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BRIEF COMMUNICATION

Protein profiles of somatic embryos and regenerated plants from NaCl selected and control cultures of orchardgrass

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Abstract

The protein profile of cells of control somatic embryos was compared to that of embryos that have become selected and maintained on 200 mM NaCl in order to detect salt inducible proteins. Two proteins (60 and 51.5 kDa) were more abundant in the selected embryos and one protein with molecular mass 18 kDa was unique to the selected embryos. Enhanced content of 27 kDa protein was observed in all somatic embryos indicating its involvement in the embryonal state. Similar pattern of salt inducible proteins in selected somatic embryos and the plantlets regenerated from such embryos was found.

Additional key words: *Dactylis glomerata*, NaCl tolerance, plant regeneration, somatic embryogenesis.

Tissue culture selection for NaCl tolerant cell/callus lines has received increasing attention (*e.g.*, Chandler and Thorpe 1986, Rains *et al.* 1986, Epstein and Rains 1987, Nabors 1990, Dix 1993, Tal 1994). In the numerous accounts of selection of salt tolerant cultures, the regeneration of NaCl tolerant plants from such cultures are still meagre particularly with gramineous species (Yano *et al.* 1982, Bhaskaran *et al.* 1983, Chandler and Vasil 1984, Bajaj and Gupta 1987, Dutta Gupta *et al.* 1992). Changes in proteins and the appearance of specific proteins in salt tolerant cells have been well documented. However, no attempt has been made to relate changes in the polypeptide patterns between the selected embryos and the plantlets regenerated from them.

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In the present work we describe the steady state changes in protein profiles which occur when cells become selected to NaCl and the appearance of these changes in the selected plantlets.

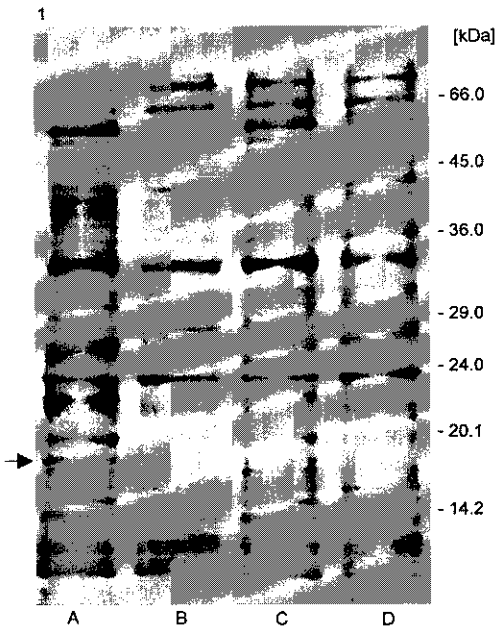
For callus initiation *Dactylis glomerata* L. genotype Embryogen-P, which produces somatic embryos in high number (Conger and Hanning 1991), was used. *In vitro* cultures were initiated from young leaf segments and maintained on SH medium (Schenk and Hildebrandt 1972) containing 30 μM 3,6-dichloro-2 methoxy benzoic acid (*dicamba*) and 0.8 % agar (SH-30). All cultures were subcultured at 4-week intervals and kept in the dark at 25 °C. Actively growing embryogenic calli were cultured on SH-30 containing 0, 50, 100, 150, 200, 250 or 300 mM NaCl to obtain a dose response curve and 200 mM was chosen for selection of tolerant calli. At this concentration, most calli turned brown within 4 weeks. However, a portion of calli remained healthy in appearance and they were subcultured every 4 weeks onto fresh SH-30 medium containing 200 mM NaCl. After 7 months, a callus which exhibited continued growth without discolouration was selected. This callus line was designated as the NaCl selected line (Dutta Gupta *et al.* 1992).

