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Studies on Callus Induction and Regeneration from Dehusked Rice (*Oryza sativa* L.) Seeds

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Abstract

The effect of varieties and media compositions on callus induction from dehusked rice seeds and subsequent plant regeneration was studied in the present investigation. Three rice varieties viz. Ranisalute, Chiniatap and Samudra Fena were studied. Among them, callus induction frequency was higher in Samudra Fena (53.33%). MS medium was used as basal medium supplemented with yeast extract (3 g/l), phenyl acetic acid (PAA) 1 mg/l, 2,4-D 1 mg/l and calcium silicate 66.66 mg/l. The medium supplemented with 2,4-D + calcium silicate showed better performance in cal1us induction (53.33%). Cal1i obtained from the same medium also showed maximum plant regeneration in all the three varieties. The variety Ranisalute performed better regarding plant regeneration (25%) in comparison with others.

Introduction

Rice belongs to the genus *Oryza,* subtribe *Oryzinae* of the family Gramineae (Roy 1985). It is the world's most important food crop after wheat and maize. A considerable improvement has already been made through traditional rice breeding. Traditional rice breeding has resulted in higher yield, improved quality, greater disease resistance and other important agronomic traits in the past and it will still play an important role in the future (Sun and Zheng 1990). Nowadays various tissue culture techniques are being applied for varietal development of cereal crops including rice in different countries (Dorosieve 1996). Among the techniques, anther culture, protoplast fusion, leaf

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culture, root culture and dehusked grain culture are important in rice tissue culture to exploit somaclonal variation for the creation of novel rice varieties (Ram and Singh 1998). Somaclonal variation, creates novel variability which can be exploited for crop improvement (Larkin and Scowcroft 1981).

In most agronomically important crop species, somaclonal variation is greater than the variation displayed among the seed progeny of the donor (Skirvin 1978). Plant variants derived from any of the cell lineage or tissue culture source is known as somacIonal variation (Larkin and Scowcroft 1981). The diverse variation characteristic of somaclones highlights the fact that somaclonal variation may be an additional tool for crop improvement rather than an interesting scientific phenomenon (Evans and Sharp 1986, Evans 1989, Bajaj 1990). Several high yielding rice varieties were developed through the application of anther culture in the People's Republic of China (Ying 1983). However, plant regeneration from callus obtained from somatic tissue is more successful than from that obtained by anther culture (Kucherenko et al. 1979 and Guo and Cao 1982).

The dehusked rice culture technique is used for callus production and plant regeneration. Its application is still limited by many factors influencing its culture efficiency such as plant genotypes (Shen et al. 1982 and Li 1991), the culture methods (Chen 1977 and Yangn and Zhou 1979), the media (Chen 1977 and Sun and Zheng 1990) and the culture conditions (Qu and Chen 1983 and Wang et al. 1977). In the backdrop of the above situation, the present study was undertaken to compare the performance of three dehusked rice varieties in respect of their callusing and regenerating ability in MS medium with various supplements.

Material and Methods

Dehusked seeds from three varieties, viz. Ranisalute, Chiniatap and Samudra Fena were used as explants. MS supplemented with different combinations of yeast extract, 2,4-D, IAA and calcium silicate were tested to observe their efficiency in callus induction from dehusked seeds of the varieties stated earlier. The combinations were : (i) MS + yeast extract (3 g/l) + PAA (1 mg/l), (ii) MS medium + yeast extract (3 g/l) + 2,4-D (1 mg/l), (iii) MS + yeast extract (3 g/l) + 2,4-D (1 mg/l) + calcium silicate (66.66 mg/l) and (iv) MS + yeast extract (3 g/l) + PAA (1 mg/l) + calcium silicate (66.66 mg/l).

MS medium supplemented with 0.8% agar, 70 g/l sucrose, 4 g/l casein hydrolysate, 1 mg/l NAA and 3 mg/l Kn was used for regeneration of the derived calli to observe the differential regeneration ability of the varieties.

The pH of the medium was adjusted at 5.6 by adding NaOH (0.1 N). Only 25 ml the of medium containing different combinations of chemical components were used in each conical flask. Conical flasks with medium were sterilized.

From each variety, only 150 seeds were taken and dehusked manually, washed in distilled water and dipped in 70% ethanol for 30 seconds. Then the seeds were dipped in a 0.2% $HgCl_2$ for 25 min, washed for three - four min with double distilled water and finally dried with autoclaved tissue paper. All experiments were done in a laminar air flow cabinet. Only ten seeds were inoculated in each flask and three replications for each treatment were maintained. The cultures were kept in dark condition for one week at 26 ± 1°C and then transferred under 16 hours photoperiod at 3000 lux and 25 ± 1_C.

After one month of inoculation, the calli that developed from inoculated seeds were transferred into test tubes containing the regeneration medium and the cultures maintained as earlier. Within one month greenish plantlets developed; the well-rooted plants were transferred onto earthen pots containing an autoclaved mixture of vermiculite and sand (1:1). Before transferring the plantlets were washed several times to remove any trace of agar.

