BIOLOGIA PLANTARUM 40 (2): 285-290, 1997/98

BRIEF COMMUNICATION

Nutrient-encapsulation of potato nodal segments for germplasm exchange and distribution

D. SARKAR and P.S. NAIK

Division of Genetics and Plant Breeding, Central Potato Research Institute, Shimla-171 001, Himachal Pradesh, India

Abstract

Nutrient-encapsulation technique using *in vitro* grown nodal segments was developed as an alternative method for distribution of potato germplasm. The nodal cuttings of two potato genotypes, Kufri Jyoti and Kufri Lauvkar, were encapsulated in calcium-free Murashige and Skoog's (MS) medium containing either 2 or 3 % sodium alginate and 0, 1, 2 or 3 % saccharose. The encapsulated segments were stored in tubes with or without semisolid MS medium, and incubated in the dark at 25 ± 1 °C for 3 to 6 weeks. Presence of saccharose in the beads was found detrimental for regrowth of new shoots. In absence of saccharose, about 82 % and 53 % encapsulated segments initiated regrowth after 3 and 6 weeks of dark storage, respectively, in tubes containing MS medium. Storage in empty tubes deteriorated the encapsulated segments, and depressed shoot formation. For potato germplasm distribution, the encapsulated segments can be transported in small tubes containing semisolid MS medium.

Additional key words: alginate capsules, microcuttings, sodium alginate, *Solanum tuberosum* L.

Potato is a vegetatively propagated crop, and transfer of plant genetic resources in the form of tissue culture is one of the safest and most efficient methods (Ng 1991, 1992). Plantlets packed in polystyrene containers (Roca *et al.* 1979), and microtubers (Estrada *et al.* 1986) are used for potato germplasm distribution. However, survival is

Received 4 December 1996, *accepted* 25 August 1997.
Acknowledgements: We thank Dr. G.S. Shekhawat, Director, Central Potato Research Institute, Shimla for providing necessary facilities, and Dr. P.C. Gaur, Head, Division of Genetics and Plant Breeding for critically reviewing the manuscript. Comments and suggestions on the manuscript from the anonymous reviewer are gratefully acknowledged.
Fax: (0177) 224460; e-mail: cpri@x400.nicgw.nic.in

reduced if plantlets are kept in the dark too long during transit (Estrada *et al.* 1986). Microtubers have the advantages of greater robustness and long survival in darkness, but the length of time required for their production, and variability in the dormancy period (Leclerc *et al.* 1995) or sprouting may cause problems where demand for the germplasm is immediate.

This communication describes an alternative method of distribution of potato germplasm through encapsulation of *in vitro* grown nodal segments. The alginate-encapsulation technique was originally developed for encapsulation of somatic embryos (Redenbaugh *et al.* 1993). Similar methods were followed for encapsulation of *in vitro*-derived vegetative propagules (Ganapathi *et al.* 1992, Bapat 1993) used for plant propagation (Piccioni and Standardi 1995) or cryopreservation of the germplasm (Dereuddre *et al.* 1991a, b).

The present study was conducted to standardise a suitable technique for nutrient-encapsulation of potato nodal segments by using different concentrations of sodium alginate and saccharose, and to evaluate the response of encapsulated segments to two different storage conditions: in tubes on culture medium and in empty tubes.

Two potato genotypes (*Solanum tuberosum* L. cvs. Kufri Jyoti and Kufri Lauvkar) used, were propagated through shoot cuttings on MS (Murashige and Skoog 1962) medium with 8.39 μM calcium D-pantothenate, 0.29 μM gibberellic acid (GA_3), 0.054 μM 1-naphthaleneacetic acid (NAA) and 3 % saccharose, and was solidified with 8 g dm^{-3} agar (*HiMedia*, Mumbai, India). The cultures were maintained in 25-mm culture tubes (with 20-d subcultures) at temperature of 25 ± 1 °C and 16-h photoperiod at irradiance of 30 μmol m^{-2} s^{-1}. Single node cuttings 5 mm long (microcuttings) were aseptically dissected.

For encapsulation, sodium alginate was used at the concentrations of 2 and 3 %. The microcuttings were immersed in calcium-free MS medium containing respective concentrations of sodium alginate and supplemented with either 0, 1, 2 or 3 % saccharose. The alginate-coated microcuttings were then dropped with a forcept in complexing solution containing the MS medium supplemented with 100 mM $CaCl_2$ and respective saccharose concentrations. For polymerization of sodium alginate, the microcuttings were held in complexing solution for 20 min. After hardening, the alginate beads were gently rinsed for 10 min in deionized water to wash away excess $CaCl_2$. The pH of all media and solutions used in the experiment was adjusted to 5.7. Then they were autoclaved at 121 °C for 20 min. All operations were done under sterile conditions.

