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Short communication
In vitro development of encapsulated somatic embryos of rice

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ABSTRACT

Somatic embryos derived from five-year old long term embryogenic cultures of rice (*Oryza sativa* L.cv; Basmati 370) were encapsulated in sodium alginate and tested for viability and development. Encapsulated embryos exhibited varying frequency of plantlet development on different media and sterile water (50-100%).

Key words : Rice, somatic embryos, encapsulation, synthetic seeds, *Oryza sativa* L.

The encapsulation of somatic embryos and other propagules for the production of artificial seeds is an emerging area with potential for large scale, low cost mass propagation, handling and transportation of plant germplasm (Redenbaugh 1991, Rao *et al.* 1998). In several plant species, regeneration of plants from encapsulated embryos has been carried out using sodium alginate as the principal hydrogel for encapsulation (Rao *et al.* 1998). However, in cereals, there have been only few reports on artificial seeds and a strong potential exists for the propagation of high yielding, individual hybrids through somatic embryogenesis and artificial seeds (Brar *et al.* 1994). Earlier, we studied the encapsulation of somatic embryos of rice (*Oryza sativa* L.) and conversion of encapsulated embryos into plants (Suprasanna *et al.*1996). In this report, results on the viability of encapsulated embryos derived from five year old long term cultures of rice cv. Basmati 370, are presented.

Somatic embryos selected from 5 year old stock cultures of the var. Basmati 370 were encapsulated as per the method described earlier (Suprasanna *et al.* 1996). Briefly, each somatic embryo was separated from the clump and mixed in 20 ml of gel matrix of 5% sodium alginate, (Sigma) in MS (Murashige & Skoog 1962) medium containing 0.5 mg/l kinetin, 1.0 mg/l abscisic acid, 1 g/l caesin hydrolysate, 3% mannitol and 3% sucrose for 3-4 min. The embryos were then dropped into a solution of 2.5% sterile calcium chloride. After 10 minutes of intermittent shaking, beads were taken out and rinsed with sterile distilled water twice. For plant development, these encapsulated somatic embryos (beads) were then placed on four media / substrates, *viz.* Ml : MS medium containing 0.5 mg/l kinetin, 1.0 mg/l abscisic acid, 1 g/l caesin hydrolysate, 3% mannitol and 3% sucrose, M2 : White's basal medium (WM) without growth regulators, M3: MS + NAA (lmg/l) and M4 : sterile distilled water alone. The cultures were incubated at 25±2°C under an illumination of 1000 Lux. Observations on survival and growth were taken using 26 beads per treatment in three replications, after 4 weeks of incubation.

The stages in the development of plantlets from encapsulated emrbyos are presented in Figure 1. Within 3-4 days of culture, encapsulated embryo showed shoot emergence and, development of complete shoots (~ 5 cm) and roots (~2 cm) was observed within 4 weeks. Plant development was observed on different substrates, M3 (100%), followed by Ml (57%) and M2 (Fig. 2). In an earlier study on the encapsulation of somatic embryos, we used MS and White's medium as the gelling matrices and WM, MS, cotton, macpeat, filter paper as various substrates and obtained maximum regeneration on White's medium (Suprasanna *et al.* 1996). In the present study. Ml was an efficient gelling matrix and NAA was the efficient substrate as compared to White's medium and sterile water. Emryogenic competence was restored and somatic embryos showed signs of secondary embryogenesis on Ml medium which is the embryogenic medium. The addition of ABA, mannitol and low levels of Kn present in Ml are conducive in maintaining the somatic embryos by inducing an osmotic stress and desiccation tolerance as has been suggested by Emons *et al.* (1993) and Suprasanna *et al.* (1995).

The encapsulated embryos placed directly in distilled water for growth did not show any germination (Fig. 2) and even after replacement of water with liquid medium containing NAA medium, growth was not resumed. It is possible that the somatic embryos needed an initial exposure with the

Abbreviations: MS - Murashige & Skoog medium (1962); NAA- naphthelene acetic acid; ABA - abscisic acid

