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RESEARCH PAPER

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In vitro Regeneration of High Yielding *Indica* Rice Varieties

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ABSTRACT

The present study was undertaken with a view to identify high frequency regeneration of plants from *In vitro* cultured tissues and the cell is a pre-requistic for successful application of tissue culture and genetic engineering technologist for crop improvements.

The highest callus induction ability was observed in Binasail (80%) is MS + 2 mg/l 2,4-D. The range of callus induction in MS medium supplemented with different concentration of 2,4-D and 0.54 mg/l BAP was 12 to 68% callus induction ranged from 36 to 80% in MS medium and different concentration of 2,4-D and 0.5 mg/l kn. The callus induction frequency in MS medium supplemented with 2,4-D and in combination with BAP was found satisfactory in all the three varieties. The range of callus induction in MS medium supplemented with different concentrations of 2,4-D and 0.5 mg/l BAP was 12 to 68%. The highest callus induction ability (68%) was observed in Binasail.

Key words: Rice, Regeneration, BAP, 2,4-D.

INTRODUCTION

Rice **(*Oryza sativa L.*)** is the world's most important food crop and a primary source of food for more than half the world's population (Khush, 2005). Rice is the world's most important cereal crop and feeds over half of the global population.It belongs to the **Poaceae** family. In Bangladesh the major cereal crops are rice and wheat although main focus is on rice production with 79.4 percent of the total cultivatable land area under rice crop as mentioned in FAO/WFP CFSAM 2008 Report. Plant biotechnology and in particular plant cell culture, is immensely important for the improvement of cereal crops like rice and wheat. The regeneration of plants from cell and tissue culture is an important and essential component of biotechnology that is required for the genetic manipulation of plants. High frequency regeneration of plants from *In vitro* cultured tissues and the cell is a pre-requistic for successful application of tissue culture and genetic engineering technologist for crop improvements. Cereals and other grass species are generally recalcitrant to tissue culture **(Vasil and Vasil, 1996).** The totipotency of a cell or tissue opens use several new contingencies in plant breeding programmes that provide gene manipulation and selection of desirable characters. Many plant breeders are now interested n tissue culture technique, because it has already proved to be a valuable method in crop improvement. Variation could play an important role for crop improvement. Somacolonal variation through tissue culture is particularly interestion in plant breeding. Some of the regenerated plants showed higher somacolonal variability in R_1 regeneration (Lutts *et al.*, 2001). The first success on plant regeneration in rice and also in monocot plants come from the report of **Nishi *et al.* (1958)** and Niizeki and Oono (1968). In the light of the study was undertaken with three high yielding rice varieties of BINA in order to achieve the objective to observe the effect of different concentrations of hormone on callus induction and regeneration.

MATERIALS AND METHODS

The experiment was conducted at the tissue culture laboratory of the plant breeding division, Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh, Bangladesh

Experimental materials are Binadhan-4 Binashail and Iratom-24. Mature rice grain embryos were used as explants for the present study. For callus induction MS medium used with different concentration of 2,4-D. *Indica* rice (*Oryza sativa* L.)/ *Indica* rice (*Oryza sativa* L) mutants were used as the experimental material for the present investigation. Binadhan-4, that is an early, high grain quality and high yielding *indica* variety. Binashail, this is long fine grain, long panicle, early, high yielding (4.2-5.0 tons/ha) *indica* variety, Iratom-24, that is height (75-80 cm), long fine grain, resistant, improved yielding *indica* variety. MS mediums, BAP were need.

Cultural methods

Embryo culture

Mature rice grains attached to endopserm were the main source of explants for embryo culture. Mature seeds of four mutants (TNDB-100, Y-1281, MR-219, DM-25) of *indica* rice were dehucked manually with a special thoroughly in running tap water. The floating dehusked seed was carried discarded. The surface sterilization of these dehusked seed was carried out in aseptic tube and immersed into 0.1% $HgCl_2$ solution for 20 minutes followed by 4-5 rinsed in autoclaved distilled water to remove traces of $HgCl_2$, which could be toxic to the explants if kept for longer duration. Sterilized mature seeds were of 2,4-D (1.0, 1.5, 2.0, 2.5 and 3 mgL^{-1}) for induction of callus. It were placed horizontally in to the test tube containing 20 ml solid medium and covered with cotton plug.

Subculture of calli

After 21-25 days old calli were sub cultured to freshly prepared MS medium supplemented with the above concentration of 2.4-D for convenient size of the calli. The culture vessels containing explants were placed under fluorescent light in a room with controlled temperature (25 ± 2°C) under 16 hour photo period with light intensity of 2000-3000 lux. Cultures were examined regularly and when showing symptoms of contamination were discarded.

