SHORT COMMUNICATION

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Comparative assessment of somatic embryogenesis and plant regeneration in dormant and non-dormant indica rice varieties

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ABSTRACT

Somatic embryogenesis (SE) and plantlet regeneration were compared in a dormant and non-dormant indica rice variety. MS and N6 media were used both for callus induction and plantlet regeneration. Both MS and N6 media emerged appropriate in facilitating increased somatic embryogenesis (%) but MS found to be better for plantlet regeneration (%). In MS, calli from individual seeds in IR64 and Swarna produced 4-9 plantlets and 2-5 plantlets, respectively, whereas, in N6 more calli appeared with no plantlet regeneration. Generation of somatic embryos from embryogenic calli is an important event since it is one of the best target tissue for undertaking rice transformation to integrate alien gene/s in rice plants.

Key words: *indica rice, in vitro culture, somatic embryogenesis, plantlet regeneration*

Cell technological approaches offer alternative in genetic improvement of crop plants since it often induce genetic variation which constitutes an important source of variability for crop improvement in an already improved variety. Among various approaches, somatic embryogenesis holds potential due to multiplication of genetically uniform plant material through clonal propagation, virus elimination, provision of source tissue for genetic transformation experiments with alien genes (Potrykus 1990), generation of whole plants from genetically modified protoplasts, etc. Frequency of callus induction and plantlet regeneration in rice are influenced by culture media composition, explants source, genotype and environment (Torbert *et al*.1998) etc. Evidences are there where various genotypes and several nutrient media were tested to regenerate rice plant using diverse explants like mature embryos (Abe and Futsuhara, 1986, Wang *et al.* 1987, Seraj *et al.*1997), scutellum-derived callus (Rashid *et al.* 1996, Tokiet *et al.* 1997, Yokoi *et al.* 1997) and immature embryos (Koetje *et al.* 1989, Seraj *et al.* 1997).

The principal objective of this study was to examine the *in vitro* behaviour of two contrast genotypes with respect to two established media compositions using mature seeds as explant and to pinpoint an efficient route for optimum somatic embryogenesis and plant regeneration which may sparehead genetic transformation work in contrast to tedious time consuming suspension culture development to obtain protoplasts as recipient of foreign genes.

Mature seeds of rice varieties used in this study were de-husked manually and surface sterilized with 0.1% Bavistin (antifungal agent) for 10 min followed by thorough rinsing with distilled water for three times. Dehusked seeds, autoclaved nutrient media (MS/N_6) and petri dishes were kept inside the laminar airflow (LAF). Seeds were further surface sterilized with 0.01% $HgCl_2$ for 15 minutes followed by three washes with sterile distilled water inside LAF. Those were blot dried and cultured onto callus induction medium (CIM) both in MS (Murashige and Skoog, 1962) and N_6 (Chu, 1975) media. Both the MS and N_6 media was supplemented with 0.8% agar and 2mg/l 2, 4- D. Sugar content of MS and N_6 was kept at 3% and 6%, respectively. Media pH was adjusted to 5.6-5.8 prior to autoclaving at 108 kPa and 121^0C for 15 min. About 500 sterilized seeds from non- dormant IR64 and dormant Swarna variety were cultured into medium in culture tube. Cultures were kept in dark at 25 ± 2^0C. After 6- 7 days, loose creamy yellow - white calli were

Table 1. Comparative assessment of different *in vitro* culture responses of two rice varieties at various hormonal concentration and combination

Designation	Medium used *	Callus induction medium	Callus induction %	Regeneration treatment medium	Regeneration %
IR64	MS	2mg/l 2,4-D	90%	2mgD l BA + 0.5mgD l NAA + 0.5mgD l IAA	50%
	N_6	2mg/l 2,4 - D	90%	(a) 2mgD l BA + 0.5mgD l NAA + 0.5mgD l IAA	-
				(b) 2% Sucrose	-
				(c) 6% sucrose + 3% sorbitol	-
Swarna	MS	2mg/l 2,4 - D	60%	2mgD l BA + 0.5mgD l NAA + 0.5mgD l IAA	5%
	N_6	2mg/l 2,4 - D	65%	(a) 2mgD l BA + 0.5mgD l NAA + 0.5mgD l IAA	-
				(b) 6% sucrose + 3% sorbitol	-
				(c) 2% Sucrose	-

* MS = Murashige and Skoog, 1962, N6 = Chu, 1975

observed in developing stage at swollen junction of radicle and mesocotyl. After about 4 weeks, callus induction and callus health were observed and calli from individual seeds were sub-cultured on freshly prepared callus maintenance medium (CMM) (i.e. CIM with half strength of 2, 4- D viz. 1mg l^{-1}). Calli were sub-cultured and CMM was changed in every two weeks in light for proliferation. Regeneration potential of the varieties were assessed by implanting calli developed from seeds on regeneration medium consists of MS and N_6 supplemented with BA (2mg l^{-1}), NAA (0.5 mg l^{-1}), IAA (0.5mg l^{-1}). Moreover, various combination of sucrose was also tried in MS and N_6 media (sucrose 2%, 6% and sorbitol 3%). All these regeneration media were incubated at 25 ±2 ^{0}C under 24 h light for somatic embryogenesis. Between the varieties, IR 64 had a

