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Research Article

IN VITRO CALLUS INDUCTION AND REGENERATION POTENTIALITY OF AROMATIC RICE (*ORYZA SATIVA* L.) CULTIVARS IN DIFFERENTIAL GROWTH REGULATORS

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Abstract

Aromatic rice (*Oryza sativa* L.) cultivars are strong aromatic rice cultivars which can thrive well in rice fields prone to flood, drought and other soil constraints. The present investigation was undertaken to determine a suitable media compositions for callus induction and regeneration using immature embryo of six aromatic grown rice cultivars of Bangladesh, namely, Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog. For callus induction different concentrations and combinations of 2, 4-Dichlorophenoxyaceticacid (2, 4-D) along with NAA were evaluated. Maximum callus induction (97.22%) was observed in Kalijira when 2 mg/L of 2, 4-D and 0.5 mg/l NAA was used and less Modhumala (66.67%) and remaining cultivars showed moderate. For regeneration initially different concentrations and combinations of 6-BenzylAminoPurine (BAP) and Indole-3-Acetic Acid (IBA) were tested. Maximum regeneration frequency (91.67%) was observed Kalijira when the optimum concentrations and combinations of 0.5 mg/l of BAP + 0.1 of mg/l IBA were used. Presently optimized regeneration method holds promise for facilitating the deployment of agronomical important trait through genetic transformation for the improvement of this important food crops.

Key Words: *Oryza sativa*; callus; embryogenic; regeneration; hormones

Introduction

Rice belongs to grass family Poaceae and it is cultivated worldwide. It is the staple food of more than half of the world population (Pathak. 1982). It is cultivated in 10.1 million ha and produces 95 per cent of the total food-grain of Bangladesh. Genetic transformation enables the introduction of novel genes directly into aromatic rice cultivars to create new genetically modified varieties (Jones *et al.*, 2005), using tissue culture protocols as their base line. These procedures require that a whole plant should be regenerated from isolated cells or tissues. Many protocols have been developed in rice and successful regeneration of plant tissue culture mainly depends on genotype, explant type, medium composition, plant growth regulator and culture environment (Khanna and Raina 1998). Both mature and immature embryos have been used extensively in tissue culture protocols, mature embryos were found to be a better choice in comparison to immature embryos (Özgen *et al.*, 1998). Immature embryos are better explant source when regeneration is considered and they require time and growth facilities (Zale *et al.*, 2004) whereas mature embryos are available throughout the year.

Aromatic rice is generally used to prepare dishes such as polau and biriani which are served on special occasions. Demand for aromatic rice in recent years has increased to a great extent for both internal consumption and export. Considerable efforts are being made to improve its productivity by using biotechnology. The aromatic rice has demand in both domestic and foreign market for attractive flavor, good taste and fine grains. Country can be benefited by earning foreign exchange by production and export of aromatic rice. But there are some limitations to cultivate aromatic rice for the farmer. Such as lack of high yielding variety, fine grain quality lack of disease or pest

resistant, stress and salt tolerance variety and proper cultural management. The conventional breeding techniques are time consuming and self in-compatibility act as barrier for distant hybridization and fertilization. The aromatic variety can be improved (disease and pest resistant variety, stress and salt tolerance variety) through tissue culture techniques viz. somaclonal variation or genetic manipulation like protoplast fusion (hybrid and cybrid) and through gene transfer.

Therefore, the experiment was undertaken considering the objectives: (1) to identify a suitable medium for callus induction using immature embryo of six aromatics rice cultivars (2) to determine a suitable media composition for plant regeneration (3) to find out the highly regenerable cultivar among six aromatic rice (*Oryza sativa* L.) cultivars from mature dehusked seeds. The maximum number of plants regenerated in a minimum time period will facilitate more effectively to explore transgenic with better expression later on. We hope that this study will be useful for local as well as for international plant breeders for improving the regeneration efficiency of transgenic cells in Rice.

Materials and Methods

Plant material

Immature zygotic embryo of Six aromatic grown rice varieties (*Oryza sativa* L.) of Bangladesh, namely, Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog were used as explants for study of *in vitro* callus induction and regeneration. Mature seed of three Indica rice genotypes (*Oryza sativa* L.) Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog were collected from Bangladesh Rice Research Institute (BRRI), Regional office, Rajshahi, Bangladesh. Seeds were sown in the department field.

