

Effect of NaCl on axillary shoot proliferation in sweetpotato

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ABSTRACT

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Micropropagation through axillary shoot proliferation is a simple technique which ensures genetic stability of the propagules. To enhance the rate of multiplication, different doses of sucrose and NaCl were used for axillary shoot proliferation in sweetpotato. Murashige and Skoog's basal medium supplemented with growth regulators [NAA (0.5 mg/l) + BA (1.0 mg/l) + GA₃ (0.5 mg/l)] and variable doses of sucrose and NaCl were used. Among the different doses tested, 4% sucrose resulted in minimum bud dormancy. However, the overall shoot multiplication rate was optimal with 3% sucrose. Although the time required to bud break was considerably stable up to 0.5 g/l of NaCl supplementation and with 3% sucrose, the mean number of shoots produced per explant improved (3.5 – 5.5 shoots/explant) with increasing NaCl level up to 1.0 g/l irrespective of the genotype tested. High rate of multiplication with supplementation of 1.0 g/l is significant for *in vitro* multiplication and also to use axillary meristem as target tissue for genetic transformation. Delayed bud break response with 2.0 g/l NaCl supplementation can be exploited for *in vitro* storage of sweetpotato.

Keywords: axillary shoot proliferation, growth regulators, sodium chloride, sweetpotato.

INTRODUCTION

Sweetpotato is a nutritionally-rich, high energy food crop grown in different agro-ecological conditions. It is gaining world wide importance as a base material for food, feed and industrial products. *In vitro* propagation methods are being routinely used in sweetpotato for the purpose of storage as well as exchange of genetic resources (Kuo, 1991; Mukherjee, 1999a). Propagation of sweetpotato through axillary or apical bud proliferation (Henderson *et al.*, 1984; Griffith and Slack, 1990; Mukherjee *et al.*, 2000) through adventitious organogenesis (Gosukonda *et al.*, 1995) or through somatic embryogenesis (Chee *et al.*, 1990; Mukherjee *et al.*, 1991) have already been reported. Multiplication through the culture of axillary buds results in 3 to 10 fold increase in shoot number in monthly culture passage. This rate may be a little slower than in other *in vitro* methods but is the simplest and stable propagation protocol and it ensures genetic stability of the propagules. A good regeneration system is prerequisite for the development of a genetic transformation system. While there have been reports on adventitious organogenesis and genetic transformation in sweetpotato (Garcia *et al.*, 1995; Egnin and Prakash, 1997), the influence of genotype often limits its wide application. Development of simple and genotype independent multiplication system will have great implications for sweetpotato biotechnology. Thus, the present investigation was undertaken to enhance the rate of *in vitro* multiplication in different genotypes of sweetpotato. The influence of different doses of sucrose and NaCl for optimizing the rate of shoot proliferation in all the tested genotypes are discussed.

MATERIALS AND METHODS

Four varieties of sweetpotato (*Ipomoea batatas* L.) viz. S 132, 187004.3, 8570 and 8516 maintained at the Regional Center of Central Tuber Crops Research Institute (ICAR) at Bhubaneswar, India were used based on the results of a preliminary screening of 111 genotypes of sweetpotato (Mukherjee, 1999b). The cultivar 187004.3 was introduced from the International Potato Center (CIP), Lima, Peru. Nodal explants (3-5 mm) collected from the field source were surface sterilized with 1% sodium

hypochlorite for 5-7 min, washed three times with sterilized water and cultured in various combinations of media. Based on the results of previous studies, the Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with growth regulators was used as the common medium for both the experiments. The growth regulators were 0.5 mg/l naphthalene acetic acid (NAA), 1.0 mg/l butyric acid (BA) and 0.5 mg/l geberyllic acid (GA_3) to study the influence of exogenous supplementation of NaCl, media combinations containing growth regulators and 3 % sucrose were used as control. The pH of the media was adjusted to 5.8 and was solidified with 8% agar throughout the duration of the study. The specific media composition with different doses of sucrose and NaCl for the different experiments are mentioned in the respective figures under the results and discussion section.

