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Effect of NaCl on In Vitro Propagation of Sweet Potato (*Ipomoea batatas* L.)

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Abstract

In vitro propagation protocols offer a better option for production of quality planting materials in a clonal crop such as sweet potato, which is a food crop of versatile uses. Propagation through axillary shoot proliferation and organogenic and embryogenic regeneration were studied in different genotypes of sweet potato. The addition of NaCl enhanced the rate of multiplication as well as yielded hardy somatic embryos. Optimal doses of NaCl in each mode of propagation were different. The hardy somatic embryos produced in NaCl medium could be stored at 8°C with or without a protective alginate covering. High-frequency germination of stored hardy somatic embryos could facilitate the production of artificial seeds. Plantlets produced with the addition of NaCl in regeneration medium were established in vivo at a high frequency (95–100%). Yield and quality of storage roots developed from artificial seed–propagated plants were comparable with those of source plants.

Index Entries: Sweet potato; organogenesis; somatic embryogenesis; artificial seeds; sodium chloride; storage roots; isozymes.

Introduction

Sweet potato (*Ipomoea batatas* L.) is an important food crop grown throughout tropical and subtropical regions for its edible tubers. In addition to excellent nutritional value, storage roots also serve as a rich source of starch for industrial uses.

Research targets today are not only geared toward increased production but production of high-quality planting materials with efficient management of resources. This shift in paradigm gives more credence for in vitro propagation of a crop such as sweet potato, a food crop of versatile uses.

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Propagation through axillary or apical bud proliferation *(1,2)*, through adventitious organogenesis *(3)*, or through somatic embryogenesis *(4–6)* has been reported in sweet potato. However, its wider application is hindered owing to low regeneration frequency. Uniformity in quantitative as well as qualitative characteristics is also important for wider application of in vitro propagation protocols for production of "true-to-type" plants. Several factors, such as genotypes, explants, nature and dose of different growth regulators, as well as other stress-inducing compounds in the regeneration medium, have been found to influence the rate of propagation in different crop species including sweet potato *(7–10)*.

Considering all these factors, the present study was undertaken to produce quality planting materials through all possible modes of propagation. We discuss the effect of NaCl on enhanced propagation through axillary shoot proliferation, organogenic and embryogenic regeneration, and production of good-quality hardy propagules with high-frequency in vivo establishment.

Materials and Methods

Plant Source and Media

Four varieties of sweet potato (*I. batatas* L.)—S 132, 187004.3, 8570, and 8516—maintained at Regional Centre of Central Tuber Crops Research Institute (ICAR) at Bhubaneswar, were used. The cultivar 187004.3 was introduced from International Potato Center (CIP), Lima, Peru. Nodal explants (3–5 mm) collected from the field source were surface sterilized with 1% sodium hypochlorite solution for 7 to 8 min, washed thrice with sterilized water, and cultured. For all the experiments, Murashige and Skoog's basal medium *(11)* supplemented with mesoinositol (100 mg/L) and sucrose (3%) was used. Basal medium supplemented with growth regulators—1 mg/L of benzyladenine (BA), 0.5 mg/L of α-napthalene acetic acid (NAA), and 0.05 mg/L of gibberellic acid (GA_3)—and NaCl (0.5–2 g/L) were used for axillary shoot proliferation studies.

Organogenic and Embryogenic Regeneration

For the study of organogenic and embryogenic regeneration, the third and fourth leaves from the shoot apex were excised and cleaned thoroughly in running tap water. Leaves were surface sterilized with 1% sodium hypochlorite solution for 5 to 6 min, rinsed three times in sterilized water, and then whole lamina was aseptically cut into 2- to 3-mm segments for initiation of callus cultures. Callus cultures were raised on MS basal medium supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D) (0.5 and 2 mg/L) based on the results of a previous study *(12)*. For shoot organogenesis from callus cultures raised in 2,4-D (2 mg/L), MS basal media supplemented with or without BA (0.2–1 mg/L) and NaCl (100–400 mg/L) were tested.