During data collection callus induction frequency was recorded considering that each callus piece originated from a single seed. Regenerated plantlets were counted based on the number of callus-producing plantlets. The frequency of callus induction and plant that of regeneration were calculated as follows:

$$\text{Callus induction frequency (\%)} = \frac{\text{Number of grains producing calli}}{\text{Number of grains plated}} \nabla\Sigma\, 100$$

$$\text{Plant regeneration frequency (\%)} = \frac{\text{Number of grains producing calli}}{\text{Number of grains plated}} \nabla\, 100$$

The study was conducted in the laboratory following Complete Randomized Design. Data on callus induction frequency was transformed by arc sin % transformation method and statistically analyzed by standard statistical package.

Results and Discussion

Callus induction : Dehusked rice seeds of three rice cultivars were tested to study their callusability in four different media compositions. It was found that the varieties, media compositions and their interaction significantly affected callus induction at 1 % level.

The results of callus induction showed similar callusability in CIM-l and CIM-4 (Table 1). The variety Rqanisalute produced maximum callus (16.67%) in CIM-3 followed by CIM-2 (13.33%). Low callus induction frequency was found in Chiniatap in all media compositions. However, it showed maximum callusibility in CIM-3 (20%). The variety Samundra Fena also produced maximum callus in CIM-3 (53.33%) and it was significantly higher than CIM-1 (3.33%) and CIM-4 (0%), but did not significantly differ from CIM-2 (43.33%) (Table 1). Pandey et al. (1994) worked with dehusked rice seeds using different level of 2,4-D in nutrient medium and they concluded that seed response for callusing was the best when 2,4-D is used as supplement at a concentration of 2.0 mg/l. In this experiment they also found that 2,4-D containing media was the best for both the callusing and plant regeneration. However, in the present study, 2,4-D was used at 1.0 mg/l in callus induction medium.

Table 1. Interaction between the genotype and growth regulators on callus induction in the dehusked seeds of three rice varieties.

Media composition		Mean value of callus induction			Mean value of each treatment
		Ranisalute V_1	Chiniatap V_2	Samundna Fena V_3	
CIM-1	MS + yeast extract + PAA	0.9064c (0%)	0.9064c (0%)	60752c (3.33%)	2.855b (1.11%)
CIM-2	MS + yeast extract + 2-4,D	18.02bc (13.33%)	6.752c (3.33%)	41.18ab (43.33%)	21.98a (19.99%)
CIM-3	MS + yeast extract + 2,4-D + calcium silicate	20.24bc (16.67%)	25.38abc (20%)	46,95a (53.33%)	30.85a (30%)
CIM-4	MS + yeast extract + PAA + calcium silicate	0.9064c (0%)	15.31c (10%)	0.9064c (0%)	5.708b (33.33%)
	Mean values of variety	10.02b (7.5%)	12.09b (8.33%)	23.95a (24.99%)	

Data in parenthesis indicate the original value of callus induction frequency, CIM= Callus inducing medium.

Mean frequency of callus induction in the media compositions (Table 1) showed that the variety Samundra Fena produced maximum callus (24.99%) and it was significantly higher than the variety Ranisalute (8.33%) and Chiniatap (7.5%). Among the media compositions, CIM-3 was found most effective in callus induction (30%) and it was significantly higher than CIM-l (1.11%) and CIM-4 (3.33%) but not significantly different from-CIM-2 (19.99%).

This observations recorded in the present study are in agreement with the findings of Pandey et al. (1994). They reported that the success of *in vitro* cultures largely depends on the nutrition media, growth regulators, variety and on the interaction between the variety and the medium. Similar reports were also made by other authors (Guo and Cao 1982).

Table 2. Plant regeneration efficiency from plated calli of dehusked seeds of three rice.

Variety	Calli induced in the medium	Number of calli plated	No. of calli regenerated plants	Frequency of plant regeneration (%)
Ranisalute	CIM-2	10	0	0
	CIM-3	24	6	25
	CIM-4	7	0	0
Chiniatap	CIM-3	18	4	22.22
	CIM-4	5	0	0
Samundra fena	CIM-l	5	0	0
	CIM-2	**11**	0	0
	ClM-3	30	5	16.67
	Total	110	15	63.89

Plant regeneration : It was observed that the plant regeneration ability of plated calli depends on the variety and the callus inducing media. Among the studied rice varieties Ranisalute regenerated maximum number of plants (25%) followed by Chiniatap (22.22%) and Samundra Fena (16.67%). It is apparent from Table 2 that calli formed on CIM-3, showed better performance in plant regeneration in all the varieties. Zhuo (1996) reported that calli derived from PAA containing medium directly regenerated into plants. The above finding is contrary to those of the present study. In the present study 2,4-D and 2,4-D in combination with calcium silicate were found more efficient than PAA both for callus induction and plant regeneration. This disagreement might be due to the use of different explants.

Different levels of absorbable silicon were tested by Liu et al. (1997) for callus induction and plant regeneration. This finding of Liu et al. (1997) agreed with the present experimental results. In this experiment the silicon-containing medium was found most efficient in callus induction and the calli derived from 2,4-D + silicon containing medium showed better performance in plant regeneration. However, silicon in combination with PAA showed a very poor performance both in callusing and plant regenerationability. This phenomenon was observed in all rice varieties studied in this experiment.

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