Selection of embryogenic callus

Embryogenic callus of indica rice (*Oryza sativa* L.) cultivars namely Kalijira, Chinigura, Radhuni Pagal, Modhumala and Mohonbhog can be described as yellowish and granular callus, compact, greenish-yellow, granular calli. These types of EC were selected and used for regeneration.

Regeneration

The green colour embryogenic calli were then transferred to fresh shoot regeneration medium; MS with BAP (2.0-2.5 mg/l), NAA (1.5 mg/l) and kinetin (1 mg/l) and incubated under light condition. The well-developed calli with shoot primordia were sub-cultured on MS shooting regeneration medium in test tube and incubated at 27°C under continuous light. Healthy shoots with defined stem were transferred to MS rooting medium and incubated at 28°C under continuous light. The plantlets with well-developed root system were planted in the pots containing autoclaved mud that was collected from rice fields. The plantlets were established in planted in several pots.

Immature embryo isolation

Immature embryos, unripe seeds were collected 12 to 15 days after anthesis from field-grown plants and surface-sterilized with $HgCl_2$ (0.1%) for 10 min. These seeds were thoroughly washed four to five times in sterile distilled water. Using fine scalpels and forceps, immature embryos were excised under a binocular microscope.

Culture media and culture conditions

The basic medium (BM) was composed of MS salts and organic com-pounds, 30g/l sucrose and 8g/l agar. The pH was adjusted to 5.7 before adding the gelling agent and media were autoclaved for 20 min at 121◦C and 1.07 kg/cm^2. Petridishes with 25 ml of medium and sealed with Parafilm were used. The immature embryos were cultured aseptically on nutrient media in the dark at 25±1°C with the scutellum side facing upwards.

Callus induction

Ten immature embryo from isolated sterilized seeds were placed individually in each Petri dish containing 25 ml of modified MS with various concentrations of 2, 4-D and NAA. The seeds were incubated in the dark at 25 ± 2ºC. Only embryogenic calli were transferred to fresh callus induction medium for multiplication. Sub-culturing of the callus was carried out once in every two weeks. The callus was observed from 2nd to 7th week. The percentage (%) of callus (total/embryogenic) induction frequency (CIF) for each group was calculated as follows:

$$\text{CIF (\%)} = \frac{\text{Total number of immature embryo that produced callus}}{\text{Total number of immature embryo plated}} \times 100$$

Results

The present investigation was carried out with six aromatic Bangladeshi high yielding cultivars Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog. Matured seeds of the studied cultivars were used for the initiation and maintenance of callus and subsequent regeneration via callus. Thus different steps of experiment and details of the results obtained from each of the experiments are described under the following heads.

Six Aromatic Rice Cultivars for Callus Induction Using Different Combinations of Growth Regulators

An investigation was made on the initiation and maintenance of callus from mature seeds of six aromatic rice cultivars. Explants were cultured in MS medium supplemented with different concentration of 2, 4-D singly and in combination with cytokinins (KIN, BAP). The growth rates of callus culture were highly influenced by different concentrations and combinations of growth regulators. The quantitative measurement of callus growth was estimated in terms of percentage of callus and degree

of callus growth. The results are presented in Table-1 and Table-2.

Effects of 2, 4-D

Different concentrations (1.5, 2.0 2.5 and 3.0 mg/l) of 2,4-D were used for producing sufficient amount of embryonic callus from mature seeds of six aromatic rice cultivars (i.e. Chinigura, Kalijira, Radhuni Pagal, Modhumala, kataribog and Mohonbhog) in MS medium. The results are presented in table-1. The Table indicates that variation of hormonal concentrations played a major role in callus induction.

Callus induction performance of matured seed in six rice cultivars was observed in MS medium supplemented with 2mg/l of 2, 4-D was found most effective in callus induction in all cultivars and it gave the highest 87.44%, 90.22%, 80.67%, 84.11%, 78.55% and 75.78% responses in Chinigura, Kalijira, Radhuni Pagal, Modhumala, kataribog and Mohonbhog respectively for 2 mg/l 2,4-D. Lowest range for Chinigura, 80.59%, for Kalijira, 83.89%, for Radhuni Pagal, 65.55%, for Modhumala, 78.67%, kataribog, for 65.22% and for Mohonbhog, 67.67% responses were observed in higher (4.0 mg/l) concentration of 2,4-D. Increasing of 2,4-D concentration above 2 mg/l the callus induction frequency decrease in all cultivars. Performance of concentration (1.5, 2.0, 2.5 and 3.0 mg/l) of 2, 4-D in different cultivars are also shown in Fig.