For embryogenic regeneration, calli raised in medium supplemented with 2,4-D (0.5 mg/L) were studied further on MS basal medium supplemented with or without BA (1 mg/L) and BA in combination with 2,4-D (0.5 mg/L) and NaCl (2.5, 5, and 10 g/L) along with 2,4-D (0.2 mg/L). The pH in all the media was adjusted to 5.7 prior to gelling with 0.8% agar (bacteriologic grade; HiMedia, India) and autoclaved for 15 min at 1.15 kg/cm^2 pressure at 121°C. All the cultures were maintained at 25 ± 1°C with a 12-h photoperiod of 30 μ Em^{-2}s^{-1} irradiance. For each treatment 25 replications were maintained.

Artificial Seed Production

For artificial seed production, isolated and single somatic embryos developed in NaCl-supplemented medium were used. Sodium alginate (2.5% [w/v]) and calcium nitrate solution (2% [w/v]) prepared in half-strength MS liquid culture medium devoid of sucrose and organics were used for encapsulation of the somatic embryos.

In Vivo Establishment

Plantlets developed through axillary shoot proliferation and organogenic and embryogenic regeneration were hardened in hydroponics (tap water + 0.01% Hoagland's nutrient) and transferred to the field *(13)*. Both encapsulated and nonencapsulated somatic embryos were stored at 8°C and were planted in vitro in MS basal medium and also in vivo in small plastic cups containing a sterilized soil:sand mixture (1:1). To evaluate storage root characteristics, plants were subsequently transferred to field conditions in bigger pots and then in the field. A field trial of regenerated plants was conducted in randomized block design with three replications following the standard package of practices. Mother clones or source plants of each genotype were also planted with the regenerated plants to serve as control. Plants were harvested 120 d after planting.

Estimation of Dry Matter, Starch, and Sucrose of Storage Roots

Freshly harvested tubers were cleaned, sliced, weighed (5–7 g for each sample with three to five replications), and dried in an oven at 70–80°C for 48–72 h. Oven-dried samples were weighed, and the difference was calculated to estimate dry matter content. Starch was estimated by the anthrone reagent method *(14)*. Sucrose contents of storage roots were analyzed following the phenol–sulfuric acid method *(15)*.

Isozyme Studies

Plants raised from artificial seeds developed from embryogenic callus cultures and the source plant or mother clones were subjected to isozyme studies. The enzyme extracts were prepared by homogenizing 0.5 g of the young leaves in a chilled mortar and pestle with the extraction buffer Tris-HCl (0.2 *M*, pH 7.4), 17% sucrose, 0.1% ascorbic acid, and 0.1% cystein HCl.

The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C. The clear supernatant was used as the enzyme extract.

Starch gel electrophoresis was carried out based on published reviews *(16)*. The buffer systems used were recommended by Geves, France, for varietal identifications in a number of crop plants *(17)*. The special apparatus and equipment required for starch gel electrophoresis were a horizontal electrophoresis system with gel trays, refrigerated at 4°C; a vacuum pump; and paper wicks (3×10 mm). The enzymes studied were acid phosphatases, esterases, and peroxidases following the specific staining reagents *(18,19)*.

Results

Of the three combinations of growth regulators tested for axillary shoot multiplication, the best response was obtained with the combination containing 0.5 mg/L of NAA, 1 mg/L of BA, and 0.05 mg/L of GA_3 for all the studied genotypes (Table 1). This combination of growth regulators considerably reduced the time required for bud break response (3–7 d) compared with the response (5–10 d) observed in other combinations of growth regulators.

