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In vitro callus induction and plant regeneration of rice (*Oryza sativa* L.) var. 'Sita', 'Rupali' and 'Swarna Masuri'

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ABSTRACT

In vitro callus induction and plant regeneration potentiality were studied from mature embryo of three Indian rice (Oryza sativa L.) varieties. Study was done by using callus induction medium (Murashige and Skoog, 1962) having different concentration of 2, 4-D viz., 1.0, 1.5, 2.0, 2.5 mg/l. All the three varieties exhibit highest frequency of callus induction at 2 mg/l 2,4-D. The callus induction frequency varied from 63.36% to 92.23%. Sita had maximum frequency of callus formation only, while Masuri showed embryogenic organogenesis (shoot formation) followed by the formation of callus and Rupali showed increase in multi root development in the early stages of callusing. Plant regeneration efficiency of in vitro grown plantlets were further observed and successfully transplanted into soil condition.

Key words: *Oryza sativa*, embryo, callusing, regeneration, 2, 4-D.

INTRODUCTION

Rice, *Oryza sativa* L. (2n = 2x = 24), an annual grass, belonging to the family Poaceae, is the staple food for the people of India and world's most important cereal crop after wheat and maize[1]. It provides half of total dietary carbohydrate, especially in Asian countries and it is suitable diet for more than three billion people, supplying 50-80% of their daily calorie intake[2]. 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[3].

Several laboratories have described regeneration of plants through callusing from various rice explants such as immature embryos, immature panicles[4], young inflorescence[5] and root[6]. Rashid *et al*.[7] studied that rice seeds have more potential for callogenesis as compared to node or tip. Successful callus induction from rice seed has been reported by several researchers[8, 9, 10]. Culture of dehusked rice embryo is a nobel technique to exploit somaclonal variation and for improvement of grain quality. Sita, Rupali and Swarna Masuri are the most important rice varieties of Asansol, Dist. Burdwan, West Bengal for their fine grain, good test and reasonably priced[11].

Therefore, this study was aimed to evaluating these three rice varieties for callus induction and regeneration efficiency under different concentrations and combinations of growth regulators. 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

Healthy seeds of *Oryza sativa* L.(family Poaceae) of three varieties viz. Sita, Rupali and Swarna Masuri MTU-7029 also known as Masuri were collected from Agricultural Blok Office, Jamuria-II, Burdwan (West Bengal), collected seeds were authenticated from Assistant Director of Agriculture.

Mature embryo culture was performed for induction of callus by somatic embryos. Mature rice seeds were dehusked manually, washed with detergent Extran (2-3% $^{V}/_{V}$) for 5-6 minutes followed by distilled water wash for 2 times. Thereafter seeds were surface sterilized with mercuric chloride (0.1% w/v) for 5 minutes and finally rinsed 3 times with sterile distilled water. Embryo portion of the undamaged seeds were aseptically transferred into the bottles (5-6 seeds in each) containing MS basal media (Murashige and Skoog, 1962) with different concentration of 2,4-D viz., 1.0, 1.5, 2.0, 2.5 mg/l, 30 g/l sucrose, 0.1 g/l myo-inositol and 8 g/l agar for studying the callogenic response of seed embryo explant. The pH of the medium was adjusted to 5.8. Cultured bottles were kept for incubation inside the culture room which is maintained at 25±1°C, 85% humidity and 10 hours light and 14 hours of dark cycle. Callus induction was noticed within two weeks of inoculated cultures. The frequency of callus induction and plant regeneration (%) were measured using the following formulas [12]:

$$\text{Frequency of callus induction (\%)} = \frac{\text{no. of explants induced callus}}{\text{no. of cultured explants}} \times 100$$

$$\text{Frequency of shoot induction (\%)} = \frac{\text{no. of culture induced shoots}}{\text{no. of culture}} \times 100$$

$$\text{Frequency of root induction (\%)} = \frac{\text{no. of shoot induced root}}{\text{no. of culture induced shoot}} \times 100$$

After proliferation of calli the Somatic embryogenic calli were identified and sub cultured in different bottles containing same media and growth regulator concentration for regeneration i.e. shoot, root or plantlet formation(Fig 5). *In vitro* raised healthy plantlets were then taken out from culture bottles and gently washed with 0.1% w/v Bavistin (BVN) followed by sterile distilled water wash to remove the adhering medium completely. Therefore, they were transferred to disposable plastic cups containing sterile soil mixture: vermiculite :: 3:1 supplemented with 0.1g/l Bavistin to prevent fungal contamination[13]. Plantlets were maintained at polyhouse at temperature ± 30°C for 7 days. Later complete plantlets were successfully established at field condition(Fig 6).

