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BRIEF COMMUNICATION

Isolation and characterization of NaCl resistant cell line of mulberry

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Abstract

The selected NaCl tolerant clones of *Morus alba* L. cv. MR_2 grow better at higher concentration of NaCl than non-selected clones. With increasing NaCl concentration the Na^+, Cl^- and proline content increased more and K^+ and Ca^{2+} content decrease less in selected clones in comparison with non-selected ones.

Key words: calcium, chloride, *Morus alba*, potassium, proline, sodium

The development of mulberry (*Morus alba* L.) cultivars tolerant to salinity may lead to increase in productivity of silk worm (*Bombyx mori* L.) in saline lands. Through cell culture techniques, salt tolerant cell lines were selected and regenerated in many plants (for review see *e.g.* Stavarek and Rains 1984). In the present study we report the procedure of salt tolerant cell line selection in mulberry. Further to understand the mechanism of salt tolerance, we estimated the Na^+, K^+, Ca^{2+} and Cl^- ions and free proline content in mulberry callus cultures.

Hypocotyl segments (1cm) from *in vitro* raised 10 d old seedlings of *Morus alba* cv. MR_2 were cultured on MS medium (Murashige and Skoog 1962) containing 1.0 mg dm^{-3} of indole-3-acetic acid (IAA) and 1.0 mg dm^{-3} of benzyladenine (BA). The cultures were incubated at 27 ± 2 °C under dark. After 45 d, soft and friable calli (500 ± 50 mg) obtained from the explants were transferred to medium with 0 - 1.0 % NaCl for selection of salt tolerant cell line. The culture conditions were the same as described above. At the end of 5^{th} week more than 75 % of these calli exhibited arrested growth at and above 0.5 % of NaCl. A small portion of calli which showed continued growth and healthy appearance at 0.5 % of NaCl were grown on the medium with the same NaCl concentration for 8 months. These clones were considered as salt tolerant. Calli maintained on the NaCl free medium were referred as non-selected clones.

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Known masses of fresh calli were inoculated to 150 cm^3 Borosil flasks containing the same medium with various concentrations of NaCl (eight replicates per treatment). After 45 d the final fresh mass of each culture was recorded. To analyze Na^+, K^+ and Ca^{2+} contents, 500 ± 50 mg of callus was digested with a 10 cm^3 of triple acid (nitric acid:sulphuric acid:perchloric acid 9:2:1 v/m) for 12 h and the extract was soluted in 50 cm^3 of de-ionised water. Na^+, K^+ and Ca^{2+} were determined using a flame photometer (*ELICO Model CL360, Elico Pvt. Ltd.*, India). Cl^- was analyzed by Versenate titrimetric method. The proline content was estimated by the method of Bates *et al.* (1973).

The selected calli showed faster growth than non-selected calli up to 0.5 % NaCl concentration (Fig. 1). This observation suggested that the salinity tolerance induced in the selected cell line was stable. Similar changes in growth pattern of NaCl selected cell lines has been reported in *Nicotiana sylvestris* (Dix and Street 1975), *Vigna radiata* (Kumar and Sharma 1989) and *Citrus aurantium* (Ben-Hayyim *et al.* 1985), *etc.*

In many plants, resistance to salinity was correlated with accumulation of Na^+ and Cl^- in their tissues for osmotic adjustment (for review see Greenway and Munns

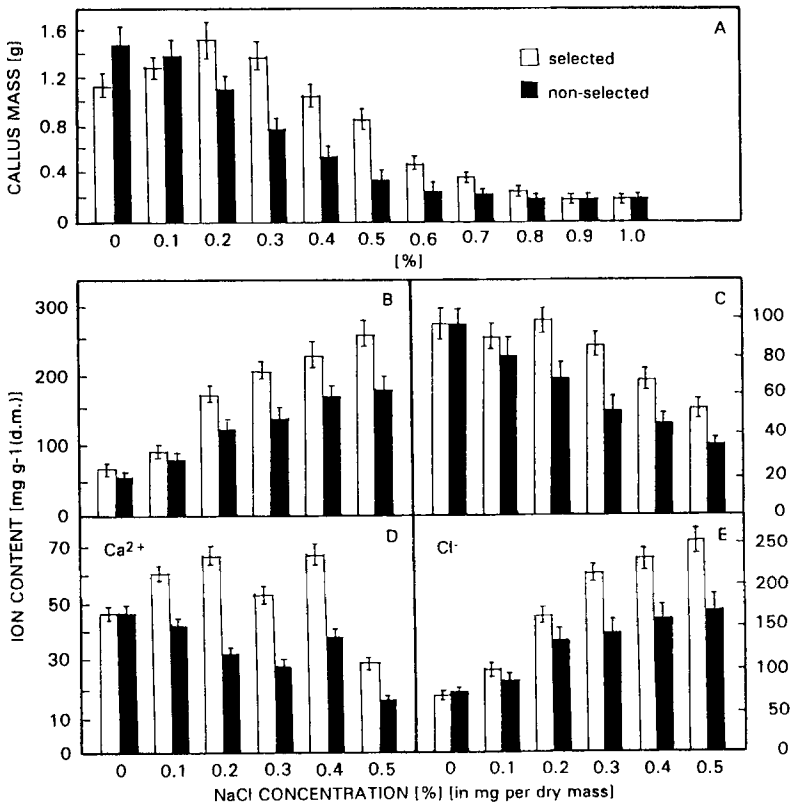

Fig. 1. Effect of NaCl concentration in the medium on growth (*A*) and Na^+ (*B*), K^+ (*C*), Ca^{2+} (*D*) and Cl^- (*E*) content in selected and non-selected mulberry cell lines (after 45 d of culture).

1980). In the present study, accumulation of Na^+ and Cl^- was increased with increasing concentration of NaCl in both seelected and non-selected cell lines. However, the content of Na^+ and Cl^- were two fold higher in a selected line at 0.5 % of NaCl (Fig. 1). Similar results were observed in *Poncirus trifoliata* (Belouly and Bouharmont 1992). The content of K^+ and Ca^{2+} decreased with the increase in concentration of NaCl in selected and non-selected cell lines. But in the selected cell line such a decreasing trend started at higher NaCl concentration (Fig. 1). This is in agreement with the previous reports concerning *Medicago sativa* (Groughan *et al.* 1978), *Nicotiana tabacum* (Watad *et al.* 1983) and *Poncirus trifoliata* (Belouly and Bouharmont 1992).

Generally plants which are grown under saline conditions show accumulation of proline and proline plays an important role in osmotic adjustment (*e.g.* Stewart and Lee 1974). In the present study the selected callus line showed two fold higher amount of proline [240 $\mu g\ g^{-1}$(d.m.)] than the non-selected cell line [108 $\mu g\ g^{-1}$(d.m.)]. Similar data has been reported in *Cicer arientinum* (Pandey and Ganapathy 1985) and *Nicotiana tabacum* (Watad *et al.* 1983). This confirmed that the cells of selected line were acclimated to salinity.

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