Effect of NaCl on Axillary Bud Culture

The percentage explant response as well as the days required to bud break were considerably stable in all four tested genotypes up to 0.5 g/L of NaCl supplementation in the medium containing all the growth regulators. However, the explant response slightly decreased at a higher (1 g/L) NaCl level. On the other hand, the mean number of shoots produced per explant concomitantly improved with increasing NaCl level (up to 1 g/L) in all the genotypes. Irrespective of the genotype tested, the shoot multiplication rate was optimal with an NaCl concentration of 1 g/L in the medium. At this concentration, the genotype 8570 recorded the highest shoot multiplication rate (5.2 ± 0.9 shoots/explant) among all the genotypes. Compared with the medium containing growth regulators alone, medium containing 2 g/L of NaCl was observed to be slightly inhibitory for shoot multiplication rate in the case of genotypes S 132 and 187004.3 but not in genotypes 8570 and 8516.

Effect of NaCl on Organogenic Regeneration

During the course of the present investigation, only the cytokinin (BA) and the stress-inducing substance NaCl were tested (Fig. 1). There was no significant improvement in the organogenic response of the leaf calli of various genotypes when the MS basal medium was enriched with different concentrations of BA. With these treatments, the callus proliferated further instead of undergoing organogenesis. However, organogenic response was significantly enhanced by NaCl (100, 200, or 400 mg/L) supplementation to the medium. The optimal response was recorded at 200 mg/L of NaCl. However, in terms of the percentage of callus showing organogenic

Table 1
Rate of Shoot Multiplication in Axillary Bud Cultures of Different Genotypes of *I. batatas* 4 wk After Culture on MS Medium Supplemented with Different Growth Regulators and Doses of NaCl

Genotype and treatment[a]	Response			
	Explant response (%)	Days to bud break	Mean shoots/explant ± SE	Mean nodes/shoot ± SE
S 132				
MS	8	10	2.00 ± 0.40	2.00 ± 0.08
MS + NAA 0.5 + BA 1	100	7	2.32 ± 0.40	3.02 ± 0.10
MS + NAA 0.5 + BA 1 + GA_3 0.05	100	4	2.64 ± 0.17	3.03 ± 0.10
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 0.5	100	4	3.2 ± 0.20	3.4 ± 0.10
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 1	96	4	3.6 ± 0.80	3.6 ± 0.13
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 2	80	6	2.7 ± 0.10	3.5 ± 0.30
187004.3				
MS	12	10	1.50 ± 0.15	2.00 ± 0.00
MS + NAA 0.5 + BA 1	100	5	2.28 ± 0.14	2.96 ± 0.20
MS + NAA 0.5 + BA 1 + GA_3 0.05	100	3	2.36 ± 0.12	3.07 ± 0.09
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 0.5	100	4	3.20 ± 0.40	3.50 ± 0.13
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 1	92	6	3.5 ± 0.10	3.50 ± 0.15
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 2	84	6	2.5 ± 0.17	3.53 ± 0.30
8570				
MS	0	0	0	0
MS + NAA 0.5 + BA 1	40	10	3.60 ± 0.80	3.61 ± 0.13
MS + NAA 0.5 + BA 1 + GA_3 0.05	68	7	4.53 ± 0.90	4.52 ± 0.22
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 0.5	68	7	4.5 ± 0.80	4.60 ± 0.22
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 1	64	7	5.2 ± 0.90	4.50 ± 0.28
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 2	60	7	4.8 ± 0.80	3.90 ± 0.26
8516				
MS	0	0	0	0
MS + NAA 0.5 + BA 1	28	10	3.71 ± 0.18	3.77 ± 0.30
MS + NAA 0.5 + BA 1 + GA_3 0.05	44	7	3.80 ± 0.24	3.94 ± 0.26
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 0.5	44	7	4.00 ± 0.30	4.00 ± 0.16
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 1	44	8	4.50 ± 0.60	4.20 ± 0.19
MS + NAA 0.5 + BA 1 + GA_3 0.05 + NaCl 2	40	8	4.20 ± 0.60	4.52 ± 0.22

[a]Growth regulators are given in milligrams/liter and NaCl in grams/liter.