The alginate capsules were stored in 17-mm culture tubes (3 capsules per tube) with or without 3 cm^3 of MS medium supplemented with 3 % saccharose and 8 g dm^{-3} agar. Storage of alginate capsules in tubes with and without medium will be referred to as storage I and storage II, respectively. In storage I, the alginate beads were plunged into the medium up 3/4 of their size. The tubes were closed with screw cap, sealed with parafilm, and kept under 16-h photoperiod (irradiance of 30 μmol m^{-2} s^{-1}) at 25 ± 1 °C for 3 d. The tubes were than transferred into the cardboard boxes, and kept under continuous dark at 25 ± 1 °C for 3 to 6 weeks. After respective storage period, the alginate capsules were cultured on semisolid (agar concentration 8 g dm^{-3})

MS medium supplemented with 2.22 μM N^6-benzyladenine (BA), 8.39 μM calcium D-pantothenate, 0.29 μM GA_3, 0.054 μM NAA and 3 % saccharose. This medium was known as "regrowth" medium. The cultures were incubated under a 16-h photoperiod (irradiance of 50 μmol m^{-2} s^{-1}) at 25 ± 1 °C for 3 weeks. Regrowth was assessed by counting the number of alginate capsules producing new shoots.

The experiment was conducted in a factorial completely randomized design (4 × 2 × 2) with 4 concentrations of saccharose (0, 1, 2 and 3 %), 2 concentrations of sodium alginate (2 and 3 %) and 2 genotypes. Each treatment comprised 30 alginate capsules each replicated 3 times. Prior to statistical analyses, the per cent regrowth data were transformed to arc sine square root, and analyses were done using analysis of variance according to Gomez and Gomez (1984).

The alginate-encapsulated nodal segments formed small, shiny, whitish beads which had an average diameter of 3 - 5 mm (Fig. 1*A*). The beads formed by 2 % sodium alginate were fragile and irregularly shaped, whereas those encapsulated by 3 % sodium alginate were round and regular. Saccharose had a significant effect on the regrowth of alginate-encapsulated nodal segments. This was observed in both the storage conditions (storage I and storage II). Neither potato genotype nor the level of sodium alginate significantly affected the regrowth of encapsulated segments.

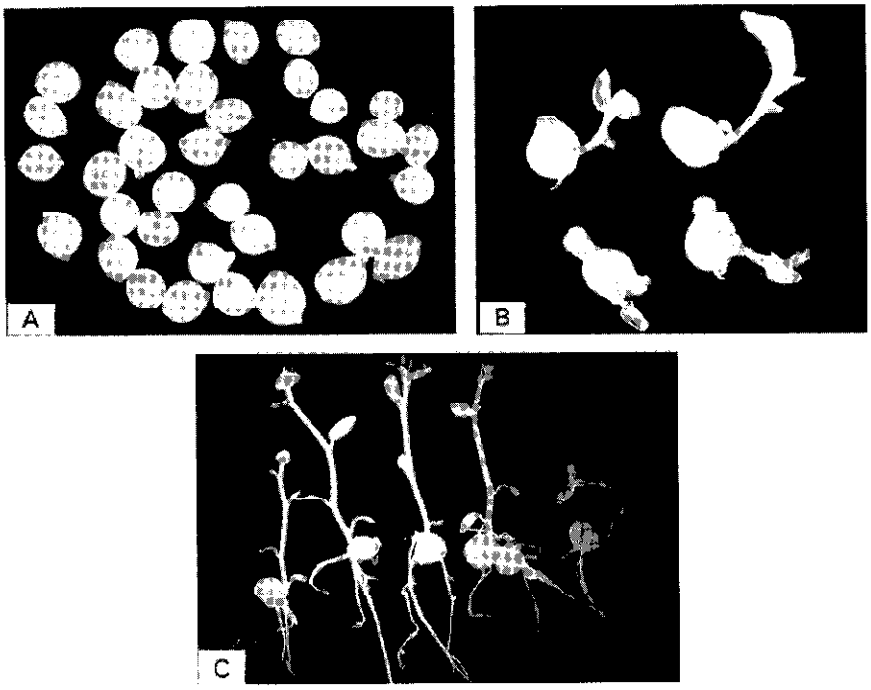

Fig. 1. Nutrient-encapsulation of nodal segments of potato cv. Kufri Jyoti. *A* - encapsulated nodal segments formed by calcium-free MS medium containing 3 % sodium alginate; *B* - 6-week-old dark-stored alginate capsules producing new shoots on regrowth medium; *C* the rooted shoots ready for subculturing or transplantation.