RESULTS AND DISCUSSION

The effect of different concentration of growth regulator (2, 4-D) on different varieties for callus induction and plant regeneration from isolated seed embryo at 21 days are shown in table 1. All the three varieties resulted optimum frequency of callus initiation at 2.0 mg/l 2,4-D concentration whereas at a high or low concentration the cultivars showed similar tendency of decrease in callus initiation. This indicate that the use of 2,4-D with 2 mgl/l was adequate for production of high amount of callus in rice. Among the three varieties sita showed 92.23% of callus induction frequency from the seed embryo which was higher than other two varieties. The other two varieties Rupali and Masuri, showed 74.44% and 80.04% of callus induction frequency in average respectively. This may happen due to different genotypic efficiency of cultivars for callus induction.

Table 1: Effect of different concentration of 2,4-D in MS media in callus induction frequency

Concentration and combination of medium	Sita		Rupali		Masuri	
	Mean of frequency (%±SD)	Degree with callus morphology	Mean of frequency (%±SD)	Degree with callus morphology	Mean of frequency (%±SD)	Degree with callus morphology
MS 1	69.49±3.1	+, Py, F	63.36±3.5	+, CrW	67.56±2.6	+, C
MS 2	81.67±2.4	++, Py, F	70.03±2.5	+, CrW	73.25±2.1	+, C
MS 3	**92.23±1.6**	**+++, Py, F**	**74.44±1.6**	**+, CrW, F**	**80.04±1.8**	**++, C, F**
MS 4	80.09±2.2	++, Py, F	67.53±2.2	+, CrW	74.51±2.0	+, C
Mean	80.87±2.3		68.84±2.4		74.84±2.1	

***Note**: MS 1= MS Media+1.0 mg L^{-1} 2, 4-D, MS 2= MS Media+1.5 mg L^{-1} 2, 4-D,*
MS 3= MS Media+2.0 mg L^{-1} 2, 4-D, MS 4= MS Media+2.5 mg L^{-1} 2, 4-D.
+= Slight callus, ++= Moderate callus, +++= Massive callus,
Py= Pale yellow, C= Creamy, CrW= Creamy white, F= Friable.

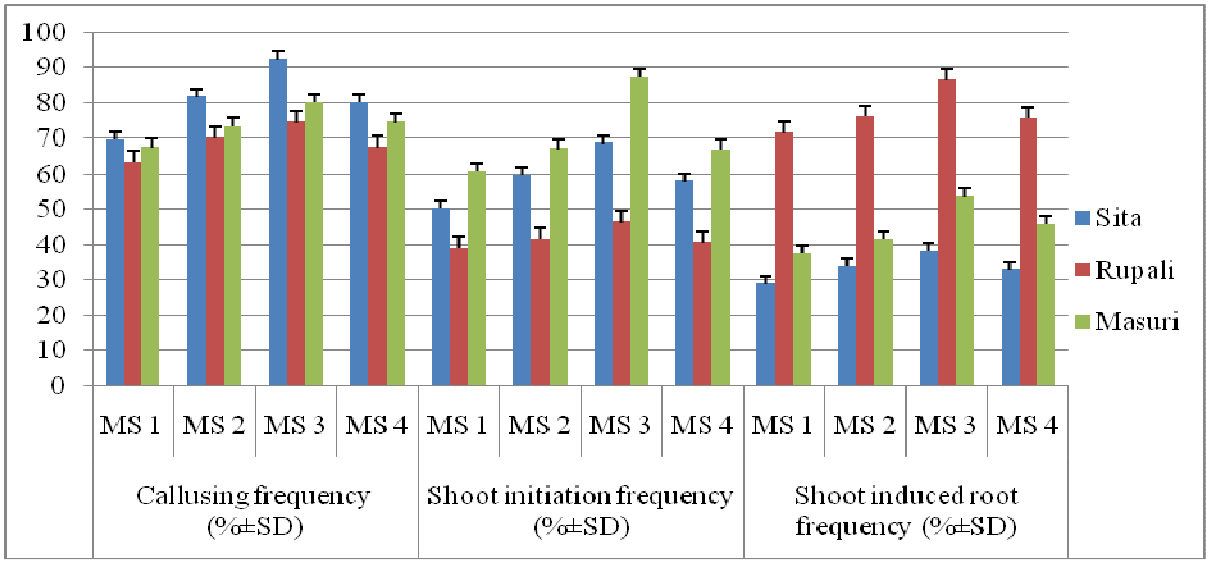

Figure 1: Effects of varieties in combination with treatments of different concentration of 2, 4-D on callus induction, regeneration and rooting parameters by using mature embryos as explant

In addition 2.0 mg/l concentration was also found to be more suitable for plant regeneration i.e. shoot initiation or plantlet formation. Frequency of shoot initiation from calli was resulting in the order of highest to lowest was Masuri>Sita>Rupali and for shoot induced root was Rupali>Masuri >Sita at the stage of day 35. This order was more or less same for all four concentrations of 2, 4-D but they only differ in their percentage of frequency. On transferring into the plant regeneration media Masuri showed maximum frequency of shoot elongation but less frequency of root proliferation(Fig 4) whereas for Rupali the tendency of highly root proliferation and a average frequency of shoot initiation(Fig 3) and for Sita the tendency of massive callus formation but a average frequency of shoot and root proliferation were found(Fig 2).