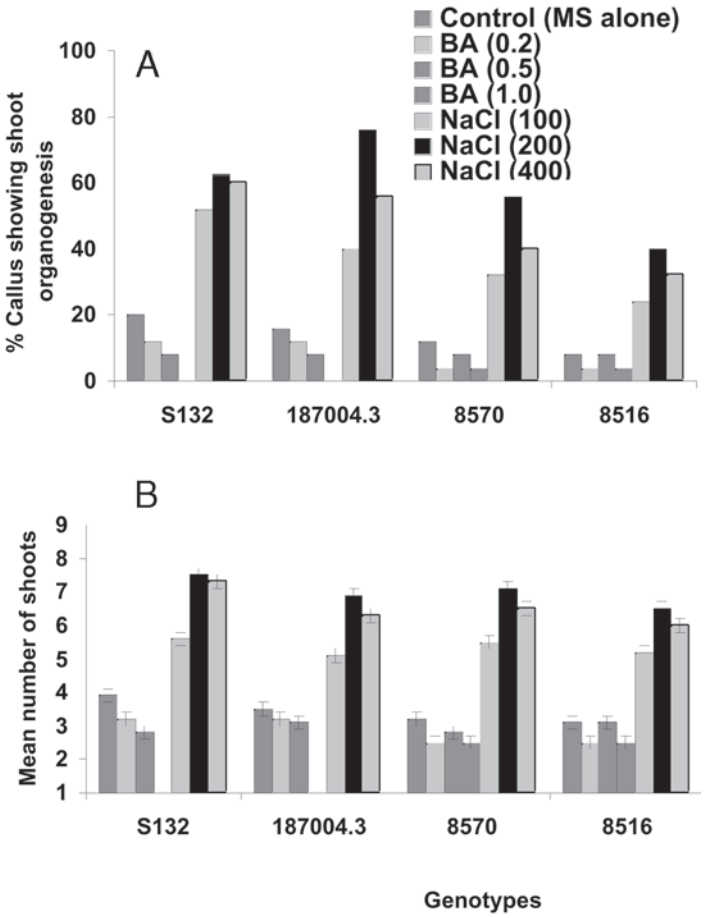

Fig. 1. Effect of growth regulator (0.2, 0.5, 1 mg/L of BA) and NaCl (100, 200, 400 mg/L) on shoot organogenesis in leaf callus of different genotypes of sweet potato. **(A)** Percentage of callus showing shoot organogenesis; **(B)** mean number of shoots/50 mg of callus inoculated ± SE. The calli used were derived from a callusing medium (MS + 2 mg/L 2,4-D). Number of replications = 25.

response (Fig. 1A) and mean number of shoot buds/50 mg of callus (Fig. 1B), the best results were obtained when the calli were cultured on MS supplemented with NaCl (200 mg/L) in all four genotypes tested. Similarly, of all the genotypes examined, the leaf calli of genotype S 132 was the most responsive (84% explant response, 7.5 ± 0.6 shoots/50 mg of callus cultured) followed by those of genotypes 187004.3, 8570, and 8516.

Effect of NaCl on Somatic Embryogenesis

Leaf explants of all four genotypes produced callus within 2–4 wk of inoculation on MS + 2,4-D (0.5 mg/L) medium. The leaf callus when separated and subcultured on MS basal medium produced somatic embryos in varying frequencies within 3–7 wk in different genotypes except 8516 (Fig. 2). The addition of the growth regulator BA alone or in combination