After respective dark incubation, the encapsulated nodal segments initiated shoot growth when cultured on regrowth medium. Within a week the capsules burst open, and new shoots emerged (Fig. 1*B*). The rooted shoots (Fig. 1*C*) were ready for subculturing or transplanting into the glasshouse within a period of 2 weeks. Higher concentrations of saccharose in the beads delayed the regrowth of encapsulated segments (Table 1). Presence of 3 % saccharose in the beads was found detrimental for regrowth of new shoots from alginate-encapsulated segments. In absence of saccharose, 80 - 83 % of alginate capsules produced new shoots after 3 weeks of dark incubation in storage I. In the same storage condition only 28 - 30 % of alginate capsules produced new shoots when the beads contained 3 % saccharose. The regrowth percentage of alginate capsules was reduced over prolonged storage in dark. Only 50 - 56 % of alginate capsules initiated shoot growth after 6 weeks of incubation in storage I when the beads lacked saccharose. Storage of beads in empty tubes (storage II) deteriorated the encapsulated segments and depressed shoot formation: only 50 - 57 % and 23 - 26 % of alginate capsules initiated shoot growth after 3 and 6 weeks of dark incubation, respectively (Table 1) when saccharose was not present in the capsules.

Table 1. Effect of saccharose and sodium alginate concentrations on regrowth of encapsulated potato nodal segments (mean percentage of alginate capsules producing new shoots and arc sine transformed means in parenthesis). Storage I - tubes with MS medium, storage II - empty tubes.

Cultivar	Saccharose [%]	Alginate [%]	Storage I 3 weeks	6 weeks	Storage II 3 weeks	6 weeks
Kufri Jyoti	0	2	83 (65.44)	54 (47.55)	54 (47.55)	25 (30.29)
		3	82 (65.21)	50 (46.28)	52 (46.28)	26 (30.97)
	1	2	71 (57.65)	41 (39.87)	40.(39.22)	16 (23.21)
		3	69 (56.20)	37 (37.25)	36 (36.58)	16 (23.21)
	2	2	44 (41.79)	24 (29.61)	26 (30.97)	5 (13.45)
		3	43 (41.16)	24 (29.24)	20 (26.51)	4 (12.31)
	3	2	30 (33.19)	15 (23.21)	12 (20.41)	3 (10.18)
		3	29 (32.49)	14 (22.30)	10 (18.28)	2 (7.48)
Kufri Lauvkar	0	2	83 (65.98)	54 (47.55)	56 (48.20)	23 (28.63)
		3	80 (63.49)	57 (48.20)	50 (45.00)	25 (30.12)
	1	2	68 (55.43)	40 (39.22)	38 (37.89)	13 (21.32)
		3	65 (53.42)	34 (35.89)	36 (36.58)	15 (23.21)
	2	2	45 (42.43)	23 (28.84)	23 (28.84)	4 (11.32)
		3	42 (40.51)	23 (28.84)	20 (26.51)	5 (12.21)
	3	2	30 (33.19)	11 (19.42)	13 (21.32)	2 (7.48)
		3	28 (31.81)	12 (20.41)	10 (18.28)	3 (9.81)
S.E.			1.85	1.29	1.68	4.50

The delayed regrowth of encapsulated segments is highly desirable for germplasm distribution. However, in the present study, increasing concentration of saccharose reduced the per cent regrowth of encapsulated nodal segments. The negative effect of saccharose on alginate-coated explants has been reported in a number of studies

(Paulet *et al.* 1993, Uragami 1993). In *Dioscorea*, Hasan and Takagi (1995) found that alginate beads with very high saccharose content (1M) caused a high rate of lethality of the coated nodal segments. Our study showed that saccharose-free alginate-MS solution was ideal for encapsulating nodal segments for potato germplams distribution. In this form the beads could be stored easily for 3 - 6 weeks thereby facilitating exchange. We did not observe any significant difference between 2 and 3 % sodium alginate for regrowth of alginate capsules. Nevertheless, we recommend the use of 3 % sodium alginate for improving the capsule hardiness and shape thereby facilitating the handling of encapsulated nodal segments. Regularly shaped beads are also less vulnerable to transportation damage during distribution. The superiority of 3 % sodium alginate for capsule hardiness and shape has been reported in woody species (Piccioni and Standardi 1995).