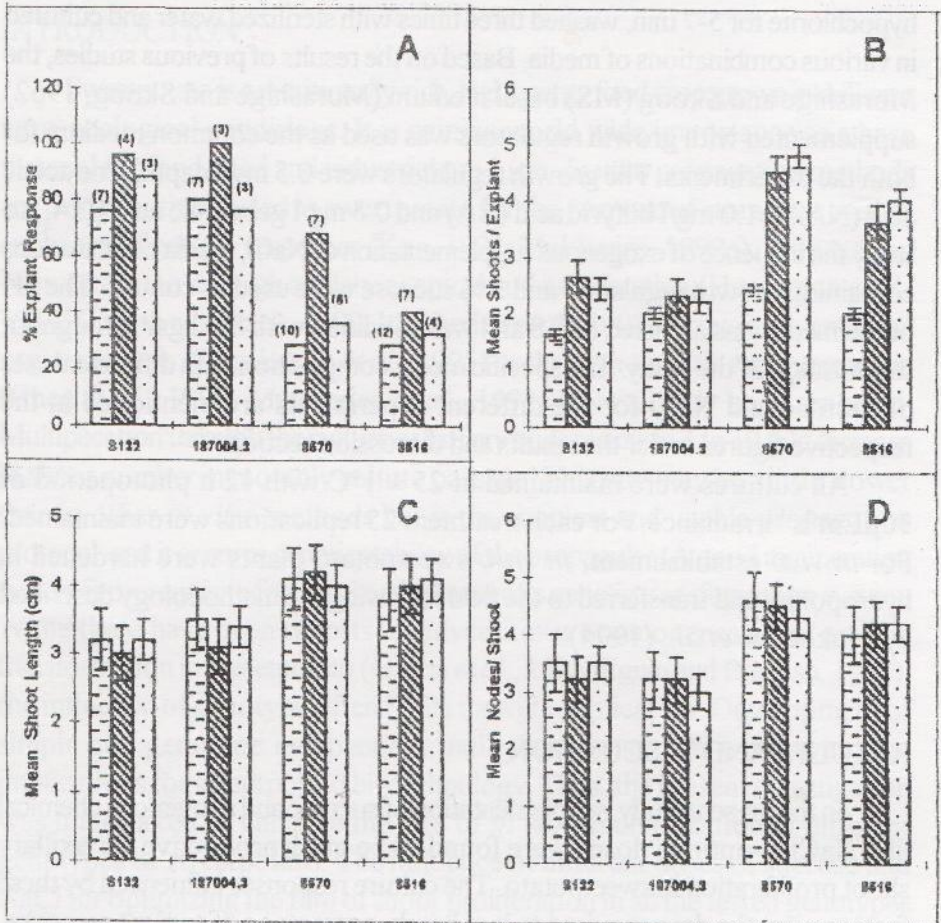
All cultures were maintained at $25 \pm 1^\circ\text{C}$ with 12 h photoperiod of $30\mu\text{Em}^{-2}\text{s}^{-1}$ irradiance. For each treatment 25 replications were maintained. For *in vivo* establishment, *in vitro* sweetpotato plants were hardened in hydroponics and transferred to the field following the methodology described by Mukherjee *et al.*, (1994).

RESULTS AND DISCUSSION

In the present study, supplementation of sucrose and exogenous chemical like NaCl at optimal doses were found to be quite productive for axillary shoot proliferation in sweetpotato. The culture responses influenced by these factors were the days required to bud break, percentage of explant response, average number of shoots produced per explant, shoot growth and quality and the average number of nodes per shoot.

Influence of different doses of sucrose

Responses of axillary buds cultured on MS medium with growth regulators along with different levels of sucrose are presented in Fig. 1 A-D. Although the time required for bud breaking was minimum with 4% sucrose the overall shoot multiplication rate was optimal with 3% sucrose (Fig. 1 A). There was no remarkable variation in shoot length or number of nodes per shoot with the variation in concentration of sucrose (Fig. 1C & D). Thus,



Sucrose concentrations - 2% - [Dotted pattern], 3% - [Diagonal lines], 4% - [Horizontal lines]

Figure 1. Effect of different concentrations of sucrose on the rate of shoot multiplication in axillary buds of four sweetpotato genotypes (S132, 187004.3, 8570 and 8516) four weeks after culture on MS + NAA (0.5 mg/l) + BA (1 mg/l) + GA₃ (0.05 mg/l) and incubated within 12 h photoperiod (30 μEm⁻²s⁻¹) at 25 ± 2°C.