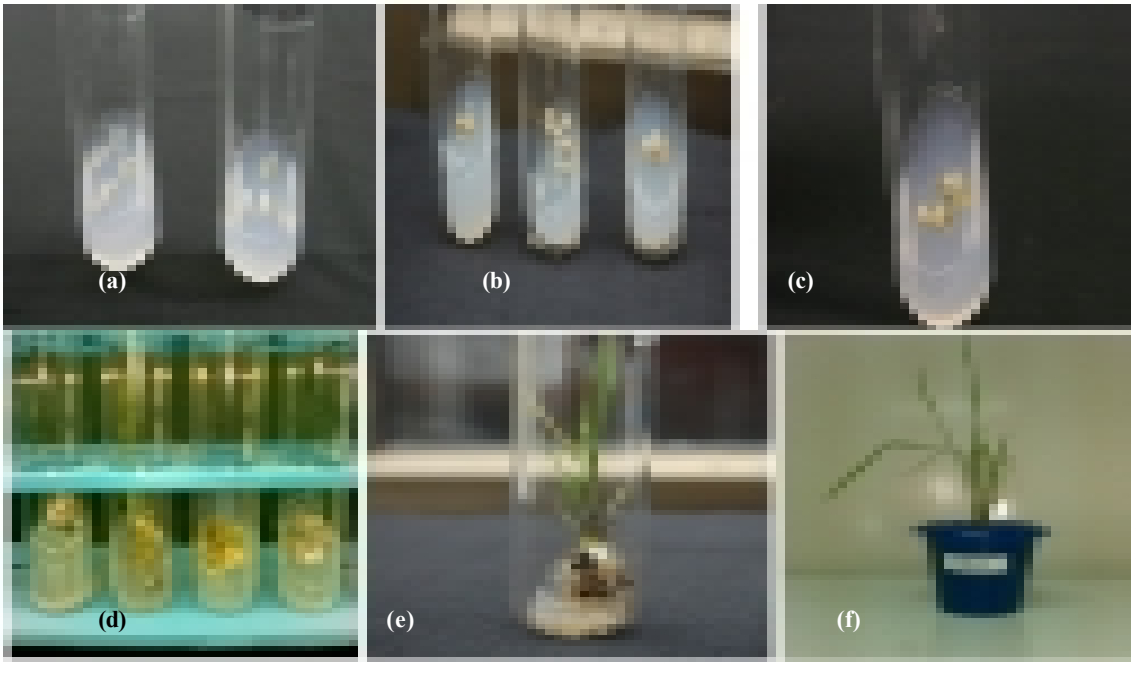

Fig 1. Mature seed sprouting for callus induction on culture medium (MS + 2, 4 – D) after 7 days of inoculation (a); Induction of callus from mature seeds of rice on MS +2, 4 –D after 21 days of inoculation (b); Proliferation of callus after 2 weeks of inoculation (c); Regeneration of plantlet from somatic embryos of mature seeds of rice (d); Regeneration of plantlet from callus on (MS +2mg l^{-1} BA +0.5mg l^{-1} NAA + 0.5mg l^{-1} IAA) regeneration medium (e); Transfer of plantlet into pot for hardening and maturity (f).

higher somatic embryo (SE) to plant conversion rate as compared to Swarna. Regenerated plantlets (8-10 cm height) with well developed roots were transferred to green house for hardening (Fig 1, a-f).

In rice callus cultures, the pathway of plant regeneration has been reported to be through organogenesis and embryogenesis (Henke *et al.* 1978, Heyser *et al*. 1983, Abe and Futsuhara, 1985). In this study, MS medium supplemented with phytohormones was found suitable for growth and maintenance of embryogenic callus and also for somatic embryo formation in culture. To the contrary N_6 was found to be suitable for growth of embryogenic callus but not suitable enough for plantlet regeneration (Table 1). Low auxin (2, 4 –D) conc. was found suitable for both callus growth and induction of embryogenic state. The presence of auxin in the pre-culture medium has often been reported to have effect on differentiation (Halperin and Witherell, 1965). In several plant species, somatic embryogenesis occured when calli raised once on 2, 4-D medium was transferred to 2, 4-D free medium, indicating the withdrawal of auxin to be conducive for callus induction (Vasil 1987). In our study, the yellowish-white calli subsequently became almost white, compact and gave rise to several somatic embryos. Our observation was supported when immature organs and meristematic tissues were considered to be more suitable for plant regeneration than mature organs (Morrish *et al.* 1987).Present result suggested that mature seed embryos as explant exhibit embryogenic potential with high frequency of plantlet regeneration. The ability of the embryogenic callus to produce somatic embryos provides the best suitable target tissue for transformation studies in rice, which is currently being pursued in most of the transgenic development research.

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