The study showed that the potato nodal segments encapsulated in alginate-MS solution placed in tubes containing appropriate semisolid medium can be used for distribution of potato germplasm. However, it would be highly advantageous if the encapsulated segments could be transported in empty tubes. This will preclude the possibility of medium spillage. Also more encapsulated segments could be accommodated in single tube thus limiting the crate size. However, in the experiment poor regrowth of alginate capsules was observed when they were stored in empty tubes. It appears that the deterioration resulted from rapid drying of beads. To avoid this, the alginate capsules can be coated with a hydrophobic copolymer which will allow slow drying of capsules. Research is currently in progress on this front.

References

Bapat, V.A.: Studies on synthetic seed of sandalwood (*Santalum album* L.) and mulberry (*Morus alba* L.). - In: Redenbaugh, K. (ed.): Synseeds: Applications of Synthetic Seeds to Crop Improvement. Pp. 381-408. CRC Press, Boca Raton 1993.

Dereuddre, J., Blandin, S., Hassen, N.: Resistance of alginate-coated somatic embryos of carrot (*Daucus carota* L.) to desiccation and freezing in liquid nitrogen. 1. Effects of preculture. - Cryoletters **12**: 125-134, 1991a.

Dereuddre, J., Hassen, N., Blandin, S.: Resistance of alginate-coated somatic embryos of carrot (*Daucus carota* L.) to desiccation and freezing in liquid nitrogen. 2. Thermal analysis. - Cryoletters **12**: 135-148, 1991b.

Estrada, R., Tovar, P., Dodds, J.H.: Induction of *in vitro* tubers in a broad range of potato genotypes. - Plant Cell Tissue Organ Cult. **7**: 3-10, 1986.

Ganapathi, T.R., Suprasanna, P., Bapat, V.A., Rao, P.S.: Propagation of banana through encapsulated shoot tips. - Plant Cell Rep. **11**: 571-575, 1992.

Gomez, K.A., Gomez, A.A.: Statistical Procedures for Agricultural Research. - John Wiley & Sons, New York 1984.

Hasan, S.M.Z., Takagi, H.: Alginate-coated nodal segments of yam (*Dioscorea* spp.) for germplasm exchange and distribution. - Plant Genet. Resour. Newslett. **103**: 32-35, 1995.

Leclerc, Y., Donnely, D.D., Coleman, W.K., King, R.R.: Microtuber dormancy in three potato cultivars. - Amer. Potato J. **72**: 215-223, 1995.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tabacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Ng, S.Y.C.: *In vitro* conservation and distribution of root and tuber crop germplasm. - In: Perrino, P., Attere, F., Zedan, H., Ng, N.Q. (ed.): Crop Genetic Resources of Africa. Vol. II. Pp. 95-106. International Institute of Tropical Agriculture, Ibadan 1991.

Ng, S.Y.C.: Tissue culture of root and tuber crops at IITA. - In: Thottappily, G., Monti, L.M., Mohan Raj, D.R., Moore, A.W. (ed.): Biotechnology: Enhancing Research on Tropical Crops in Africa. Pp. 135-141. International Institute of Tropical Agriculture, Ibadan 1992.

Paulet, F., Engelmann, F., Glaszmann, J.-C.: Cryopreservation of apices of *in vitro* plantlets of sugarcane (*Saccharum* sp. hybrids) using encapsulation/dehydration. - Plant Cell Rep. **12**: 525-529, 1993.

Piccioni, E., Standardi, A.: Encapsulation of micropropagated buds of six woody species. - Plant Cell Tissue Organ Cult. **42**: 221-226, 1995.

Redenbaugh, K., Fujii, J.A.A., Slade, D.: Hydrated coating for synthetic seed. - In: Redenbough, K. (ed.): Synseeds: Applications of Synthetic Seeds to Crop Improvement. Pp. 35-46. CRC Press, Boca Raton 1993.

Roca, W.M., Bryan, J.E., Roca, M.R.: Tissue culture for the international transfer of potato genetic resources. - Amer. Potato J. **56**: 1-10, 1979.

Uragami, A.: Cryopreservation of cultured cells and organs of vegetables. In: Cryopreservation of Plant Genetic Resources. Pp. 111-135. Japan International Cooperation Agency, Tokyo 1993.