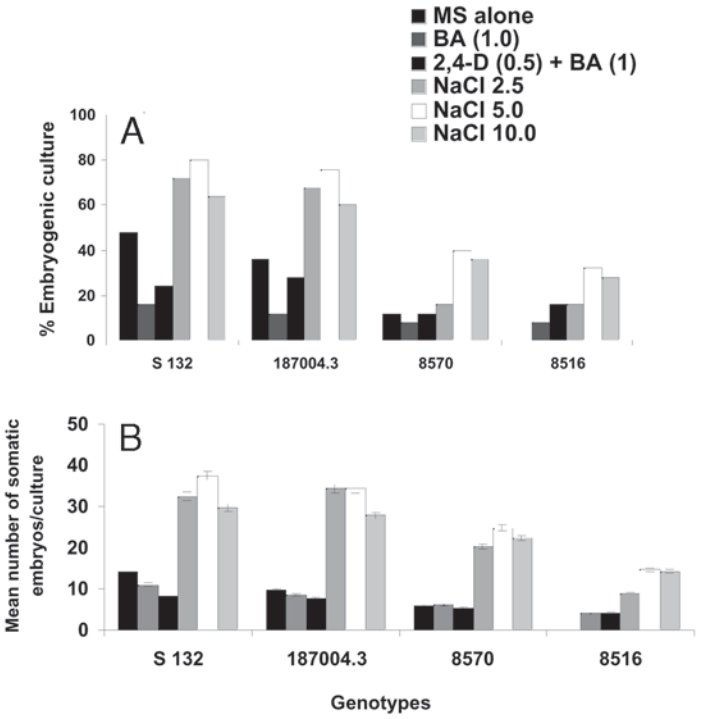

Fig. 2. Effect of growth regulators (1 mg/L of BA, 0.5 mg/L of 2,4-D + 1 mg/L of BA) and NaCl (2.5, 5.0, 10 g/L) on somatic embryogenesis in leaf calli of different genotypes of sweet potato. **(A)** Percentage of embryogenic cultures of 25 replicates; **(B)** mean number of somatic embryos/responsive cultures ± SE. The calli used were derived from a callusing medium (MS + 0.5 mg/L of 2,4-D).

with 2,4-D did not improve the embryogenic frequency as compared to MS basal medium alone in the case of S 132, 187004.3, and 8570. However, embryonic production did occur in genotype 8516 with the addition of BA (1 mg/L) or BA (1 mg/L) + 2,4-D (0.5 mg/L).

The embryogenic response in terms of both percentage of embryogenic culture and number of somatic embryos/responsive culture (Fig. 2A,B) improved with the addition of NaCl as compared to the control media (MS alone for genotypes S 132, 187004.3, and 8570 and MS + 0.5 mg/L of 2,4-D + 1 mg/L of BA for genotype 8516). Of the three doses of NaCl tested, 5 g/L consistently produced the most somatic embryos per culture (Fig. 3A) for all the tested genotypes. Depending on the genotype, the mean number of somatic embryos/ culture could be enhanced by two- to fourfold when compared to control.

The embryos formed were also predominantly single (Fig. 3B) on media supplemented with NaCl. The process of embryonic development and plantlet formation could be enhanced by isolation and transfer of the heart-to-torpedo stages of somatic embryos to fresh MS medium. A high-frequency (90–100%) plantlet formation was recorded from stored encapsulated (Fig. 3C,D) and nonencapsulated somatic embryos. Plantlets