- A = % explant response of 25 replicates, values in parentheses are number of days required to bud break;
- B = mean number of shoots/explant ± standard error (SE);
- C = mean shoot length (cm) ± SE;
- D = mean number of nodes/shoot ± SE.

optimal sucrose concentration for maximum culture response was recorded to be 3% for all the tested genotypes. Influence of genotype was also elicited at a particular concentration of sucrose. Bud dormancy was only three days for the genotypes S 132 and 187004.3, but was almost double (6 days) for the genotype 8570. Higher concentration of sucrose was more effective for the genotypes 8570 and 8516. Positive response at higher concentration of sucrose was also noticed for the mean number of shoots produced per explant in the genotypes 8570 and 8516 in contrast to the genotypes S 132 and 187004.3. Percent explant response also varied among the genotypes, and was as high as 100% in 187004.3 and as low as 44% in 8516. However, mean number of shoots produced per explant was minimum (2.5 ± 0.3) in the genotype 187004.3 and was maximum (4.5 ± 0.2) in 8570 in the medium containing 3% sucrose. Despite genotype influences, 3% sucrose was found optimal in maintaining the metabolic integrity of the cultured tissues or explants of sweetpotato under *in vitro* conditions.

Effect of NaCl supplementation

The percentage explant response as well as the days required to bud break were considerably stable in the four tested genotypes up to 0.5 g/l of NaCl supplementation but the explant response slightly decreased at higher (1.0 g/l) NaCl level (Fig. 2 A-D). On the other hand, the mean number of shoots produced per explant concomitantly improved with increasing NaCl level (up to 1.0 g/l) in all the genotypes (Fig. 3.). Irrespective of the genotype tested, the shoot multiplication rate was optimal with NaCl concentration of 1.0 g/l in the medium. At this concentration of NaCl, the genotype 8570 recorded the highest shoot multiplication rate (5.2 ± 0.2 shoots/explant). Compared to the control medium, the medium containing 2.0 g/l NaCl appeared to have some inhibitory effect on shoot multiplication in the case of the genotypes S132 and 187004.3 but it was not so in the case of 8570 and 8516.

A high frequency (90-100%) *in vivo* establishment was recorded for sweetpotato when the plantlets were obtained with a supplement of NaCl. The influence of genotype was also pronounced with NaCl treatment. Bud dormancy was double (6 days) in 187004.3 genotype with 1-2 g NaCl supplementation as compared to the control medium (3 days). Similarly, percent

