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Plant regeneration from scutellum- derived callus of Assam rice collection *(Oryza sativa* L.)

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Abstract : ***In vitro*** **plant regeneration from callus induced from embryos of mature seeds of 8 ARC lines along with *japonica* line Taipei 309 were studied. Observations on callus induction were carried out on MS medium containing 2mg 2,4-D L^{-1}. The callus induction frequency varied from 72.0% to 96% and regeneration frequency from 0 to 26.66% depending upon the genotype used. Of the nine lines tested regeneration were produced in considerable numbers in five lines only. (ARC 7140, ARC 12194, ARC 12451, ARC 14763, ARC 15759). This study regarding enhancement of somatic embryogenesis could be used for further genetic transformation studies.**

Key words : ***Assam Rice Collection (ARC), in vitro culture, callus.***

Introduction

Production and maintenance of embryogenic calli and subsequent plant regeneration in higher frequency are important aspects of tissue culture. Callus induction and efficient plant regeneration for *Oryza sativa* L. *japonica* varieties had been reported widely (Abe and Futsuhara, 1986; Mikami and Kinoshita, 1988). However, plant regeneration from the major cultivated *indica* varieties is generally poor (Abe and Futsuhara, 1984; Kavi Kishor and Reddy 1986). Even with the *indica* group, there are significant variations in the *in vitro* culture response among different genotypes (Peng and Hodegs, 1989). Among the several explants, seed cultures have been used to compare the capacity for callus growth and plant regeneration. Because these are comparatively easy to establish (Abe and Futsuhara, 1986). So the present study was undertaken to identify the regenerataion potential of Assam Rice Collection.

Materials and Methods

Eight Assam rice cultures of *indica* rice (ARC 7140, ARC 11979, ARC 12194, ARC 12451, ARC 14763, ARC 15759, ARC 18023, ARC 18214 and one *japonica* check, Taipei 309) were used in this study. The mature seeds of paddy were dehusked and surface sterilized with 70 percent ethanol for 60 seconds followed by treatment in 0.1% mercuric chloride solution for 10 minutes. After sterilization, seeds were washed with sterile distilled water. MS medium (Murashige and Skoog, 1962) was used for both callus induction and plant regeneration. Sterilized seeds were incubated under aseptic condition on the callus induction medium containing 2mg 2,4 -D L^{-1} and the direct embryo contact with the medium was avoided. Five mature seeds per genotype with three replications, totally 15 seeds for each genotype were tested. The culture tubes were incubated in dark 25$\pm$2°C for 3 days and then kept in the light room under 16 hr light

Table 1. Percentage of regenerability shown by Assam Rice collection.

Geno-type	Number of seeds inoculated	Callus induction (mean)	Rhizo-genic	Embryo-genic	Callus Induction Percent	Regeneration percent			Regeneration percent per explant
						Shoot	Root	Both	
ARC 7140	15	13.8	8.0	5.8	92	1	1	1	6.66
ARC 11979	15	10.8	8.0	2.8	72	1	0	-	-
ARC 12194	15	13.5	7.5	6.0	90	8	4	4	26.66
ARC 12451	15	13.8	3.0	10.8	92	4	3	3	20.00
ARC 14763	15	13.8	2.0	11.8	92	2	1	1	6.66
ARC 15759	15	11.4	2.5	8.9	76	4	3	3	20.00
ARC 18023	15	10.8	2.0	8.8	72	-	-	-	-
ARC 18214	15	13.5	8.0	5.5	90	-	-	-	-
Taipei 309	15	14.4	1.0	13.4	96	-	-	-	-

and 8 hr dark photoperiod. Callus formation occurred after 7 days of incubation.

The scutellum derived calli obtained from seed explants were maintained by subculturing at an interval of three to four weeks on solid MS medium supplemented with 2mg 2,4–DL^{-1}. Embryogenic calli (smooth, white and knobby appearance) and Non embryogenic calli (yellow, translucent and wet) (Nabors *et al.*, 1983) and rhizogenic calli (root forming, white calli) were assorted from the calli mass before subculture. For callus regeneration, the culture tubes were incubated under controlled environmental condition in the growth room. The cultures were subjected to light and dark cycle of 16/ 8 hrs per day. The light (2000 lux) was provided by a cool, white lamp (Philips 40w). The cultures were maintained at a temperature of 25$\pm$2°C and 70 percent relative humidity (RH). The media containing MS+50ml tryptophan (0.5 gm/ 500ml) +0.1mg IAA+2.5mg kinetin + 30gm sucrose was used for regeneration. Hardening of plantlets were done initially in water for 4 to 5 days followed by soil.

Results and Discussion

Seeds when cultured on MS medium without any growth regulator or 0.5mg kinetin, germinated and developed roots and shoots. However, callus induction was observed when seeds were cultured on MS medium containing 2, 4- D (2 mg/L) with 7 days after incubation. Callus developed from scutellum which subsequently developed into subculturable mass of callus after three to four weeks. Calli were induced mainly from the epithelium of the scutellum as was observed by Nishimura and Maeda (1977). Pant and Ghosh (1984) reported the importance of scutellar tissues for callus induction and regeneration of plantlets in *Oryza sativa* cv Kiran. The highest callus induction (96%) was obtained in *japonica* check (Taipei 309) followed by ARC 7140 (92%), ARC 12451 (92%), ARC 14763 (92%), ARC 12194 (90%) and ARC 18214 (90%). This clearly shows the genetic difference with respect to

callus induction and subsequent proliferation as was observed by Azira and Bhalla (2000). Embryogenic calli were characterized by their morphological appearance. The light yellow coloured nodular and friable calli were considered as embryogenic whereas smooth, watery and whitish calli was considered as non-embryogenic. Putative embryogenic calli were further confirmed through histology. There was a positive relationship between the number of somatic embryos per unit section and the extent of nodular structures on the surface of the calli. Finally, the embryogenic calli were confirmed by germinating somatic embryos on the regeneration media. Callus pieces showed green spots when transferred to regeneration medium.

In general, during rice tissue culture, *japonica* rice varieties have been found to be highly amenable for regeneration even from older sub-cultured calli. Taipei 309 which is a *japonica* type variety, has been extensively used as a model system for regeneration and also for genetic transformation studies (Bitten court *et al.,* 1995). The present study revealed that Taipei 309 have exhibited poor plant regeneration. Out of 8 ARC lines, only 5 lines have regenerative capacity (Table.1). The line ARC 12194 has given high regeneration frequency (26.66%) which may be attributed to the presence of some genes from *indica* rice varieties. The poor regeneration response of other three lines (ARC 11979, ARC.18023, ARC 18214) might be ascribed to established fact that *indica* rice varieties are recalcitrant.

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