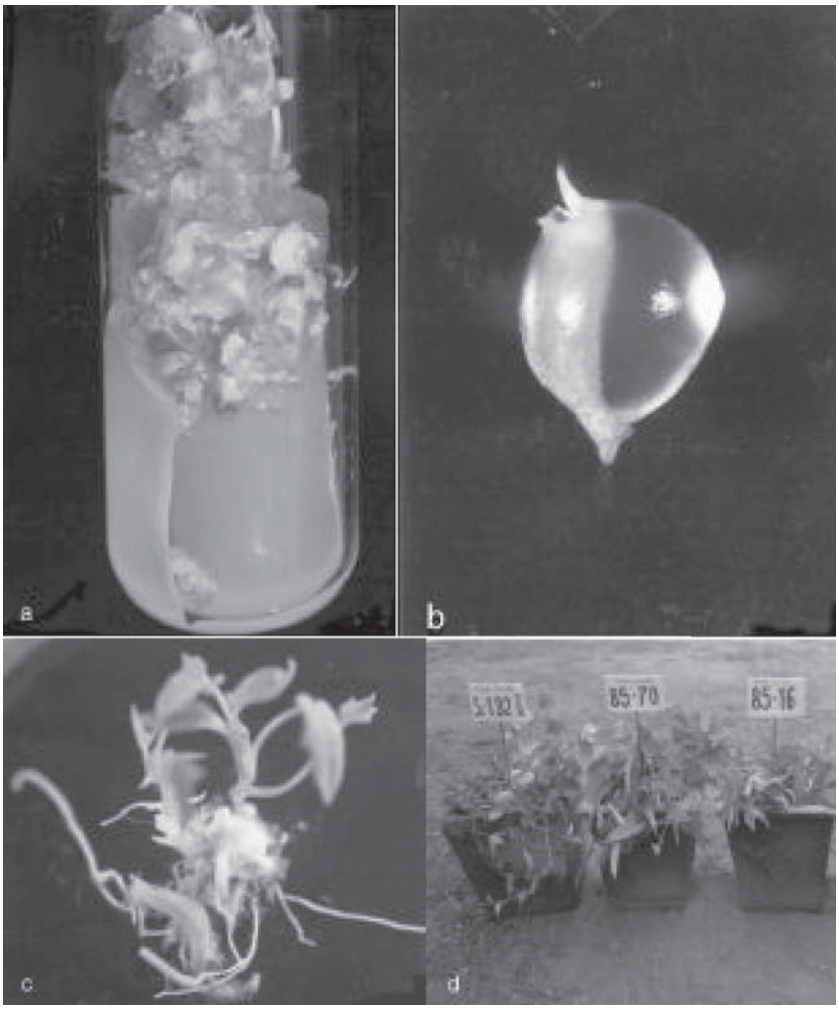

Fig. 3. Enhancement of somatic embryogenesis and plantlet formation from somatic embryos in leaf callus cultures of sweet potato. **(A)** Enhanced somatic embryogenesis in leaf callus and bipolar differentiation of embryos developed on MS + NaCl (5 g/L) 4 wk after culture (magnification: ×1.6); **(B)** single somatic embryo encapsulated with sodium alginate stored at 8°C and germinating on plain MS medium 1 wk after plantation (magnification: ×4.5); **(C)** plantlet developed from stored encapsulated somatic embryos (artificial seed); **(D)** plantlet developed from somatic embryos and artificial seeds of different sweet potato genotypes established in potted soil.

developed through all modes of propagation with NaCl supplementation in regeneration medium were established in the fields at a very high frequency (90–100%).

Evaluation of Biochemical Characteristics of Storage Roots of Regenerated Plants

A comparative evaluation of yield and the biochemical constituents dry matter, starch, and sucrose revealed a low degree of variation among

Table 2
Evaluation of Yield and Biochemical Characteristics of Mother Clones and Regenerants Developed from Artificial Seeds

Genotype[a]	Yield range (g/plant)	Yield range (t/ha)	Dry matter (%)	Starch (%)	Sucrose (%)
S 132					
MC	240–265	20–22	40.50 ± 0.70	29.30 ± 0.20	2.50 ± 0.20
RM	260–275	22–23	40.30 ± 0.90	29.00 ± 0.50	2.50 ± 0.50
VR1	290–300	24–25	40.80 ± 0.70	29.20 ± 0.50	2.60 ± 0.30
VR2	280–300	23–25	41.00 ± 0.80	29.50 ± 0.70	2.80 ± 0.40
VR3	275–300	23–25	41.50 ± 0.70	30.60 ± 0.50	3.10 ± 0.30
VR4	275–300	23–25	41.60 ± 0.70	30.30 ± 0.70	3.10 ± 0.30
8570					
MC	280–300	23–25	25.30 ± 1.20	16.40 ± 0.50	2.10 ± 0.10
RM	295–310	24–26	25.30 ± 0.70	16.80 ± 0.70	2.30 ± 0.50
VR	325–335	27–28	26.30 ± 0.70	17.50 ± 0.50	2.40 ± 0.10
187004.3					
MC	255–275	21–23	38.90 ± 0.90	26.90 ± 0.90	2.40 ± 0.10
RM	270–280	22–23	39.30 ± 0.70	27.30 ± 0.70	2.50 ± 0.30
8516					
MC	360–390	30–32	23.70 ± 0.90	14.50 ± 0.50	2.30 ± 0.20
RM	385–400	32–33	24.00 ± 1.20	14.80 ± 0.70	2.40 ± 0.20

