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In vitro callus induction in rice (*Oryza sativa* L.)

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***In vitro* callus induction from embryos of matured seeds of four rice varieties *viz.*, ASD 16, ADT 43, Basmati 370, Pusa Basmati and Pokkali were studied. Observations on callus induction were carried out on six different callus induction medium having different concentration of 2,4-D *viz.*, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The callus induction frequency varied from 58.33 % to 96.67 %. This study will be useful for selecting suitable callus induction medium for callus induction.**

Key words: *Rice, Embryo, Callus induction, 2,4-D*

Rice (*Oryza sativa* L.) is the world most important cereal crop after wheat and maize. Rice has 24 species, of which 22 are wild and two *viz*. *Oryza sativa* and *Oryza glabrrima* are cultivated (Ray, 1985). It provides one-third of total dietary carbohydrate, especially in Asian countries and it is stable diet for more than three billion people, supplying 50 to 80 per cent of their daily calorie intake (Khush, 2005). A considerable improvement has been done through traditional rice breeding. Rice breeding has made significant progress towards higher yield, improved quality, greater disease resistance and other important characters of agricultural importance in the past and even in future, it will still play an important role (Sun *et al*., 1990).

Dehusked rice seed culture is a valuable technique to exploit somaclonal variation. But its application is limited by many factors which influence culture efficiency, such as plant genotype (Liu *et al*., 1997), the culture methods, the media (Sun *et al*., 1990) and the culture conditions. Production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means and to exploit somaclonal variation (Monirul Islam *et al*., 2005). The objectives of this study were to find a suitable medium and culture condition for callus induction and this will also useful for callus based stress studies like salinity and drought.

Materials and Methods

Mature seeds of five rice varieties *viz*., ADT 43, ASD 16, Pusa Basmati, Basmati 370 and Pokkali were used. MS nutrient medium (Murashige and Skoog, 1962) was used as basal medium and it was solidified with 8.5 g/l agar. The agar was slowly dissolved in boiled distilled water without forming any clumps and three per cent sucrose used as carbon source in nutrient medium. For callus induction MS medium was supplemented

with different concentrations of 2, 4-D *viz.*, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l and P^H of the medium was adjusted to 5.8.

Rice seeds were manually dehusked and washed with sterile water, and then the seeds were transferred to the laminar airflow chamber. The seeds were kept in 70 per cent ethanol for one minute. Then seeds were washed with sterile distilled water three times and were immersed in 0.1 per cent mercuric chloride for 15 minutes. Again the seeds are washed thoroughly three to four times with sterilized distilled water to remove all the trace of mercuric chloride and were blot dried using sterilized tissue paper. Surface sterilized seeds were cultured with the help of sterilized forceps into the test tube containing callus induction medium. Cultures were incubated in dark at 25±1°C. Callus induction was noticed within two weeks of inoculated cultures. Callus induction frequencies were recorded.

The frequency of callus induction was calculated according to the following formula:

$$\text{Callus induction frequency (\%)} = \frac{\text{No. of seeds produced calli}}{\text{No. of seeds inoculated}} \times 100$$

Table 1. Callus induction from five rice varieties

Varieties	Callus induction frequency (%)					
	M1	M2	M3	M4	M5	M6
ADT 43	80.00	70.00	91.33	92.00	83.33	71.33
ASD 16	81.67	84.17	94.17	96.67	86.67	80.83
Pusa Basmati	81.48	79.26	86.67	94.81	85.18	76.26
Basmati 370	80.83	81.67	93.33	94.17	89.17	77.50
Pokkali	75.00	63.33	71.67	83.33	76.67	58.00

M1=MS + 0.5 mg/l 2,4-D; M2=MS + 1.0 mg/l 2,4-D; M3=MS + 1.5 mg/l 2,4-D; M4=MS + 2.0 mg/l 2,4-D; M5=MS + 2.5 mg/l 2,4-D; M6=MS + 3.0 mg/l 2,4-D

Result and Discussion

The effects of varieties on callus induction from dehusked rice seeds are shown in Table 1. Callus induction in rice was found highly variable and genotype specific. Among the five studied varieties, the variety ASD 16 produced 94.81 per cent callus from the inoculated seeds, which was higher than other four varieties. In all the treatments Pokkali had poor callus induction and ASD 16 had the best induction. This may be due to callusing efficiency was found to be genotype dependant. It was also confirmed by Rashid *et al*., 2003 who reported that rice varieties differed in degree of callusing. Rasheed *et al.*, 2005 resulted that tissue culture generates a wide range of variation, which is resulted with incubation time and cultivar specific. For callus induction MS medium supplemented with different concentrations of 2,4-D was used. In that 2 mg/l 2,4-D showed high callus induction percentage in all the varieties and followed by 1.5 and 2.5 mg/l 2,4-D. It was also confirmed by Pandey *et al*., 1994; shankhdhar *et al*., 2002; Tam and Lang, 2003; Naqvi *et al*., 2005. Jaseela *et al*., 2009 reported that 60-100 per cent of the cultured seeds formed callus at all the

concentrations of 2,4-D used and among the different auxin analogues used to induce somatic embryogenesis 2,4-D is the most efficient and therefore used in majority of embryogenic and tissue culture systems and also they proved 2 mg/l 2,4-D to be the most favorable for callus induction and callus proliferation. The role of 2,4-D in cell division is to increase the rate of cell division and this attributes to the increased amount of callus.

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