Table-1: Effect of different concentrations of 2, 4-D on callus induction from mature embryo of six rice cultivars using MS medium

Genotypes	Range of days and percentage of callus induction with degree of callus growth and callus morphology												Mean production acres
	Concentration of 2,4-D mg/l												
	1.5			2.0			2.5			3.0			
	Range	%	Degree with callus morphology	Range	%	Degree with callus morphology	Range	%	Degree with callus morphology	Range	%	Degree with callus morphology	
Chinigura	10	81.66	+++Py,C	11	87.44	+++Py,C	10	85.66	+++Py,C	11	80.59	+++Py,C	91.55±2.07
Kalijira	11	84.11	+++CrW,C	11	90.22	+++CrW,C	13	88.44	+++CrW,C	12	83.89	++CrW,C	93.42±3.00
Radhuni Pagal	10	69.89	+++CrW,C	12	80.67	+++CrW,C	11	77.44	+++CrW,C	11	65.55	++CrW,C	76.14±3.54
Modhumala	12	80.22	++CrW,C	13	84.11	+++Crw,C	13	82.11	+++Crw,C	14	78.67	+Crw,C	82.28±2.32
kataribog	13	68.78	+++Cre	12	78.55	+++Cr,C	10	75.67	+++Cr,C	12	65.22	+++Cr,C	78.06±1.77
Mohonbhog	12	67.78	+++Cre	13	75.78	+++Cr,C	10	71.58	+++Cr,C	13	67.67	+++Cr,C	75.07±1.85

Cr, Cr,W, Py, Y, BrY, C, N, L, Dr, M indicates Creamy, Creamy White, Pale Yellow, Yellow, Bright Yellow, Compact, Nodular Loose, Dry and moist callus, respectively. +, ++, +++ indicates proe, average and massive growth of callus, respectively.
*In each treatment 36 explants were used.

Table 2: Effect of different kinds and concentrations of 2 mg/l of 2, 4-D with NAA for callus induction from mature embryo of six rice cultivars using MS medium.

Genotypes	Range of days for callus induction with percentage and degree of callus growth											
	Concentration and combination of phytohormone											
	T_1			T_2			T_3			T_4		
	Range	%	Degree	Range	%	Degree	Range	%	Degree	Range	%	Degree
Chinigura	6-7	88.89	+++	5-6	94.44	+++	5-6	86.11	+++	4-6	69.44	++
Kalijira	7-8	94.44	+++	5-7	97.22	++++	5-6	88.89	+++	4-6	66.44	++
Radhuni Pagal	7-9	66.67	+++	6-7	77.78	+++	5-7	61.11	++	5-7	36.11	++
Modhumala	8-10	61.11	+++	7-8	66.67	+++	5-7	52.77	++	6-7	38.89	++
Kataribog	1-8	63.89	+++	6-7	72.25	+++	5-6	58.33	++	6-7	41.67	++
Mohonbhog	8-9	60.11	+++	7-8	72.22	+++	5-7	55.56	++	5-7	41.67	++

T_1 = 2.0, 2, 4-D + 0.25 NAA T_2 = 2.0, 2, 4-D + 0.5 NAA T_3 = 2.0, 2, 4-D + 1.0 NAA T_4 = 2.0, 2, 4-D + 1.5 NAA
In each treatment 20 explants were used.

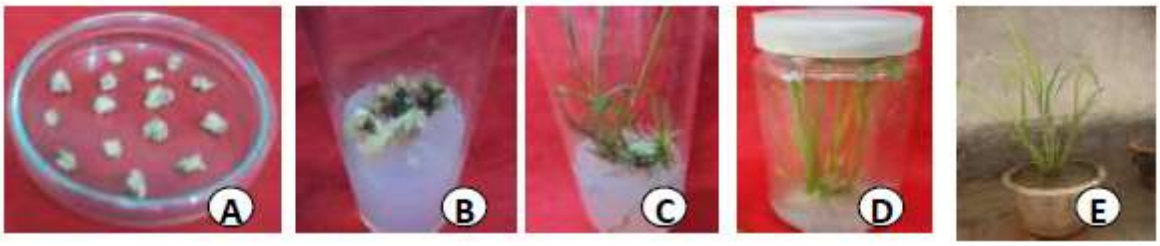

Fig. 1: Plant regeneration from calli derived immature embryos in aromatic rice cultivar Chinigura **[**(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

