants (a) and cotyledonary explants (b), cultured for 3-weeks on MS-medium, supplemented with NaCl and NaCl with proline. The represented as percentages of control.



Results in Table 1 show that the cotyledonary explants had a substantially higher rate of shoot regeneration than the hypocotyl explants. Sodium chloride decreased the percentage regeneration of cotyledonary explants and completely inhibited regeneration at high levels (100 and 150 mM).

Proline application to the salinized media enhanced shoot regeneration in the cotyledonary and hypocotyl explants especially at high levels of NaCl Table 1.

Mathur *et. al.* (23) found that the capacity for regeneration of plantlets from internodal segments of *Kickxia ramosissima*, was reduced in the presence of NaCl. He also found that proline supplemented singly at 100 μM to the medium with 120 mM NaCl counteracted the inhibitory effect of NaCl. Amino acid which stimulated the somatic embryogenesis. Proline was shown to be the best amino acid which stimulated the sometic embryogenesis (37,38). Glutamine and asparagine also enhanced the shoot regeneration of soybean (14,16,33). They concluded that organic nitrogen sources were important for efficient plant regeneration. Proline may also serve as an important source of nitro-

Table 2: Proline content [μg g^{-1} (f.m)] of hypocotyl and cotyledonary explants, cultured for 3 weeks on MS-II, supplemented with different levels of NaCl, or NaCl with proline (100 mg^{-1} dm^{-3}).

	Proline contents (μg g^{-1}(f.m))			
	Hypocotyl explants		Cotyledonary explants	
NaCl (mM)	00	Proline	00	Proline
00	27.50 ± 0.83	33.21 ± 2.64	39.03 ± 2.08	52.68 ± 1.74
25	52.71 ± 2.38**	59.06 ± 1.32**	63.75 ± 2.55**	71.52 ± 2.05**
50	76.51 ± 0.92**	83.06 ± 2.54**	80.97 ± 2.56**	96.89 ± 2.74**
100	89.54 ± 0.64**	105.48 ± 1.75**	103.29 ± 2.91**	116.97 ± 2.34**
150	105.49 ± 2.00**	114.63 ± 1.61**	127.03 ± 3.07**	133.24 ± 2.34**
LSD at 5%	2.76	3.71	4.83	4.78
LSD at 1%	3.94	5.28	6.87	6.81

** Highly significant as compared with control.

gen in plant metabolism (8) and as a readily available source of energy and reducing power (35).

The dry mass of hypocotyl explants (Figure 1a) were more affected by decrease in the dry weights as occured, NaCI increased in the culture medium. A slight increase about (18.9 and 34%) in dry wt of hypocotyl explants at 25 and 50 mM NaCI with proline, respectively. While at high levels of NaCI the dry mass of hypocotyl were affected less than those of NaCI only. In case of cotyledonary explants, the proline added increased the dry mass at all NaCI levels especially at 100 mM and 150 mM Figure 1b.

Addition of proline in the absence of stress resulted in an improvement in the growth of both hypocotyls and cotyledons. At high NaCI levels (100 and 150 mM), exogenously supplied proline caused a significant increase in dry weight of the cultured hypocotyls and cotyledons Figure 1.

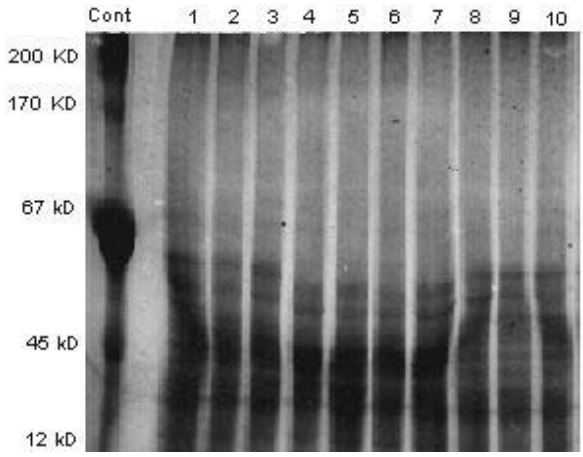


Figure 2: SDS-PAGE of soluble protein fractions from cotyledonary explants callus cultures grown at different levels of NaCI (00, 25, 50, 100 and 150 mM NaCI) and NaCI plus proline. Lanes (1, 3, 5, 7 and 9) correpond to different levels of NaCI respectively. Lanes (2, 4, 6, 8 and 10) correspond to NaCI plus proline. Proteins (about 4 mg) were solublized in Laemmli buffer, electrophoresed on an 10% polyacrylamide gel and stained with Coomassie blue. Mr* 10^{-3} of standard protein are shown on the left.

The adverse effect of NaCI could be due to sodium toxicity (20,24) and/or water deficit (1).

