



## Genotype Affected Callus Induction and Plant Regeneration from Dehusked Rice (*Oryza sativa* L.) Seeds

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### Abstract

Callus induction and plant regeneration ability of the dehusked rice genotypes are important tools for varietal improvement. This investigation presents the comparative callus induction and plant regeneration as obtained from the dehusked seeds of rice cultured on MS (Murashige-Skoog) medium. In the experiment conducted in a Complete Randomized Design under laboratory conditions, dehusked seeds of Ranisalute, Chiniatap, Somudrafena, Chiniguri-2 and Katchra rice cultivars grown in South West Bangladesh were tested on autoclaved and agarified MS nutrient medium with four replications to study their callus induction and subsequent plant regeneration ability. Chiniguri-2 yielded the highest calli and plant regeneration.

**Keywords:** Dehusked rice, callus, MS medium

### 1. Introduction

Rice (*Oryza sativa* and *Oryza glaberrima*) is the world's most important food crop. So its importance as staple food emphasizes its improvement undoubtedly. A considerable improvement has been achieved through traditional rice breeding (Sun *et al.*, 1990). Now a days, however, 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 culture, root culture and dehusked grain culture are important to exploit novel rice varieties (Ram and Singh, 1998). Several high yielding rice varieties were developed through the application of anther culture in China (Ying,

1983). But plant regeneration from callus is more successful than anther culture (Guo and Cao, 1982). Moreover, plants regenerated from calli show desirable genetic changes such as disease resistance, salt tolerance, and other physiological properties (Kucherenko *et al.*, 1979). Additionally, calli induced by dehusked rice seeds can be successfully used for plant regeneration. These facts suggest that there is a greater possibility of developing novel rice varieties through the appropriate use of dehusked rice induced calli. However, dehusked rice culture technique is still limited by many factors which influence culture efficiency, such as plant genotype (Li, 1991), the culture methods (Yang and Zohu, 1979), the media (Sun *et al.*, 1990) and the culture conditions (Qu and Chen, 1983).

In this investigation, the comparative callus induction and plant regeneration ability of Ranisalute, Somudrafena, Chiniguri-2, Chiniatap and Katchra rice cultivars cultured on MS medium were evaluated.

## 2. Materials and Methods

Autoclaved and agarified (8 g L<sup>-1</sup> agar) MS nutrient medium (Murashige and Skoog, 1962) containing all needed inorganic major and minor elements, vitamins, amino acids etc. with a pH of 5.7 which were used for callus induction and plant regeneration. Ten healthy, sterilized (70% ethanol/0.2% HgCl) and dehusked rice seeds of the Ranisalute (C1), Somudrafena (C2), Chiniguri-2 (C3), Chiniatap (C4) and Katchra (C5) rice cultivars were inoculated in a cotton plugged conical flask with 25 ml of the MS medium having four replications. With the same number of replication, plant regeneration medium contained 5 ml of the agarified MS medium in 15 mL sterilized test tubes. For callus induction, agarified MS medium was supplemented with 30 g L<sup>-1</sup> sucrose. For plant regeneration, agarified MS medium was supplemented with casein hydrolysate (4 g L<sup>-1</sup>) and sucrose 70 g L<sup>-1</sup>.

After inoculation of seeds, the cultures were kept in dark condition for one week at 26±1°C. Then the cultures were transferred under 16 hour's photoperiod at light intensity of 3000 Lux. The temperature maintained through out the culture period was 25±1°C. After that, the induced calli were transferred into 15 mL test tubes with the maintenance of the same culture temperature, photoperiod and light intensity. Within one month, greenish plantlets were emerged from the calli. Well rooted plantlets were transferred to the earthen pot containing an autoclaved mixture of vermiculite and sand (1:1). Prior to transfer, the plantlets were washed several times with distilled water to remove the trace of agar. For calculation, calli emerged from one seed was considered as one and the regenerated plants were counted based on the

number of callus producing plantlets and the data on callus induction and plant regeneration were collected at the end of one week and the 4th week of a culture month, respectively.

Statistical analysis was carried out by a computer package MSTAT-C and LSD was used for comparison of the treatment means.