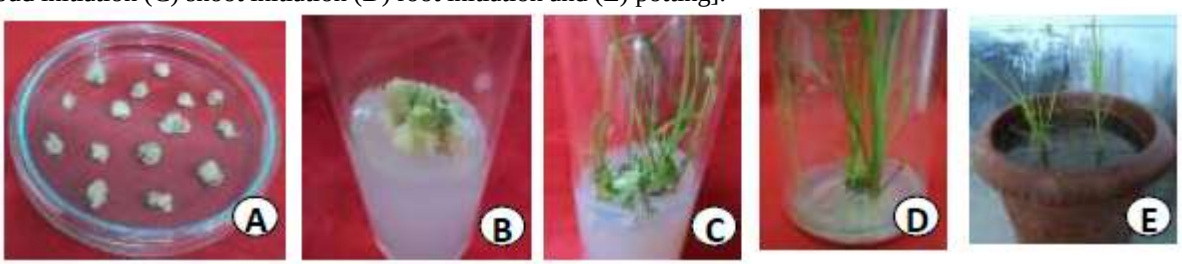

Fig. 2: Plant regeneration from calli derived immature embryos in aromatic rice cultivar Kalijira **[**(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

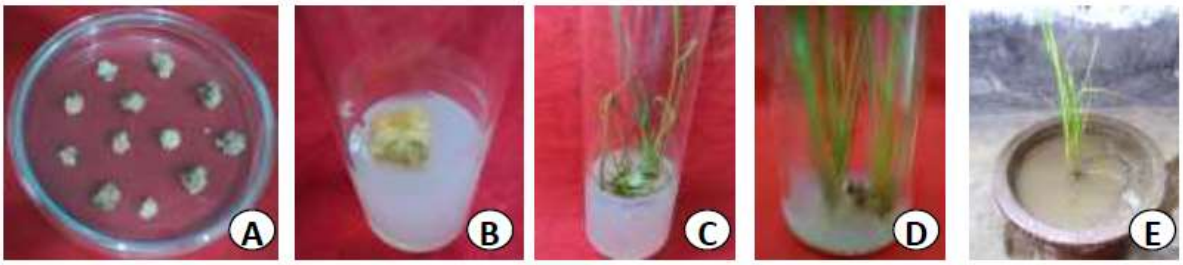

Fig. 3: Plant regeneration from calli derived immature embryos in aromatic rice cultivar Radhuni Pagal **[**(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

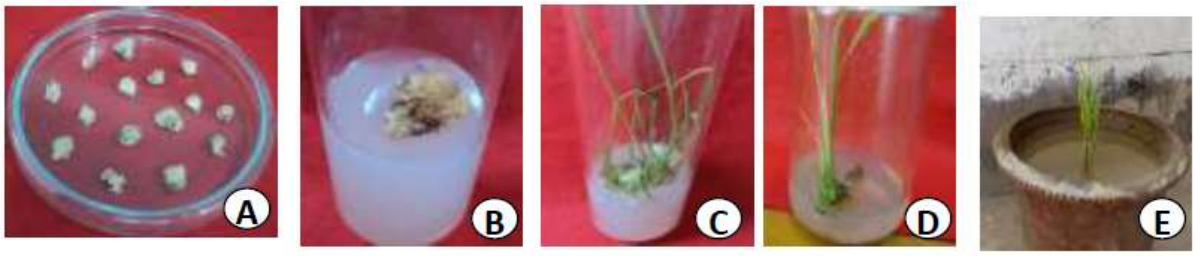

Fig. 4: Plant regeneration from calli derived immature embryos in aromatic rice cultivar Modhumala [(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

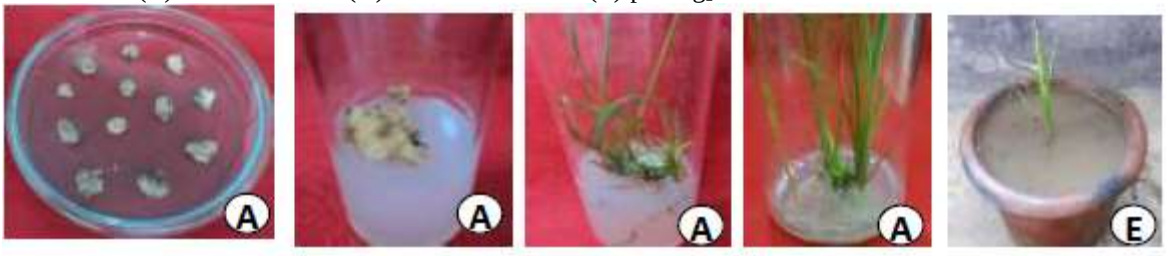

Fig. 5: Plant regeneration from calli derived immature embryos in aromatic rice cultivar Kataribog [(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