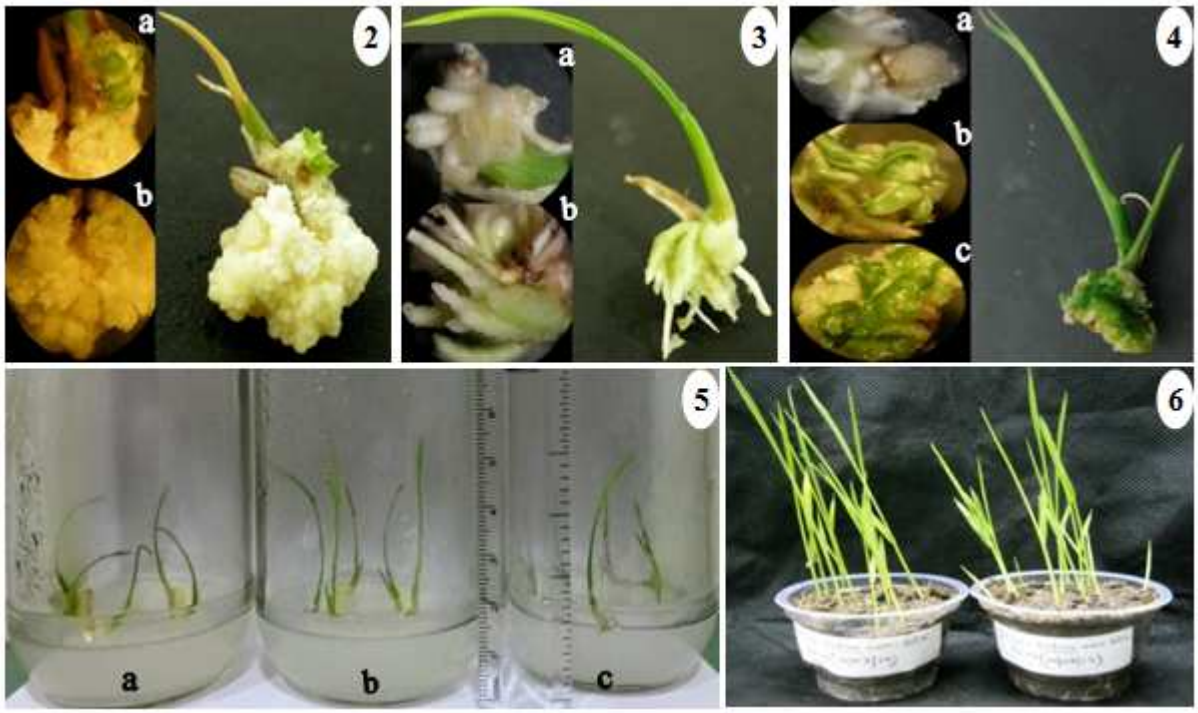

Figure 2-4. Callus initiation, somatic embryogenesis, shoot and root proliferation of different varieties (2=Sita, 3=Rupali, 4=Masuri). a, b and c: Photographs taken under 4X magnification of stereo microscope (Model: Olympus, Magnus) at different day interval; 5. Normal plantlet regeneration from callus on same concentration of medium; 6. Hardening of regenerated plants in pot culture

This finding agreement with previous report by Mannan *et al.*[14], Revathi *et al.*[15], Alam *et al.*[16]. Similar observations were also reported in rice by Verma *et al.*[13], where they showed that different varieties of rice produced high amount of callus cultured on MS medium supplemented with 2.0 mg/l 2, 4-D.

CONCLUSION

This present study reports a successful *sativa* rice high frequency regeneration porotocol from mature seed embryo through embryogenic callus formation in three diverse and highly productive locally adapted cultivars Sita, Rupali and Swarna Masuri. Thus finding of this study will be very useful for producing high frequency callus induction that is the prime step for crop improvement or rapid propagation through biotechnological approaches which provide a simple *in vitro* protocol for generating high frequency callus formation and its subsequent regeneration potentiality. These findings can also be utilized for designing transformation experiment for further cultivar improvement for disease and pest resistant, high yielding enhanced nutritive value, stress and salt tolerance through tissue culture and gene transfer techniques.

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