[a]MC, mother clone; RM, regenerant like that of mother clone; VR, variant regenerant.

some of the regenerants and their respective mother clones (Table 2). The regenerants VR1 to VR4 of S 132 and VR of 8570 with a higher range of yield were found to be qualitatively as good as source plants or mother clones. These variant regenerants were subsequently screened using isozyme markers to confirm the stability of those variations. Profiles of the isozymes esterases, peroxidases, and acid phosphatases were studied to compare the regenerants with their respective source plants. Uniformity was noticed in isozyme profiles of regenerants and source plants.

Discussion

The observed results regarding the influences of genotype on the rate of in vitro propagation are more or less in agreement with those of earlier studies *(1,5,12,20)*. From the results, it can be concluded that rate of multiplication can be enhanced significantly (5- to 10-fold) by exogenous supplementation of NaCl in all the genotypes.

Variation in organogenic regeneration in different genotypes, as observed in the present study, is a common feature in the case of sweet potato *(1,6)*. However, supplementation of NaCl in regeneration medium enhanced the organogenic regeneration in all the tested genotypes.

Sweet potato cell cultures undergo complex developmental sequences as they respond to different growth regulators. Various auxins and cytokinins have been used for callus formation and embryogenesis. The auxin

2,4-D was the most commonly used auxin although the doses used for embryogenesis varied *(4,5,12,21)*. The positive influence of cytokinin along with auxin during embryogenic regeneration in certain sweet potato clones is in agreement with other studies *(22)*.

Furthermore, our results in sweet potato revealed that supplementation of an exogenous chemical such as NaCl could be more effective for enhancing the embryogenic response, by two- to fourfold, when used along with 2,4-D. The positive influence of NaCl on embryogenesis in rice *(8)* and wheat *(23)*, and KCl and NH_4NO_3 in sweet potato *(4,9)* also supports the present findings on enhanced rate of propagation with exogenous supplementation of a stress chemical such as NaCl.

Singulation of somatic embryos has its advantages for production of artificial seeds. Improvement in production and development of single somatic embryos either by physical fragmentation or by the addition of exogenous chemicals such as NH_4NO_3 and KCl and also by regulating gases such as ethylene, CO_2, and oxygen have previously been studied in sweet potato *(4,9,24)*. More recently, diagnostic separation of competent embryos through a computer vision system have also been explained *(25)*. The production of predominantly single and sturdy somatic embryos with mere supplementation of an exogenous chemical such as NaCl in the culture medium in the present study seems to be a simple and convenient approach. Those sturdy somatic embryos could tolerate cold-temperature (8°C) stress and could show good conversion frequencies (90–100%) into plantlets with equal success of field establishment.

Uniformity in qualitative and quantitative characteristics is the most important aspect of in vitro propagation protocols. Homology in the banding patterns of isozymes in the present study that were studied as markers to characterize different varieties in sweet potato *(26)* ensures that regenerated plants are as good as source plants.

The results on enhanced rate of propagation with optimal doses of NaCl are highly significant for utilization of in vitro protocols judiciously for moderate to rapid propagation of sweet potato, to obtain quality planting materials, to develop novel and cost-effective artificial seed production techniques, and also to isolate salt-tolerant lines of popular genotypes of the high-energy food crop.

