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Isolation of sodium chloride tolerant cell lines and plants in finger millet

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Abstract

Sodium chloride tolerant cell lines of finger millet were isolated from embryogenic cultures growing on MS medium supplemented with picloram (2 mg l^{-1}), kinetin (0.1 mg l^{-1}) and sodium chloride (1 %) at the end of 6 passages. The sodium chloride tolerant cell lines showed better growth in comparison with control at all concentrations of sodium chloride tested, with optimum growth at 0.25 % NaCl. When the tolerant lines were grown for 3 passages in absence of NaCl, the growth was lower than that of the tolerant lines tested immediately at the end of 6 passages of selection. NaCl tolerant calli had more Na^+ in comparison with control and they regenerated plants in presence of 1 % NaCl, while the control lines failed to differentiate. When screened in a hydroponics system with 1 % NaCl, the tolerant plants grew to maturity while the control plants failed to grow.

Introduction

One of the major constraints limiting plant productivity in many parts of the world is the presence of excess amounts of salts in the soil. Although conventional methods of plant breeding have contributed towards selection of salt tolerance, tissue culture selection may act as a supplement for the isolation of salt tolerant cells and plants of many crop species such as *Nicotiana tabacum* (Nabors *et al.* 1975, 1980), *Medicago sativa* (Croughan *et al.* 1978), *Saccharum officinarum* (Liu and Yeh 1984), *Sorghum bicolor* (Bhaskaran *et al.* 1986), *Pennisetum purpureum* (Chandler and Vasil 1984) and *Oryza sativa* (Vajrabhaya *et al.* 1989). The advantage of this technique is that the large population of cells screened *in vitro* may have inherent variability which may include characters enhancing tolerance to specific stress. In the present study, we report isolation of NaCl tolerant cell lines and plants from embryogenic cultures of finger millet.

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Material and methods

Material: Finger millet (*Eleusine coracana* Gaertn.) cv. CO-12 (obtained from TNAU, Coimbatore) was used as the source material. Embryogenic callus cultures were initiated from shoot apices of germinating seeds as described before (Eapen and George 1989) on MS medium (Murashige and Skoog 1962) supplemented with 4 mg l^{-1} of picloram and 0.5 mg l^{-1} of kinetin (Kn). The callus was subcultured for 4 passages on medium containing picloram (2 mg l^{-1}) and Kn (0.1 mg l^{-1}) (CM medium) and calli growing on 5th passage were used for experiments with NaCl.

Selection of NaCl tolerant cell lines: To study the effect of different concentrations of NaCl on callus growth, CM medium was supplemented with 0.25, 0.5, 0.75, 1 and 2 % of NaCl. Growth values (GV) based on fresh and dry masses were calculated at the end of 40 d of culture. Initial dry mass was computed while final dry mass was taken after drying the tissues at 65 °C for 24 h.

$$\text{Growth value (GV)} = \frac{\text{Final mass}}{\text{Initial mass}}$$

Toxic concentration of NaCl (1 %) was selected based on GV. Callus cultures from 5th passage were grown on CM medium supplemented with 1 % NaCl for 6 passages each lasting 40 d. 250 - 300 mg of tissues were inoculated in each tube with 20 replicates. At each passage growing tissues were subcultured, while necrotic brown tissues were discarded. At the end of six passages in NaCl, the growth of NaCl tolerant lines was tested on CM medium supplemented with 0.25, 0.5, 0.75, 1 and 2 % NaCl. In a separate experiment, the NaCl tolerant lines were grown for another three passages on CM medium devoid of NaCl and finally tested on different concentrations of sodium chloride.

Plant regeneration: For plant differentiation, NaCl tolerant calli selected at the end of 6 passages in presence of NaCl were transferred to MS medium devoid of phytohormones with or without NaCl (1%). Plants regenerated in presence of NaCl from tolerant cell lines and control regenerated plants obtained in absence of NaCl were transferred to hydroponic system (6 plants each) with half strength Hoagland's (1950) medium with or without NaCl (1 %) for 1 week and then in full strength medium with or without NaCl for 6 weeks. The medium was changed every week and electrical conductivity of the medium was around 11 mS cm^{-1} throughout the culture period.

Ion content analysis: To estimate the content of sodium ions (Na^+) in the tolerant and control calli, tissues were dried overnight at 65 °C, weighed (100 mg), charred in *Biological Material Oxidiser* (BMO) and extracted in warm HCl with deionized water (1:10). Sodium was determined by atomic absorption spectrometry. Analysis was done on triplicate samples.

Results and discussion

Incorporation of NaCl in the medium caused a decline in the growth values of embryogenic callus. In presence of 0.25 % NaCl, GVs based on fresh and dry masses showed a reduction of 45 and 52 %, respectively. Further decrease in GV was evident with increasing concentrations of NaCl in the medium (Fig. 1).