Embryogenic callus pieces both from control and selected cultures were allowed to grow for a total period of 56 d on NaCl free SH-30 medium (control cultures), whereas selected cultures were maintained on SH-30 medium containing 200 mM NaCl. The cultures were incubated in the dark at 25 °C and transferred to fresh medium at 14-d intervals.

For plant regeneration, selected somatic embryos were transferred to SH medium without *dicamba* (SH-0) and with 200 mM NaCl, control somatic embryos were transferred to SH medium without NaCl and *dicamba* (SH-0). All cultures were placed in the light (irradiance 30 - 60 μmol m^{-2} s^{-1}, cool white fluorescent bulbs). After 21 d, the leaves of regenerated plants were frozen and stored in liquid nitrogen for further protein analysis.

The frozen embryos and leaves of plantlets were ground in a mortar with liquid nitrogen. The frozen powder was quickly added to extraction buffer containing 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ethylene glycol-bis-(β-aminoethyl-ether)-N,N,N'N'-tetraacetic acid (EGTA), 100 mM dithiothreitol (DTT), 10 μg cm^{-3} leupeptin, 10 μg cm^{-3} α-2-macroglobulin and 100 mM Tris-HCl, pH 8.5. The buffer to sample ratio was 1:1 (v/m). The mixture was centrifuged for 10 min at 13 000 *g*. The supernatants were incubated with 1 mg cm^{-3} protamine sulphate for 10 min at room temperature on a gyratory shaker. After centrifugation for 5 min at 13 000 *g* the extract was saved. For SDS (sodium dodecyl sulphate) electrophoresis the extract was diluted with 2 × Laemmli sample buffer (Laemmli 1970) and placed in a boiling water bath for 3 min. Supernatants obtained after centrifugation in an Eppendorf centrifuge for 5 min were used for electrophoresis. Soluble protein was assayed according to the procedure of Bradford (1976).

One dimensional gel electrophoresis was performed using the procedure of Laemmli (1970). Slab gels (1.5 mm thick) with a 10 to 15 % polyacrylamide gradient in the running gel and a 4 % polyacrylamide stacking gel were used for electrophoresis. Equal amounts of protein were loaded on each lane. The gels were run at 30 mA for 3 to 4 h and then stained with 0.05 % Coomassie brilliant blue



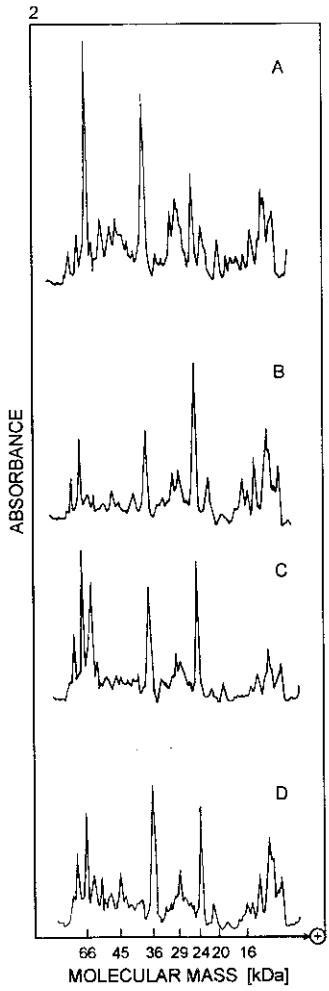


Fig. 1. SDS-PAGE of equal amounts of total cellular protein on 10 - 15 % polyacrylamide gradient gel. *Lanes A* and *B* represent protein extracted from cells of selected and control embryos. *Lanes C* and *D* represent the protein profile of cells of selected and control plants, respectively. Molecular masses are indicated on the right; the newly synthesized protein is indicated by the *arrow*.

Fig. 2. Densitometer tracing of the gel in Fig. 1. *Arrows* indicate major protein differences among selected embryos (*A*), control embryos (*B*), selected plants (*C*), and control plants (*D*).