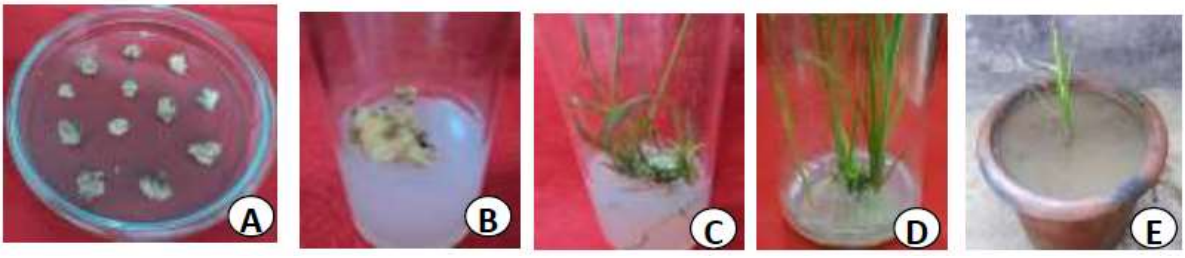

Fig. 6: Plant regeneration from calli derived immature embryos in aromatic rice cultivar **Mohonbhog.**[(A) 20 days old callus (B) bud initiation (C) shoot initiation (D) root initiation and (E) potting].

A comparative study has been made in search of the cultivar that shown the best callus induction frequency ultimately. The figure shown that among the cultivars aromatic rice Kalijira was found the best for callus induction and followed by Chinigura, Radhuni Pagal, Modhumala, kataribog and Mohonbhog was less efficient in callus induction**.**

Effect of 2, 4-D in combination with NAA

Although singly 2, 4-D (Auxin) gave the highest result of callus induction in rice, some worker have showed a good result in other cereal crops (e.g. wheat) using 2, 4-D in combination with low concentration of NAA. So in this experiment 2, 4-D was tested in combination with NAA. Here effect of 2.0 mg/l of 2, 4-D in combination of four concentrations (0.25, 0.5, 1.0 and 0.5 mg/l) of NAA was latest on callus induction in MS medium. The results are shown in table-2. The results were observed using NAA along with 2, 4-D. However, required days to callus initiation (i.e., the period between the inoculation of the explants and appearance of callus visible to the naked eyes) were decrease (5-7 days).

The trend of callus induction efficiently was similar to single used of 2, 4-D for the studied cultivars. Here also Kalijira has shown better efficiency than Chinigura, Radhuni Pagal, Modhumala, kataribog and Mohonbhog were less efficient in callus induction. However in comparing NAA with 2, 4-D the effect of calli induction was found better than 2, 4-D singly in most of the cases. The highest percentage of callus induction from immature embryos of were found in T_2 (2.0 mg/l 2, 4-D+0.5 mg/l NAA) and the range of callus induction were 72.22%-97.22%.

Callus morphology

Under the experiment various texture color and nature of callus was observed. The texture and color of callus did not show any remarkable variations among the cultivars (i.e. the texture and color of matured seed derived calli of six cultivars were more or less similar). The matured seed derived calli were Creamy White (CrW) to Creamy (Cr). The nature of mature seed derived calli was compact but fragile and to be very dry with increasing the concentration of 2, 4-D and root. Different types of callus gave Fig.1, Fig.2, Fig.3, Fig.4, Fig.5 and Fig.6 in Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog respectively.