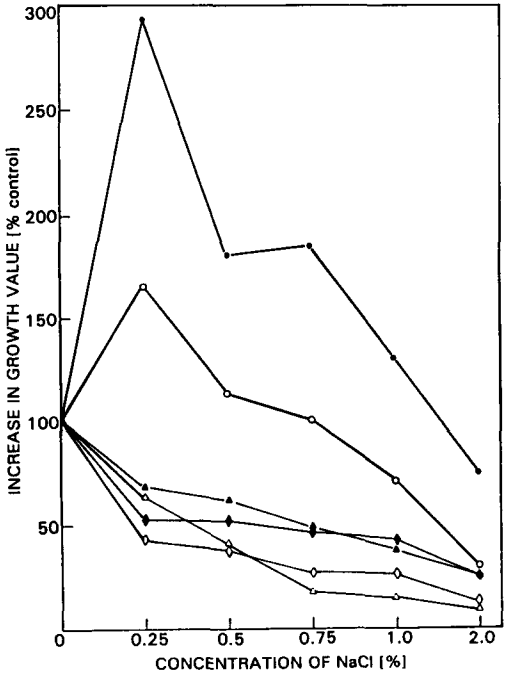

Fig. 1. Effect of different concentrations of NaCl on growth value based on fresh (*open symbols*) or dry masses (*closed symbols*) of embryogenic cultures of *E. coracana* cv.. CO-12 grown on MS + picloram (2 mg l^{-1}) + Kn (0.1 mg l^{-1}) at the end of 40 d of culture. Comparison of control calli (*rhombus*), tolerant cell line tested after 6 passages of growth on 1 % NaCl (*circles*) and tolerant cell line tested after growing on non-stress medium for 3 passages and then back on stress medium (*triangles*).

The result showed that tolerant cell lines not only grew well in presence of 1 % NaCl, but also required 0.25 % NaCl for its optimal growth (Fig. 1). Similar requirement for NaCl for optimum growth was shown by NaCl tolerant lines of *Solanum melongena* (Jain *et al.* 1987), *Pennisetum purpureum* (Chandler and Vasil 1984) and *Saccharum officinarum* (Liu and Yeh 1984). In general, tolerant cell lines had a higher GV compared to the control in presence of all concentrations of NaCl tested (Fig. 1). When the level of NaCl was upto 0.5 %, the calli of tolerant lines grown on non-stress medium for 3 passages showed slightly higher GV compared to the control. But this value was much lower than what obtained when tolerant lines were tested immediately after selection without transfer to media devoid of NaCl. As

the concentration of NaCl increased both the control and tolerant cell lines grown in the absence of NaCl for 3 passages showed almost similar pattern of growth (Fig. 1). Such a loss of tolerance by salt tolerant lines when grown in the absence of NaCl has been reported for *Pennisetum purpureum* (Chandler and Vasil 1984) and *Nicotiana tabacum* (Hasegawa *et al.* 1980) while salt tolerance remained stable in the absence of salt in some cases (Tyagi *et al.* 1981, Kochba *et al.* 1982, Rangan and Vasil 1983).

Table 1. Regeneration potential (average No. of plantlets per 500 mg of tissue ± S.E.) of NaCl tolerant and control cell lines of *E. coracana* cv. CO-12 on stress and non stress medium.

Treatment	Medium	No. of plantlets
Control calli	MS	725.00 ± 2.00
Control calli	MS + NaCl (1 %)	0
Tolerant cell line	MS	265.00 ± 48.76
Tolerant cell line	MS + NaCl (1 %)	338.88 ± 41.40

The germination potential of the somatic embryos of tolerant cell lines was determined by transferring the embryogenic tissues growing in the 6th passage in stress medium to MS basal medium with or without NaCl. Control calli failed to germinate on MS medium with 1 % NaCl, while tolerant calli produced an average of 388 plants per 500 mg of tissue (Table 1). The tolerant cell lines differentiated in the control medium also, but the number of plants was less when compared to control calli and the germination potential of the somatic embryos from the tolerant line was low. In *Pennisetum purpureum*, however, the regenerated NaCl tolerant plants did not retain its tolerance (Chandler and Vasil 1984) while in *Oryza sativa* (Vajrabhaya *et al.* 1989), *Citrus sinensis* (Ben-Hayyim and Goffer 1989), *Nicotiana tabacum* (Nabors *et al.* 1980) and *Linum usitatissimum* (McHughen 1987) the tolerance persisted in the regenerated plants and was heritable in some cases.

When grown in the hydroponic system all the control plants failed to grow in the medium with NaCl while the tolerant plants (66.7 %) flowered and set seeds.

The ion content analysis of the control calli as well as tolerant cell lines showed that the latter accumulated more Na^+ (19.2 ± 0.3 mg g^{-1}) than former (4.4 ± 0.2 mg g^{-1}). However, a low Na^+ content has ben reported in tolerant lines of *Citrus* (Ben-Hayyim and Kochba 1983). Pandey and Ganapathy (1984) reported that their unselected and selected *Cicer arietinum* cells showed no difference in Na^+ or Cl^- accumulation at low levels of salt, but selected lines accumulated more Na^+ and Cl^- at high levels of salt. NaCl tolerant cell lines and plants have been shown to contain more Na^+ in comparison with control (Tal *et al.* 1978, Chen *et al.* 1980, Liu and Yeh 1984, Liu and Li 1991).

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