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In Vitro Callus Induction and Plant Regeneration in Seed Explants of Rice (*Oryza Sativa* L.)

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Abstract: The effect of different concentrations of 2,4D in the presence and absence of calcium silicate on rice seed culture was investigated in three rice genotypes. Callus induction medium was supplemented with the following concentrations of growth regulator: 2,4D 1 mgL^{-1} + Calcium Silicate 60 mgL^{-1}, 2,4D 2.5 mgL^{-1}, 2,4D 2.0 mgL^{-1}, 2,4D 1.5 mgL^{-1}. For plant regeneration MS medium fortified with Casein Hydrolysate 4 gL^{-1}, NAA 1 mgL^{-1}, Kinetin 3 $mgL^{-1}$was applied. Both genotype and growth regulators significantly affected callus induction and plant regeneration. The variety Pajam and the medium containing 2,4D 1 mgL^{-1} + Calcium Silicate 60 mgL^{-1} and 2,4D 2.5 mgL^{-1} were found most efficient for callus induction. Variety Kalizira showed better performance in plant regeneration; calli of this variety, derived from the medium fortified with 2,4D 2.5 mgL^{-1}produced 80% regenerated plants.

Key words: Rice, dehusked seed, callus, regeneration

INTRODUCTION

Oryza sativa (*Oryza sativa* L. 2n=2x=24*)* is an annul grass. It belongs the family Gramineae. Rice is the world most important food crop after wheat and maize. 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[13]. During the past few decades techniques of tissue culture, like anther culture, protoplast fusion, leaf culture, root culture and dehusked grain culture are being employed in rice breeding to exploit somaclonal variation for the creation of novel rice varieties[9] .

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[11,4], the culture methods[2,17], the media[2,13] and the culture conditions[8,15]. 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. So, in this paper a comparative study of the effects of different concentrations of 2,4D is presented in the presence and absence of calcium silicate on callus formation and plant regeneration from dehusked seeds of three local rice genotypes.

MATERIALS AND METHODS

Dehusked seeds from the rice varieties Pajam, Lucky and Kalizira were used for callus induction.

Agarified MS[6] medium was selected as basal medium both for callus induction and plant regeneration. Callus induction medium was supplemented with the following concentrations of growth regulator: 2,4D 1 mgL^{-1} + Calcium Silicate 60 mgL^{-1}, 2,4D 2.5 mgL^{-1}, 2,4D 2.0 mgL^{-1}, 2,4D 1.5 mgL^{-1}. For plant regeneration MS medium fortified with Casein Hydrolysate 4 gL^{-1}, NAA 1 mgL^{-1}, Kinetin 3 $mgL^{-1}$was applied. In both cases the P^H of the medium was adjusted to5.6.

Rice seeds were manually dehusked and washed with tap water, and then the seeds were transferred to the laminar airflow cabinet. The seeds were kept in 70% ethanol for 30 sec. After that the seeds were rinsed in 0.2 % $HgCl_2$ solution for 10 min. The materials then washed 3 times with autoclaved double distilled water to remove all the trace of $HgCl_2$. Finally the materials were kept in sterilized tissue paper to soak the remaining water. Surface sterilized seeds were cultured with the help of sterilized forceps into the conical flask containing 25 ml of callus inducing medium. In each flask, 10 seeds were inoculated and for each treatment, 30 seeds were cultured.

The cultures were kept in the dark for one month at 25 $\pm$ 1°C, and then they were transferred to a light of 2000 Lux under 16 hour's photoperiods with the same temperature regime. Callus induction was noticed within

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two weeks of inoculation of cultures. After two months of inoculation of seeds, calli were transferred into the plant regeneration medium and were kept in a light of 2000 Lux under 16 hour's photoperiods at 25 ± 1°C. Within one month greenish plantlets were obtained from the plated calli.After eight weeks of inoculation of rice seeds, callus induction frequency was calculated. All the calli originated from a single seed was considered as one.

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

Callus induction frequency (%) =

$$\frac{\text{No. of seeds produced calli}}{\text{No. of seeds cultured}} \times 100$$

Plant regeneration from plated calli was calculated with the following formula:

Plant regeneration (%) =

$$\frac{\text{No. of calli produced plants}}{\text{No. of plants plated}} \times 100$$

The experiment was laid out following Complete Randomized Design (CRD) with three replications and the data were analyzed in computer using statistical package MSTAT-C program.

RESULTS AND DISCUSSIONS

Callus induction:

Effect of variety: The effects of varieties on callus induction from dehusked rice seeds are shown in Table1. Callus induction in rice was found highly variable and genotype specific. Among the three studied varieties, the variety Pajam produced 100% callus from the inoculated seeds, which was significantly higher than other two varieties. Frequency of callus induction from the seeds of variety Lucky and Kalizira (85% and 90% respectively) did not differ significantly.

Effect of growth regulators: For callus induction MS medium supplemented with different concentration of growth regulators were used. The results obtained are presented in Table 2. Among the combinations, 2,4D 1 mgL^{-1} + Calcium Silicate 60 mg L^{-1} and 4D 2.5 mgL^{-1} were found to be best for callus induction (100%) and the results were significantly higher than 2,4D 2.0 mgL^{-1} and 2,4D 1.5 mgL^{-1}.

Effects of growth regulator and variety interaction: Results of variety and growth regulator interaction effect on callus induction are given in Table 3. The rate of callus induction in this case varied from 53.33% to 100%. Maximum calli (100%) were produced from the combinations of 1,2,3,4,5,6,7 and 10, which were significantly higher than the combinations of 8,9,11 and 12.