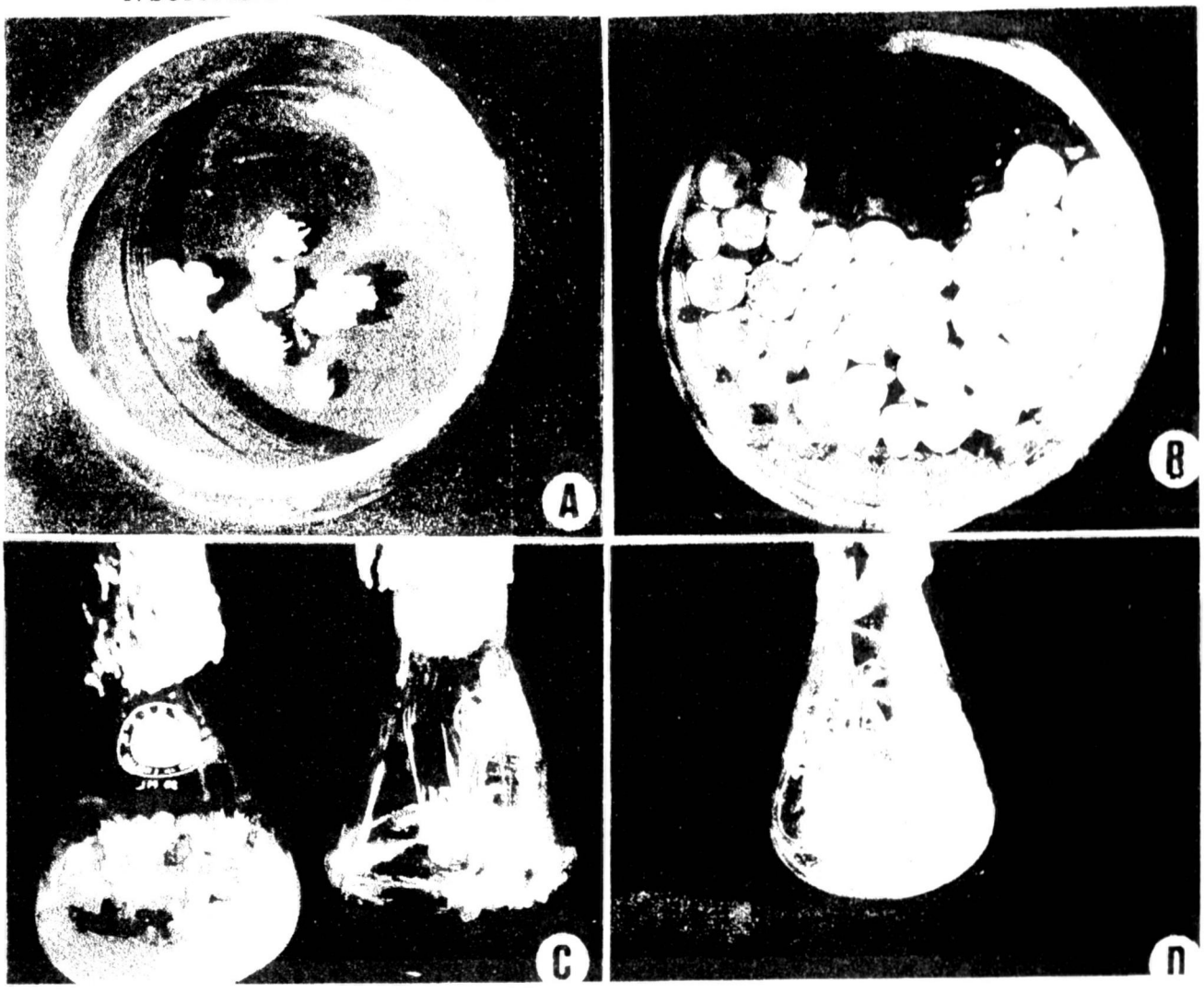

Fig. 1. A. Somatic embryo clumps used for isolating single embryos for encapsulation.
B. Somatic embryos encapsulated in 5% sodium alginate
C. Encapsulated embryos growing on M1 medium showing secondary embryogenesis (left) and M3 medium showing elongated shoots.
D. Plantlet development from encapsulated embryos on M3 medium.

nutrient medium to maintain viability after which subsequent development can occur on water or other substrates. Patnaik *et al.* (1995) observed that it was possible to retrieve the plants from encapsulated buds, only when the gel matrix was made using MS medium with necessary additives as compared to distilled water which failed to support growth of encapsulated buds. Such a capsule gel can serve as a reservoir of nutrients acting as an artificial endosperm for encapsulated explants. In papaya, when water alone was used as the solvent for sodium alginate, 20% germination frequency was achieved in somatic embryos (Castillo *et al.* 1998) and 36 - 80% germination was noted in case of encapsulated cardamom shoot tips (Ganapathi *et al.* 1994). Piccioni and Standardi (1995) tested de-ionized water and nutritive media for encpasulation gel in six woody species. It was found that the former helped the regrowth and shoot production as compared to the latter.

Synthetic seed technique can be useful for the storage and maintenance of the germplasm if the regeneration ability is maintained and restored in long term culture periods. High frequency somatic embryogenesis, synchronous embryo development and maximum conversion of embryos into plants are generally considered the critical factors for realizing the potentials of artificial seeds (Rao *et al.* 1998).

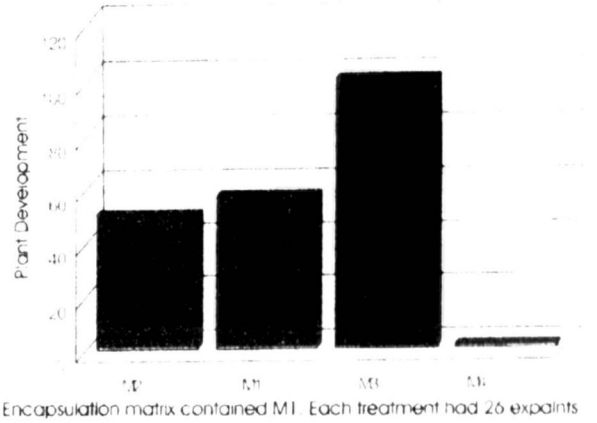

Fig. 2. **Plant development from encapsulated somatic embryos of rice on different substrates.**

One of the practical applications of somatic cell culture in future could be the production somatic embryos as planting material for hybrid rice (Zapata *et al.* 1995). Somatic embryogenesis *via* synthetic seeds could offer a potential way to exploit hybrid vigour without having to produce new hybrid seeds. However synthetic seed research in rice has just began and technological developments for high frequency embryogenic systems in *indica* rice cultivars and refinements in the encapsulation technique are required before potentials of synthetic seed research can be utilized.

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