References

1. Henderson, J. H. M., Phills, B. R., and Whatley, B. T. (1984), in *Handbook of Plant Cell Culture*, vol. 2, Sharp, W. R., Evans, D. A., Ammirato, P. V., and Yamada, Y., eds., Macmillan, New York, pp. 302–326.
2. Griffiths, H. M. and Slack, S. A. (1990), *Phytopathology* **80(10),** 1061.
3. Gosukonda, R. M., Porobodessai, A., Blay, E., Prakash, C. S., and Peterson, C. M. (1995), *In Vitro* **31,** 65–71.
4. Chee, R. P., Leskovar, D. I., and Cantliffe, D. J. (1992), *Am. Soc. Hort. Sci.* **117(4),** 663–667.
5. Mukherjee, A., Debata, B. K., and Naskar, S. K. (1998), *J. Sci. Ind. Res*. **57,** 709–715.
6. Mukherjee, A. (1999), in *Biotechnology and Its Application in Horticulture*, Ghosh, S. P., ed., Narosa, New Delhi, pp. 267–294.

7. Stuart, D. A. and Strickland, S. G. (1984), *Plant Sci. Lett.* **34,** 165–174.
8. Karim, N. H. and Zapata, F. J. (1994), *Indian J. Plant Physiol.* **37,** 242–245.
9. Bieniek, M. E., Harrell, R. C., and Cantliffe, D. J. (1995), *Plant Cell Rep.* **41,** 1–8.
10. Norton, M. A. and LaBonte, D. R. (1996), *Hort. Sci.* **31(4),** 630.
11. Murashige, T. and Skoog, F. (1962), *Physiol. Plant* **15,** 473–497.
12. Mukherjee, A., Debata, B. K., and Naskar, S. K. (1998), *Cytobios* **96,** 109–118.
13. Mukherjee, A., Nair, N. G., and Rajendran, P. G. (1994), *J. Root Crops* **20(2),** 123, 124.
14. McCready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. (1950), *Anal. Chem.* **22,** 1156–1158.
15. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Anal. Chem.* **28,** 350–356.
16. Shaw, C. R. and Prasad, R. (1970), *Biochem. Genet.* **4,** 297–320.
17. Bourgoin Greneche, M. and Lallemand, J. (1995), Electrophoresis and its application to the description of varieties. A presentation of the techniques used by the GEVES, UPOV Gazette and News Letter; **76,** 17–24.
18. Brewbaker, J. L., Upadhya, M. D., Makinen, Y., and McDonald, T. (1968), *Physiol. Plant* **21,** 930–940.
19. Brewer, G. J. (1970), in *An Introduction to Isozyme Techniques*, Academic, New York, London, p. 186.
20. Lizarraga, R., Panta, A., Espinoza, N. N., and Dodds, J. H. (1992), in *Tissue Culture of Ipomoea Batatas,* CIP Research guide No. 32, CIP, Lima, Peru.
21. Jarret, R. L., Salazar, S., and Fernandez, Z. R. (1984), *Hort. Sci.* **19,** 397–398.
22. Sehgal, C. B. (1975), *Beitr. Biol. Pflanz*. **51,** 47–52.
23. Galiba, G. and Yamada, Y. (1988), *Plant Cell Rep*. **7,** 55–58.
24. Harrell, R. C., Bieniek, M., and Cantliffe, D. J. (1992), *Biotech. Bioeng*. **39(4),** 378–383.
25. Padmanabhan, K., Cantliffe, D. J., Harrell, R. C., and McConnell, D. (1998), *Plant Cell Rep*. **17,** 685–692.
26. Kim, D. S., Oh, S. K., Chin, M. S., and Ray, J. H. (1996), *Korean J. Crop Sci.* **41(3),** 332–339.