Regeneration response in different rice cultivars

Regeneration response were tested for Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog in different treatments using MS medium, results were in **Table-3.** The efficiency of regeneration response is tested for Chinigura using different treatments in MS medium. Here also the highest percentages of regeneration were obtained in T_4 (0.5 mg/l BAP + 0.1 mg/l IBA) using MS (83.33%) while T_2 (0.1 IBA) shows the lowest results (41.33%). The efficiency of regeneration response is tested for Kalijira using different treatments in MS medium. The percentage of plant regeneration, mean number of shoots per cultures, their standard error and root induction percentage of shoot induced cultured were estimated and are presented in Table 3. The results shows that here T_4 (0.5 mg/l BAP + 0.1 mg/l IBA) performs the best and gave 91.67% while T_2 (0.1 IBA) shows the lowest results (41.67%) value in shooting. The range of shoot number and mean performance was 2-6 and 3.87 for T_4 treatment and also lowest for T_2 (0.1 IBA). The regeneration response of Radhuni Pagal was tested from matured seed derived calli in MS medium. The percentage of plant regeneration, mean number of shoots per culture, their standard error and root induction percentage of shoot induced cultured were estimated and are presented in Table-3**.** The results reveal that calli gave the highest percentage in the combination of 0.1 mg/l IBA and 0.5 mg/l BAP (T_4) shows best results (83.33%) while T_2 (0.1 IBA) shows the lowest results (33.33%). The range of shoot number (4-8) and mean performance (5.6) also shown better for T_4 treatment. The regeneration responses were also tested for Modhumala in MS medium. The results are shown in **Table-3**. The results shows that here also T_4 (0.5 mg/l BAP + 0.1 mg/l IBA) performs best and gave 66.67% while T_2 (0.1 IBA) shows the lowest results (16.67%) value in shooting. The range of shoot number and mean performance was 2-6 and 3.87 for T_4 treatment and also lowest for T_2 (0.1 IBA). The efficiency of regeneration response is tested for Kataribog using different treatments in MS medium. The results are presented in Table-3. The results indicates that the highest percentage of regeneration was obtained using T_4 (0.5 mg/l BAP + 0.1 mg/l) and gave 66.67% regeneration and the lowest results obtained for T_2 (0.1 IBA) (25.00%). The range of shoot number and mean performance was 2-7 and 4.38 for T_4 treatment and lowest for T_2 (0.1 IBA). Regeneration response were tested for BRRI dhan57 in different treatments using MS medium, results were in Table-3. The result indicates that the trend of regeneration similar to other studied cultivars. Here also the highest percentages of regeneration were obtained in T_4 using MS (66.67%). In respect of all cultivars Mohonbhog gave the highest percentage of regeneration response than another five rice cultivars. In general root formation occurred about a week earlier than were about 100% of the regenerated callus**.** Different types of shoot formation and the root formation gave Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5, and Fig. 6 in Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog respectively. However, on transference to MS medium without any growth regulator (T_1/control), yellowish nodular calli obtained from different cultivars became dark green or blackish and no further mrophogenic changes were noticed. In some cases external portion of calli started to became blackish and consequently the calli became narcotic.

Table-3: Regeneration efficiency of six rice cultivars from callus derived from mature seeds in MS medium using different treatments.

Treatments	Chinigura				Kalijira				Radhuni Pagal			
	Shoot induction (%)	Number of shoot		% of shoots inducted root	Shoot induction (%)	Number of shoot		% of shoots inducted root	Shoot induction (%)	Number of shoot		% of shoots inducted root
		Range	$\overline{X}$ ±SE			Range	$\overline{X}$ ±SE			Range	$\overline{X}$ ±SE	
T_1	0	-	-	-	0	-	-	-	0	-	-	-
T_2	41.33	1-4	2.80±0.73	100	41.67	2-3	2.60±0.24	100	33.33	1-3	2±0.41	100
T_3	50.00	3-5	3.83±0.48	100	50.00	3-5	3.67±0.33	100	50.00	2-4	2.5±0.42	100
T_4	83.33	4-8	6.63±0.39	100	91.67	3-8	6.30±0.30	100	83.33	4-8	5.6±0.52	100
T_5	58.33	3-7	4.12±0.64	100	66.67	3-6	4.00±0.53	100	58.33	2-5	3.4±0.37	100
T_6	50.00	2-6	3.83±0.54	100	50.00	2-5	3.00±0.73	100	41.67	1-4	2.6±0.50	100
T_7	41.67	2-5	4.00±0.45	100	50.00	2-5	3.20±0.37	100	41.33	2-4	3.0±0.31	100
T_8	66.67	3-8	4.37±0.60	100	66.67	3-8	4.75±0.59	100	66.67	2-7	4.1±0.69	100
T_9	58.33	3-7	4.28±0.60	100	58.33	2-7	4.14±0.34	100	58.33	2-6	4.0±0.43	100
Mean (%)	56.25				59.37				54.16			
	Modhumala				Kataribog				Mohonbhog			
T_1	0	-	-	-	0	-	-	-	0	-	-	-
T_2	16.67	0-1	1.50±0.50	100	25.00	1-2	1.67±0.33	100	25.00	1-2	1.67±0.33	100
T_3	25.00	1-2	1.67±0.33	100	41.67	1-3	2.20±0.37	100	33.33	1-3	1.75±0.48	100
T_4	66.67	2-6	3.87±0.48	100	66.67	2-7	4.38±0.56	100	66.67	2-4	3.50±0.42	100
T_5	33.33	1-2	1.50±0.29	100	41.67	2-4	3.20±0.37	100	41.67	1-4	2.6±0.51	100
T_6	33.33	1-3	1.75±0.49	100	50.00	2-6	3.83±0.60	100	50.00	2-5	3.33±0.42	100
T_7	41.67	1-3	2.40±0.24	100	41.67	2-5	3.4±0.50	100	41.67	2-4	3.20±0.20	100
T_8	50.00	2-5	3.67±0.42	100	58.33	2-5	3.71±0.36	100	58.33	2-4	2.85±0.34	100
T_9	58.33	2-5	3.28±0.42	100	58.33	2-3	2.42±0.20	100	58.33	2-5	3.42±0.43	100