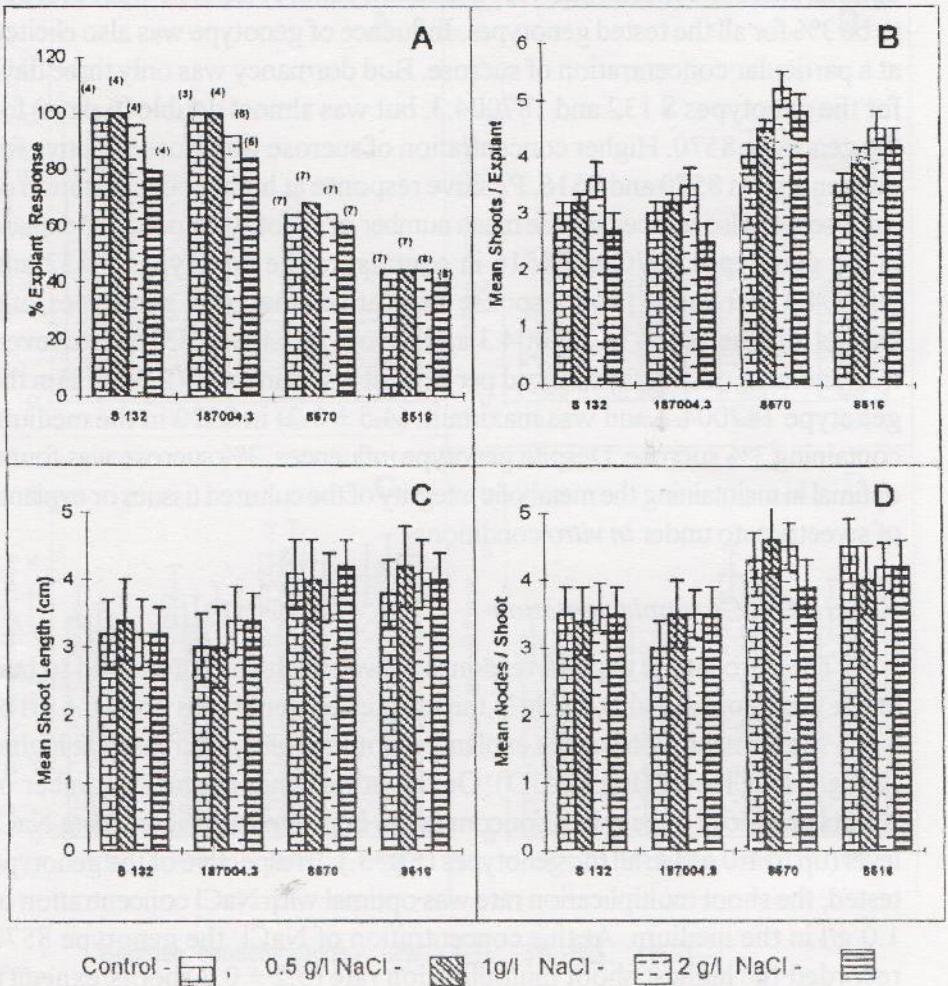


Figure 2. Effect of different concentrations of NaCl on the rate of shoot multiplication in axillary bud cultures of four sweetpotato genotypes (S132, 187004.3, 8570 and 8516) four weeks after culture on MS + NAA (0.5 mg/l) + BA (1 mg/l) + GA₃ (0.05 mg/l) + sucrose within 12 h photoperiod (30m Em⁻²s⁻¹) at 25 ± 2°C.

- A = % explant response of 25 replicates, values in parentheses are number of days required to bud break;
 B = mean number of shoots/explant ± standard error (SE);
 C = mean shoot length (cm) ± SE;
 D = mean number of nodes/shoot ± SE.