Transfer of calli to regeneration medium

When calli attained in convenient size, they removed aseptically from the conical flask sterilized petridsh. The calli were again cultured on freshly prepared Murashige, T. & Skoog, F. (1962) medium containing 3 different hormonal supplements (0.50 ml-1, NAA 9. 11. 13 mgl-1 Kinetin) for shoot induction from callus. The culture vessels containing explants were placed under fluorescent light in a room with controlled temperature (25 ± 2°C) under 16 hour photoperiod with light intensity of 2000-3000 lux. All cultures were cheeked daily and when showing symptoms of contamination were discarded.

RESULTS AND DISCUSSION

The mature embryos of three rice varieties were placed on MS medium supplemented with five different concentrations of 1,4-D (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) to see callus induction performance of these three genotypes (Table 1). The mature embryos of three aromatic rice varieties were cultured on MS medium supplemented with five different concentrations of 2,4-D (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) in combination with a single concentration of BAP (0.5 mg/l).

Table 1. Effect of different concentrations of 2,4-D and BAP in MS medium on callus induction from 25 mature embryos of each in three rice varieties.

Variety	Supplements mg/L		No. of explants showing callus induction	Callus induction %	Days to Callus induction
	2.4-D	BAP			
Binadhan-4	1.0	0.5	7	28	15-20
	1.5	0.5	16	64	
	2.0	0.5	15	60	
	2.5	0.5	8	32	
	3.0	0.5	4	16	
Binasail	1.0	0.5	7	28	15-20
	1.5	0.5	14	56	
	2.0	0.5	17	68	
	2.5	0.5	7	28	
	3.0	0.5	3	12	
Iratom-24	1.0	0.5	6	24	15-20
	1.5	0.5	17	68	
	2.0	0.5	16	64	
	2.5	0.5	8	32	
	3.0	0.5	3	12	

The callus induction performances of these varieties were evaluated and the results are presented in Table 1. Among the three varieties, the highest callus induction ability (68%) was observed in Binasail in combination of 2.0 mg/l 2,4-D + 0.5 mg/l BAP and in Iratom-24 it was 1.5 mg/l 2,4-D + 0.5 mg/l BAP in all the varieties callus induction took 15-20 days (Plate1).

Table 2. Effect of different concentrations of 2,4-D and Kn in MS medium on callus induction from 25 mature embryos of each in three rice varieties.

Variety	Supplements (mg/L)		No. of explants showing callus induction	Callus induction %	Days to Callus induction
	2.4-D	Kn			
Binadhan-4	1.0	0.5	10	40	20-25
	1.5	0.5	17	68	
	2.0	0.5	19	76	
	2.5	0.5	15	60	
	3.0	0.5	12	48	
Binasail	1.0	0.5	11	44	20-25
	1.5	0.5	16	64	
	2.0	0.5	20	80	
	2.5	0.5	15	60	
	3.0	0.5	11	44	
Iratom-24	1.0	0.5	10	40	20-25
	1.5	0.5	14	56	
	2.0	0.5	18	72	
	2.5	0.5	13	52	
	3.0	0.5	9	36	

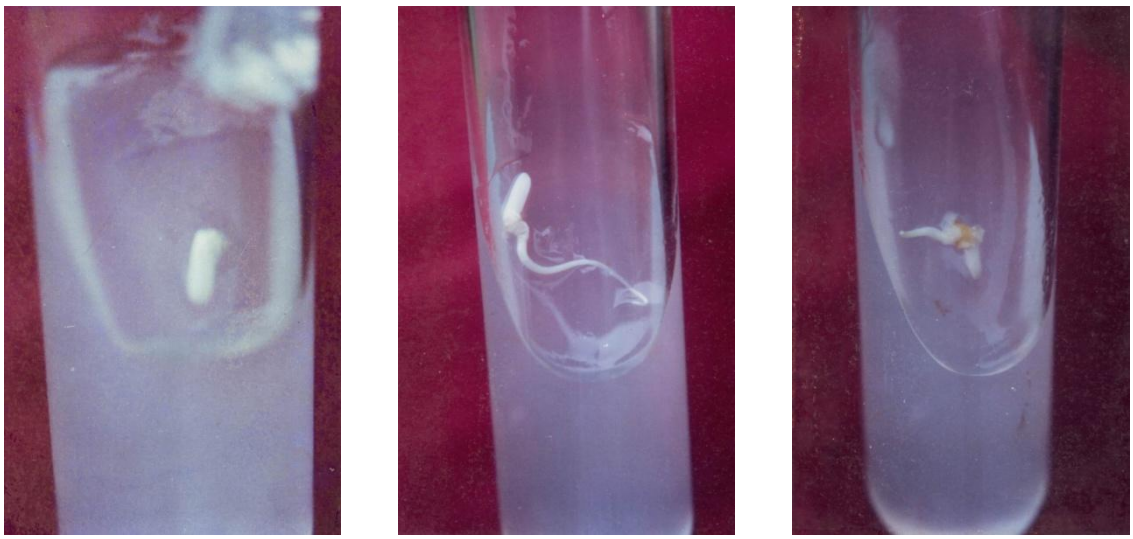

Plate 1. Callus induction of different concentrations of 2,4-D and Kn in MS medium on callus induction from 25 mature embryos of each in three rice varieties