## 3. Results and Discussion

### 3.1. Callus induction

During the culture period, dehusked rice seeds of the test cultivars appreciably produced calli (Table 1 and Fig. 1). Although, calli induction was significant ( $p \leq 0.01$ ), Chiniguri-2 yielded the maximum calli (39.19%) which was closely followed by Somudrafena and Khatchra (about 36%). The lowest calli (14.06%) was observed in Ranisalute. The cultivars Ranisalute and Chiniatap did not differ significantly with respect to callus induction and the frequency of callus induction of these two varieties was remarkably lower than those of the other three cultivars. Similarly, callus induction of Somudrafena, Chiniguri-2 and Katchra did not differ significantly having 36.02, 39.19 and 36.18% callus induction, respectively. These sorts of callus induction suggest that the calli formations were influenced by the respective genotypes tested. As for calli formation, these findings also revealed that there was an inter cultivar variation. These findings are in good agreement with those reported by Chung (1980) and Chen (1982) who clearly stated that calli formation varies among genotypes.

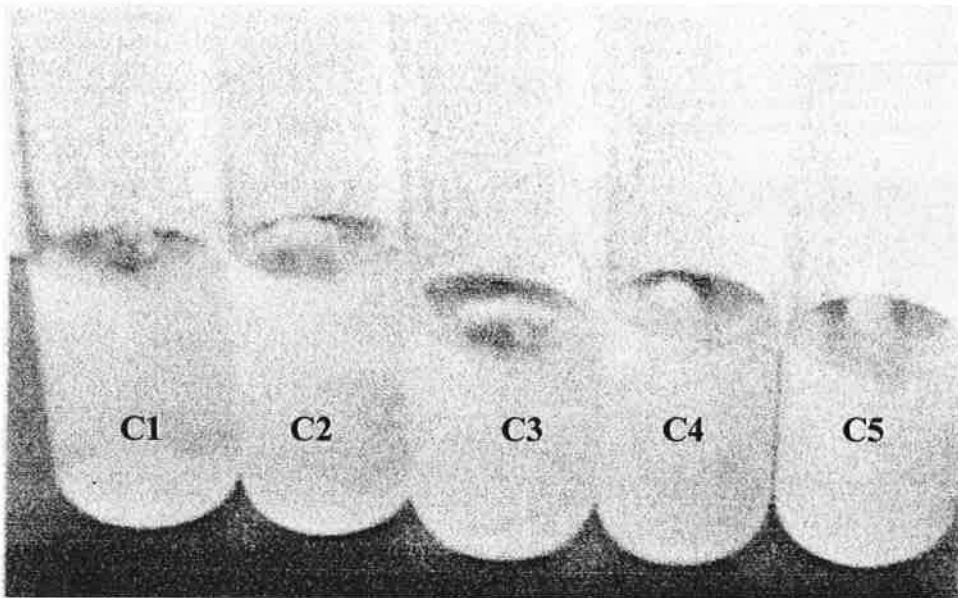
### 3.2. Plant Regeneration

As shown in Table 2 and Fig. 2, calli derived from all the cultivars tested for plant regeneration responded significantly ( $p \leq 0.01$ ) in plant regeneration and ranged from 20 to 66.66%. But the minimum plant regeneration (only 20%) was observed from the calli of the cultivar Chiniatap.

**Table 1.** Effect of variety on callus induction.

Cultivar	Seeds inoculated	Callus induction (%)
C1: Ranisalute	40	14.06
C2: Somudrafena	40	36.02
C3: Chiniguri-2	40	39.19
C4: Chiniatap	40	16.09
C5: Khatchra	40	36.18
CV%	-	5.69
LSD <sub>(0.01)</sub>	-	3.43
Level of significance	NS	*