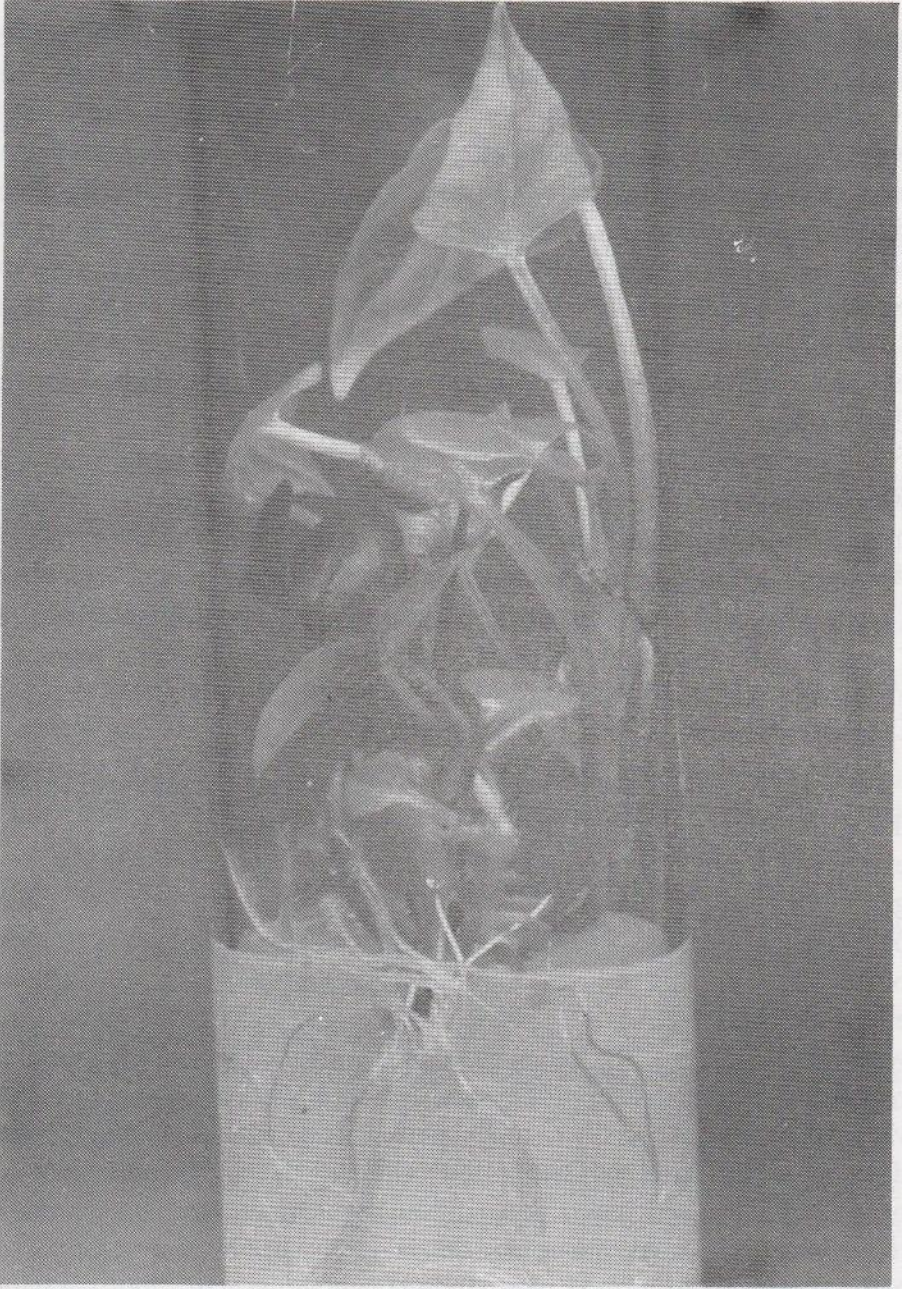


Figure 3. Multiple shoots produced from axillary bud with NaCl treatment in sweetpotato

explant response did not improve with NaCl treatment except for the 8570 genotype. However, the mean number of shoots per explant improved with NaCl treatment in all the genotypes. The number of shoots produced was highest (5.4 ± 0.5) in 8570 followed by 8516 (4.6 ± 0.4). Variations in mean shoot length was elicited among the genotypes at different doses of NaCl (Fig. 2C). Positive influence of NaCl in enhancing the number of nodes only registered in the genotypes 187004.3 and 8570 (Fig. 2D). Overall response was better in 8570 and 8516 with NaCl treatment as compared to S132 and 187004.3. These results are significant to be able to isolate salt-tolerant lines of 8570 and 8516 which have already been released for mass cultivation in Orissa state.

Mukherjee (1999a) revealed that the production of somatic embryos with higher doses of NaCl has not affected the genetic integrity of the propagules. The results regarding the influences of genotype on the rate of *in vitro* propagation more or less agreed with earlier studies of Henderson *et al.* (1984), Ng (1986), Lizzaraga *et al.* (1992) and Mukherjee *et al.* (1998).

CONCLUSION

From the results, it can be concluded that shoot regeneration efficiency, though influenced by genotype, can be enhanced significantly (5-10 fold) by exogenous supplementation of NaCl. Despite genotypic influence, all the genotypes (both the highly responsive as well as the less responsive) have performed well with NaCl supplementation. Production of the highest number of shoots per explant in a less responsive genotype like 8516 revealed the efficacy of NaCl treatment (Mukherjee, 1999b). The high rate of proliferation with 1.0 g/l NaCl, and the longer bud dormancy with 2.0 g/l is encouraging results for storage of micropropagules. Growth inhibition in sweetpotato *in vitro* using ABA especially to inhibit axillary bud development have been discussed earlier (Jarret and Gawel, 1991). Growth inhibition through extension of bud dormancy with higher doses of NaCl could be an excellent approach to get genetically stable dormant propagules. Delayed bud break response could also be recorded at 8th photoperiod (Mukherjee *et al.*, 2000) or with supplementation of 2% sucrose in the medium. However, shoot quality was

not as good as those obtained with NaCl and sucrose. Hence, supplementation of exogenous chemical like NaCl is quite effective for propagation as well as for storage purposes.

Results of the present study are highly significant to provide genetically stable propagules for judicious utilization by rapid multiplication program, for *in vitro* storage and also for possible use of axillary meristem as a target tissue for genetic transformation of sweetpotato.

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