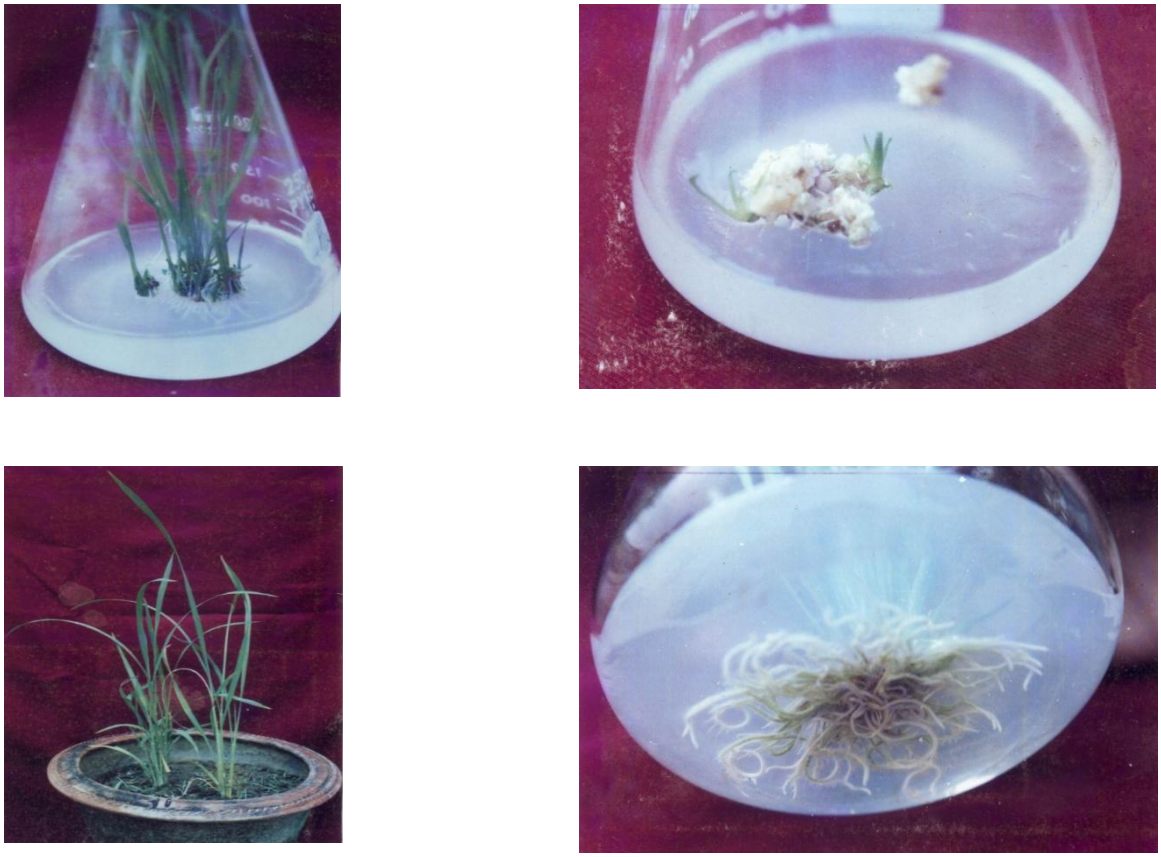

Plate 2. Regeneration of shoot and root from embryonic callus.

Table 3. Effect of Genotypes × Treatment (MET) interaction on root induction, no. of roots/plants and root length of three rice varieties.

Treatment mg/l MET	Characters		
	% of root induction	No. of roots/plant	Root length (cm)
Binadhan-4 × 2.0	79.0 b	11.5 b	1.87 d
Binasail × 2.0	80.0 b	12.0 ab	2.50 c
Iratom-24 × 2.0	82.5 b	9.8 bc	2.95 a
Binadhan-4 × 2.5	88.5 a	13.5 a	3.22 a
Binasail × 2.5	91.0 a	14.0 a	2.85 ab
Iratom-24 × 2.5	89.5 a	12.6 a	2.50 c
Binadhan-4 × 3.0	82.0 b	9.0 c	2.72 b
Binasail × 3.0	74.0 d	9.8 bc	1.90 dc
Iratom-24 × 3.0	78.0 bc	7.5 c	2.15 d

Common letter (s) in column did not differ significantly at 5% level.