R-250 in 40 % methanol + 10 % acetic acid. Destaining was carried out in 10 % methanol + 7 % acetic acid. Following electrophoresis, gels were photographed and scanned at 560 nm with a *LKB* densitometer. The photograph and densitogram in this paper are representatives of three experiments.

Analysis of protein patterns of somatic embryos and regenerated plants from control and selected cultures revealed the presence of 27 or 28 proteins with molecular masses from 70 to 11 kDa (Figs. 1,2). The major polypeptides in the control embryos had molecular masses of 66, 37, 29, 27, 22, 19 and 14 kDa (Table 1). Protein profiles from the selected embryos showed increased content of 60 and 51.5 kDa polypeptides. The findings are not in agreement with previous studies on tobacco and maize (Singh *et al.* 1985, Ramagopal 1986) where a reduction of high molecular mass polypeptides in NaCl adapted cells was found. However, a similar pattern of increase was noted in barley seedlings (Maslenkova *et al.* 1992). On the other hand, the intensity of the 66 and 22 kDa bands slightly decreased. A unique band with a molecular mass of 18 kDa was detected in selected embryos. It comprised 2.1 % of the total extractable proteins. These results suggest that the 18 kDa polypeptide is newly synthesized. Recent studies demonstrated that a 26 kDa protein, referred to as osmotin was intimately associated with salt adaptation (Singh *et al.* 1987, Kononowicz *et al.* 1992). In the present work, we were unable to detect this specific protein. Our selected line lost either the need or the ability to osmotic adjustment under NaCl stress (Dutta Gupta *et al.* 1995). This may account for the absence of an osmotically regulated protein.

Table 1. Polypeptide composition of the somatic embryos and regenerated plants from selected and nonselected cultures. Values indicate percentage of the total extractable proteins based on the scanning of stained gels.

Polypeptide [kDa]	Control embryos	Selected embryos	Control plants	Selected plants
60.0	2.5	13.5	4.5	12.6
51.5	*1.1*	*9.1*	*4.4*	*4.9*
37.0	9.2	11.1	11.3	12.6
29.0	4.4	5.6	4.4	4.4
27.0	4.4	6.4	1.4	1.9
22.0	13.9	6.7	7.7	12.8
19.2	3.9	2.1	3.8	0.3
18.0	-	2.1	-	2.0

Experiments were performed to determine whether these salt inducible proteins also exist in the regenerated plantlets. Proteins in cells of plantlets regenerated from control embryos were comparable to those in control embryos. However, the 27 kDa polypeptide is prominent only in the cells of the somatic embryos regardless of whether the embryos were from NaCl selected or nonselected cultures. This protein may be one that is related to the embryonal state. This interpretation is in agreement with previous protein analysis of *Dactylis* (Hahne *et al.* 1988). One polypeptide with molecular mass of 51.5 kDa, faintly expressed in the control embryos, was increased in the regenerated plants. Salt inducible polypeptides with molecular masses 60 and 18 kDa identified in selected embryos were common in selected plants in nearly equal amount (Table 1).

In conclusion, the pattern of salt inducible polypeptides indicate that the traits selected in somatic embryos are also expressed in regenerated plants. The long term tolerance of these plants to NaCl and sexual transmission of the trait remains to be investigated.

References

Bajaj, Y.P.S., Gupta, R.K.: Plants from salt tolerant cell lines of napier grass (*Pennisetum purpureum* Schum.). - Indian J. exp. Biol. **25**: 58-60, 1987.

Bhaskaran, S., Smith, R.S., Schertz, K.: Sodium chloride tolerant callus of *Sorghum bicolor* (L.) Moench. - Z. Pflanzenphysiol **112**: 459-463, 1983.

Bradford, M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Chandler, S., Vasil, I.K.: Selection and characterization of NaCl tolerant cells from embryogenic cultures of *Pennisetum purpureum* Schum (napier grass). - Plant Sci. Lett. **37**: 157-164, 1984.