Proline is taken up by cells (28) and can an osmoticum within cells (35). Proline has been reported to play an important role in protecting enzymes against denaturation (26,27) and in regulating the cytosolic acidity (40,41).

The proline accumulation (Table 2) increased significantly as salinity level raised in the culture media and also more accumulated in the cotyledonary explants compared with the hypocotyl explants. These results are in agreement with reports by several investigators

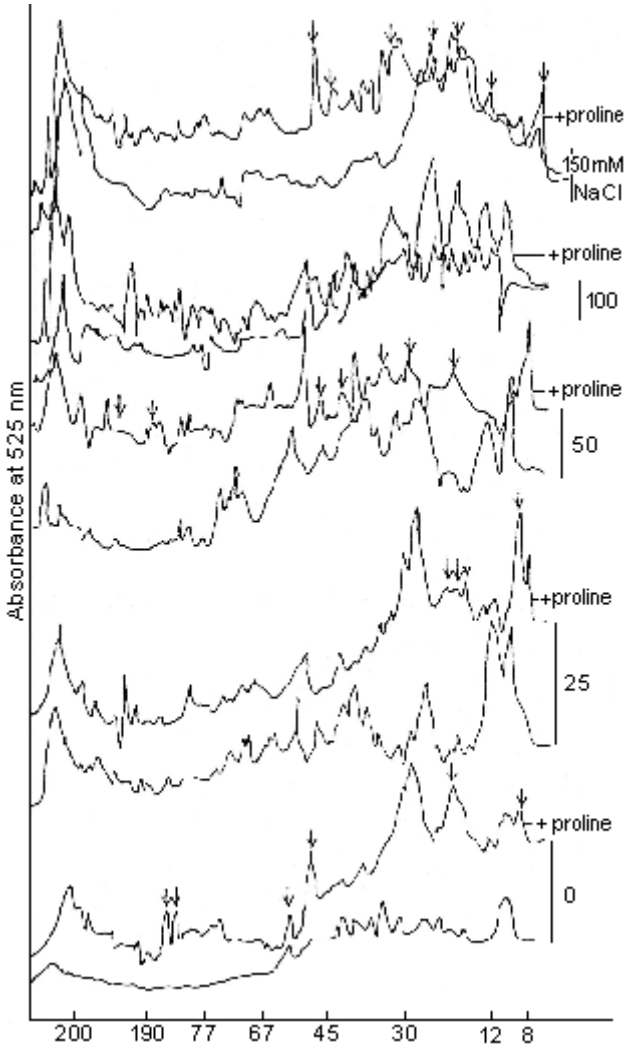


Figure 3: Scan of SDS-PAGE electrophoretic profile of tomato cultures.

(9,10,42). The higher levels of proline serve as compatible osmotica within the cytoplasm to buffer against high vacuolar ion concentrations (17), of function as water structure regulators and aid in the solubility of proteins and other biopolymers under condition or reduced water capacity (31). Proline has also suggested to serve as an important source of nitrogen in plant metabolism (8) and as readly available source of energy and reducing power (35).

The protein pattern of cultures grown on (MS-II) media, supplemented with different levels of NaCI and NaCI with proline, were analyzed by gel electrophoresis (Figure 2). Densitometer profiles (Figure 3) of accumulated proteins revealed that proline induced changes in proteins. The characteristic changes in proteins are described below.

(1) Proteins of Mr 190, 90 and 26 kDa, accumulated in the culture media supplemented with proline and were not detectable in untreated culture, control cells.

(2) Proteins of 170, 58, 45, 26, 12 and 8 kDa accumulated in the cultures grown at (25 mM NaCI). Exogenous proline addition enhanced the biosynthesis of extra polypeptides of Mr. 35-30 KDa and 67 kDa.

(3) A protein of 67 kDa was apparently induced as NaCI increased in the medium at 150 mM NaCI. In cultures supplemented with proline, newly synthesised proteins of Mr. 52 and 40 kDa were recorded in addition to those produced at 150 mM NaCI.

(4) The major proteins of Mr 26 kDa appeared in the cultures treated with NaCI and NaCI with proline.

Proline enhanced the biosynthesis of proteins in salt-stressed plants (18). These authors concluded that proline stabilized the protein synthesizing machinery or produced alteration in the regulation of key enzymes to combat stress.

Our results indicate that a major protein accumulated of 26 kDa in salt-stressted cultures and in salt-stressed cultures with proline. These results are in agreement with La Rosa *et al* (21), Bressan *et al.* (7) and Ben-Hayyin *et al.* (6). They concluded that the prolonged adaptation of cells to salinity, resulted in increase of this protein Mr 26 kDa and called Osmotin.

Exogenously added proline to medium containing high levels of NaCI enhanced the accumulation of proteins of Mr 52 and 40 kDa. These newly synthesized proteins may explain the role played by proline in counteracting the inhibitory effect of salt stress. This suggestion needs more investigation.

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