Induction of roots

Cultured on freshly prepared MS medium containing hormonal supplements of 0.5 mg/l NAA and 3 different concentrations of MET (Multi-effect-Trizole) for observing the rooting

response of regenerated shoots. Root induction (%), number of root/shoot and length of roots were recorded and presented in Table 3.

Organogenesis via callus

Plants can be regenerated from *in vitro* culture via shoot and subsequent root morphologenesis and thus regeneration systems lead to complete plants. For induction of shoots from calli of three rice varietires (Binadhan-4, Binasail and Iratom-24) were cultured in MS medium supplemented with auxin and different concentrations of cytokinin, The combinations such as (i) 0.5mg/l NAA + 5.0mg/l Kn (ii) 0.5mg/1 NAA + 7mg/l Kn (iii) 0.5mg/1 NAA+ 9mg/l Kn (i) 0.5mg/1 NAA + 11mg/lKn 0.5mg/1 NAA + 13mg/l Kn were used for this purpose. Regenerations capacity of callus to various combinations of NAA and Kn in MS medium are presented in the Table 4.

Table 4. Effect of different concentrations of NAA and Kinetin in MS medium o shoot regeneration from 20 no of callus induction of each in three rice varieties.

Variety	Supple-ments (Mg/l) NAA Kn	No. of callus showing shoot regeneration	shoot regenerat ion (%)	No of shoots/ callus (Mean SD)
Baridhan-4	0.5 5	5	25 fg	1.8 ± 0.014
	0.5 7	7	35 de	2.6 ± 0.021
	0.5 9	10	50 b	4.7 ± 0.011
	0.5 11	9	45 c	5.5 ± 0.009
	0.5 13	5	25 fg	3.4 ± 0.017
Binasail	0.5 5	4	20 g	2.81 ± 0.014
	0.5 7	7	35 de	2.5 ± 0.012
	0.5 9	10	50 bc	4.8 ± 0.019
	0.5 11	13	65 a	5.1 ± 0.020
	0.5 13	6	30 ef	3.6 ± 0.015
Iratom-24	0.5 5	5	25 f	2.0 ± 0.008
	0.5 7	6	30 ef	2.5 ± 0.016
	0.5 9	8	40 cd	3.8 ± 0.025
	0.5 11	11	55 b	4.5 ± 0.028
	0.5 13	5	25 fg	3.2 ± 0.019

Common letter (s) in column did not differ significantly at 5% level.

From the results (Table 4) it was observed that shoot proliferation was distinctly variable among the varieties in the regeneration medium at different concentrations of auxin and cytokinine. Among the varieties Binasail showed the highest (65%) shoot regeneration followed by Iratom-24 (55%) in MS + 0.5 mg/l NAA+ 11.0 mg/l Kn. But Binadhan-4 showed the highest (50%) shoot formation when Kn was used at the rate of 9.0 mg/l in MS +0.5mg/l in MS +0.5mg/l NAA. The prolificacy of shoot was to be recorded the highest in the hormonal combination of MS +0.5mg/lNAA+11.0mg/l Kn, It was found that shoots/ callus of Binadhan-4 was 5.0. in Binasail 5.2 and in Iratom-24 it was 4.9 in number(Plate2).

All the varieties showed the lowest shoot regeneration percentage both in 0.5 mg/l NAA+ 5.0 mg/l Kn and 0.5mg/l NAA+ 13.0 mg/l Kn combinations. In all the cases green spot like structures wee appeared first at the surface of calli within 15-20 days after transferring to

regeneration medium. From these green spots regeneration occurred. After shoot differentiation weak and narrow root induction was observed. Azira and Bhalla (2000) earlier reported plant regeneration ability of rice. They observed that shoots per callus varied depending on the genotype and hormonal concentration. This result similar with the findings of Anju *et al.,* (1999) who proved that cytokinine and auxin were essential for organogenesis. Present investigation revealed that NAA in combination with 0.5 mg/l Kn to regenerate plantlets.

SUMMARY AND CONCLUSION

The range of callus induction in MS medium supplemented with different concentrations of 2,4-D was 50.0 to 80%. The highest callus induction ability was observed in Binasail (80%) in MS + 2 mg/l 2,4-D. The range of callus induction in MS medium supplemented with different concentrations of 2,4-D and 0.5 mg/l BAP was 12 to 68%. The highest callus induction ability (68%) was observed in Binasail in combination of 2.0 mg/l 2,4-D + 0.5 mg/l BAP and in Iratom-24 it was 1.5 mg/l 2.4-D + 0.5 mg/l BAP.

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