Chandler, S.F., Thorpe, T.A.: Variation from plant tissue cultures: biotechnological application to improving salinity tolerance. - Biotech. Adv. **4**: 117-135, 1986.

Conger, B.V., Hanning, G.E.: Registration of Embryogen-P orchard grass germplasm with a high capacity for somatic embryogenesis from *in vitro* cultures. - Crop Sci. **31**: 855, 1991.

Dix, P.J.: The role of mutant cell lines in studies on environmental stress tolerance: an assessment. - Plant J. **3**: 309-313, 1993.

Dutta Gupta, S., Auge, R.M., Denchev, P.D., Conger, B.V.: Growth, proline accumulation and water relations of NaCl-selected and nonselected callus lines of *Dactylis glomerata* L. - Environ. exp. Bot. **35**: 83-92, 1995.

Dutta Gupta, S., Joshi, P.A., Hovanesian, J.C., Conger, B.V.: Ultrastructural characterization of somatic embryos regenerated from NaCl selected and nonselected calli of *Dactylis glomerata* L. - Protoplasma **170**: 177-185, 1992.

Epstein, E., Rains, D.W.: Advances in salt tolerance. - In: Gabelman, W.H., Loughman, B.C. (ed.): Genetic Aspects of Plant Mineral Nutrition. Pp. 113-125. Martinus Nijhof Publishers, Dordrecht - Boston - Lancaster 1987.

Hahne, G., Mayer, J.E., Lorz, H.: Embryogenic and callus-specific proteins in somatic embryogenesis of the grass, *Dactylis glomerata* L. - Plant Sci. **55**: 267-279, 1988.

Kononowicz, A.K., Nelson, D.E., Singh, N.K., Hasegawa, P.M., Bressan, R.A.: Regulation of the osmotin gene promoter. - Plant Cell **4**: 513-524, 1992.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. - Nature **227**: 680-685, 1970.

Maslenkova, L.T., Miteva, T.S., Popova, L.P.: Changes in the polypeptide-patterns of barley seedlings exposed to jasmonic acid and salinity. - Plant Physiol. **98**: 700-707, 1992.

Nabors, M.W.: Environmental stress resistance. - In: Dix, P.J. (ed.): Plant Cell Line Selection. Pp. 167-186. VCH Verlagsgeselschaft, Weinheim 1990.

Rains, D.W, Croughan, S.S., Croughan, T.P.: Isolation and characterization of mutant cell lines and plants: salt tolerance. - In: Vasil, I.K. (ed.): Cell Culture and Somatic Cell Genetics of Plants. Pp. 537-547. Academic Press, New York 1986.

Ramagopal, S.: Protein synthesis in a maize callus exposed to NaCl and mannitol. - Plant Cell Rep. **5**: 430-434, 1986.

Schenk, R.U., Hildebrandt, A.C.: Medium techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. - Can. J. Bot. **50**: 199-204, 1972.

Singh, N.K., Handa, A.K., Hasegawa, P.M., Bressan, R.A.: Proteins associated with adaptation of cultured tobacco cells to NaCl. - Plant Physiol. **79**: 126-137, 1985.

Singh, N.K., Bracker, C.A., Hasegawa, P.M., Handa, A.K., Buckel, S., Hermodson, M.A., Pfankoch, E., Regnier, F.E., Bressan, R.A.: Characterization of osmotin: a thaumatin-like protein associated with osmotic adaptation in plant cells. - Plant Physiol. **85**: 529-536, 1987.

Tal, M.: *In vitro* selection for salt tolerance in crop plants: theoretical and practical considerations. - *In Vitro* cell. dev. Biol. **30** (3): 175-180, 1994.

Yano, S.I., Ogawa, M., Yamada, Y.: Plant formation from selected rice cells resistant to salts. - In: Fujiwara, A. (ed.): Plant Tissue Culture. Pp. 495-496. Abe Photo Printing Co., Tokyo 1982.