\*= Significant at 1% level of probability



C1- Ranisalute, C2- Somudrafena, C3- Chiniguri-2, C4- Chiniatap, C5- Khatchra

**Fig. 1.** Callus induction from dehusked rice seeds of the test rice cultivars.

On the contrary, the calli derived from the cultivars Ranisalute, Chiniguri-2 and Khatchra showed comparatively better performance on the plantlets formation (50, 66.66 and 50 %

respectively. However, it is remarkable that Sumodrafena which gave the maximum callus produced only 40% plantlets. The calli obtained from Chiniguri-2 gave rise to the maximum plant

regeneration (66.6%). These sorts of variability in plant regeneration among the cultivars agree well with the findings of Guo and Cao (1982) and Wu and Chen (1987). They confirmed that dehusked rice seeds affected plant regeneration in the different genotypes although genotype to genotype variation was remarkable. The rice cultivars used in the current investigation have shown differential potentiality for both callus induction and plant regeneration. The plants in

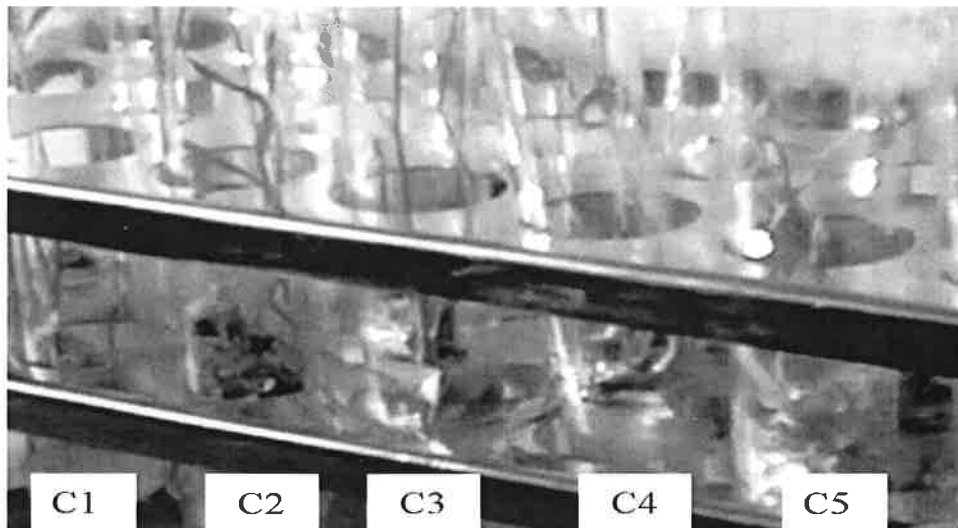
Fig. 3 display the well grown regenerated plants derived from the calli of the Chiniguri-2 cultivar on the autoclaved vermiculite/sand mixture. This remarkable growth of the regenerated plants recognizes the efficacy of the popularly used vermiculite/sand mixture for growing regenerated plants and shows the genotype dependent efficiency of the Chiniguri-2 rice cultivar on callus induction.

**Table 2.** Plant regeneration from plated calli

Cultivar	No. of calli plated	No of plant regenerated	Plant regeneration (%)
C1: Ranisalute	10	5	50.00
C2: Somudrafena	24	16	40.00
C3: Chiniguri-2	20	8	66.66
C4: Chiniatap	10	2	20.00
C5: Khatchra	12	6	56.00
CV%	27.18	14.8	6.90
LSD	1.56	2.04	4.55
Level of significance	**	*	*

\*= Significant at 1% level of probability

\*\*= Significant at 5% level of probability



*Cultivars: C1- Ranisalute, C2- Somudrafena, C3- Chiniguri-2, C4- Chiniatap, C5- Khatchra*

**Fig. 2.** Regenerated plants from the calli of cultivars



Fig. 3. Reregnerated plants (Chiniguri-2) grown on vermiculite-sand mixture.

#### 4. Conclusions

This study revealed that quantitative information on callus induction and plant regeneration could be obtained from the MS medium based cultures of dehusked rice seeds. Additionally, it is evident that callus induction and plant regeneration depended on the genotypes. Further, the cultivars responded well to both callus induction and plant regeneration may be utilized for exploiting somaclonal variant plants as well as in breeding program through tissue culture to reduce the huge requirement of seeds and breeding materials needed for the development of new varieties. However, field study based confirmation on the callus induction and plant regeneration efficiency of the cultivar Chiniguri-2 need to be studied.

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