Regeneration of plated calli: When the dehusked rice seeds were cultured on callus inducing medium, a, soft friable creamish callus was formed within 4 – 5 weeks of culture. The produced calli of convenient size were transferred on MS medium supplemented with Sucrose70gL^{-1} Casein hydrolysate 4gL^{-1}, NAA 1mgL^{-1}, Kinetin 3mgL^{-1}. Plantlets with 5 – 7 cm long shoots and very well developed roots were formed in 6 – 8 weeks. Table 4 shows the results of organogenesis of plated calli. Various varieties showed marked differences regarding composition of the culture medium for morphogenesis of plated calli. Some of the calli formed only roots without shoots, but others produced both roots and shoots. All these types of morphogenesis were regarded as organogenesis. Results indicate that calli derived from all the combinations showed reasonable potentiality for organogenesis (80% - 90%). However significant differences observed in producing whole plantlets containing both shoot and root. Among the three rice varieties Kalizira produced good amount of regenerated whole plantlets for all combinations of growth regulators. Although for this variety calli derived on the medium supplemented with 2,4D 1.0 mgL^{-1} produced maximum green regenerated whole plants (80%) and which was significantly higher than all other combinations studied. 53.85% of calli derived from mentioned variety formed on the medium supplemented with 2,4D 2.0 mgL^{-1} produced only roots. Calli of the variety Pajam produced on T_1, T_3 and T_4 combinations of growth regulators showed high organogenic potentiality but most of them differentiated only into roots.

The present investigation revealed that both genotype and media composition and their interaction largely affect on callus induction and subsequent plant regeneration. This revelation is in agreement with the findings of Pandey *et al*[7]. Pandey reported that the success of *in vitro* culture largely depends on the nutritional media, growth regulators, and genotype and on the interaction of genotype X medium. Similar reports were also made by other authors[10,16,1,3].

Many authors stressed the role of 2,4D in rice tissue culture. Thus Pandey *et al.*[7] worked on dehusked rice seeds, using different level of 2,4D in nutrient medium and

Table 1: Effect of varieties on callus induction

Varieties	No. of seeds inoculated	Frequency of callus induction, %
Pajam	120	100.00 a
Lucky	120	85.00 b
Kalizira	120	90.00 b

Table 2: Effect of different concentrations of growth regulators on callus induction

Concentrations of growth regulators	No of seeds inoculated	Frequency of callus induction, %
2,4D 1mgL^{-1} + Calcium Silicate 60 mgL^{-1}	90	100.00 a
2,4D 2.5 mgL^{-1}	90	100.00 a
2,4D 2.0 mgL^{-1}	90	93.30 b
2,4D 1.5 mgL^{-1}	90	73.30 c

Table 3: Effect of variety and treatment interaction on callus induction

Calli derived from the combination	No. of seed inoculated	Frequency of callus induction, %
T_1V_1	30	100.00 a
T_1V_2	30	100.00 a
T_1V_3	30	100.00 a
T_2V_1	30	100.00 a
T_2V_2	30	100.00 a
T_2V_3	30	100.00 a
T_3V_1	30	100.00 a
T_3V_2	30	86.70 b
T_3V_3	30	93.33 ab
T_4V_1	30	100.00 a
T_4V_2	30	53.33 d
T_4V_3	30	66.70 c

Here ,V_1= Pajam, V_2= Lucky, V_3= Kalizira and , T_1=2,4D 1mgL^{-1} + Calcium Silicate 60 mgL^{-1}, T_2=2,4D 2.5 mgL^{-1}, T_3=2,4D 2.0 mgL^{-1}, T_4=2,4D 1.5 mgL^{-1}

Table 4: Plant regeneration from the plated calli.

Calli derived from the combination	No. of calli inoculated	Frequency of organogenesis %	Frequency of calli forming both shoot and root %	Frequency of calli forming only roots %
T_1V_1	35	91.42	12.50 c	75.00
T_1V_2	15	80.00	8.33 c	83.33
T_1V_3	30	96.67	41.38 b	31.03
T_2V_2	40	87.50	51.42 b	28.57
T_2V_3	12	83.33	80.00 a	10.00
T_3V_1	20	80.00	6.25 c	75.00
T_3V_3	15	86.67	38.46 b	53.85
T_4V_1	12	96.00	8.33 c	83.33

they concluded that 2,4D at the concentration of 2.0 mgL^{-1} gave the best response for callus formation. Result of the present experiment also showed that 2,4D containing media responded well both for callusing and plant regeneration. Although in the present study 2,4D was used at different doses (2.5, 2.0, 1.5, 1.0 mgL^{-1} respectively).

Effect of absorbable silicon was reported by Liu *et al*[5] in rice anther culture. Different level of absorbable silicon viz. 0, 60 and 120 mgL^{-1} were tested by them in callus inducing medium and 60 mgL^{-1} was found efficient both for callus induction and plant regeneration.

Wahed[14] also reported that the use of Calcium Silicate at the rate of 60 mgL^{-1} in the medium produced both maximum calli and green regenerated plants. The findings of the present investigation are also in agreement with the views of Wahed. In this experiment Calcium Silicate 60 mgL^{-1}+ 2,4D 1mgL^{-1} containing medium produced 100% calli for all the varieties studied and the plated calli formed on this medium showed high organogenic potentiality. However maximum of these calli only differentiated into roots. This dissimilarity may be due to use of different varieties.

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