In each treatment 40 explants were used, T_1 = 0, T_2= 0.1 IBA, T_3= 0.1 BAP + 0.1 IBA, T_4= 0.5 BAP + 0.1 IBA, T_5= 1 BAP + 0.5 IBA, T_6= 0.5 BAP + 0.1 IAA, T_7= 0.5 BAP + 0.5 IBA, T_8= 3 KIN + 0.5 NAA, T_9= 3 KIN + 0.5 IAA (mgl^{-1})

Discussion

Tissue culture technique is recognized as a novel means to generate genetic variability (Larkin and Scowcroft 1981) and has been proposed as an excitant supplementary technique for plant which can accelerate the breeding programs through the use of new expended genetic variability. Successful application of tissue culture method involved the establishment of a more or less de-differentiated cells or tissue under defined culture condition, proliferation of a number of cells and the subsequent regeneration of plants (Larkin and Scowcroft 1981). In Bangladesh little systematic work has been reported of using tissue culture for improvement of rice. Therefore, a research work was undertaken to develop *in vitro* culture protocol on some Bangladeshi rice cultivars.

The present study on callus induction was conducted with six kinds of aromatic rice cultivars i.e. Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog. These cultivars are high yielding variety, fine grain quality lack of disease or pest resistant, stress and salt tolerance variety and proper cultural management. So this demandable cultivar has to bring under experiment. Various tissue culture techniques are being applied for varietals development of cereal crops including rice in different countries (Dorosieve. 1996). For callus culture there are many reports with many species. On the country, for monocot plants it has been believed that callusing induction is very difficult, because they are recalcitrant *in vitro* manipulation. *Yel* rice was the first in them which response positively to callus (Niizeki and Oono 1968). Now a day's information is available on callus induction and shoot, root differentiation from different explants in crop includes rice.

In the present study initiatives were made to observed the efficiency of callus production and plant regeneration from callus in six rice cultivars viz., Chinigura, Kalijira, Radhuni Pagal, Modhumala, Kataribog and Mohonbhog using MS medium supplemented with different growth regulators because callus can be induced and grown on both MS and N6 medium (Rashid et al. 2004) but MS medium was found to be more effective in callus induction among the rice cultivars (Azria and Bhalla 2000; Niroula *et al*., 2005).

For concentration (1.0, 2.0, 3.0 and 4.0 mgl^{-1}) of 2, 4-D were tested for early and high percentage of callu induction in six aromatic rice cultivars. The result reveal that concentration of 2, 4-D and genotype had great variability for early induction and high production of callus. Regarding use of concentration of 2, 4-D, 2.0 mg/l was found best for high amount (90.22%) of callus induction in case of Kalijira. With the increase of concentration of 2, 4-D above 2.0 mg/l the callus induction efficiency was reduce (20.25%) in all cultivars. The indicate that the used of low concentration of 2, 4-D was enough for production of high amount of callus in rice. Similar results in rice were also reported by others (Rashid et al. 2003; Islam *et al*., 2013; Islam *et al*., 2014).

The effect of combinations NAA along with (2, 4-D, 2.0 mg/l) on callus induction was also tested in MS medium. Callus induction was found more effective in Kalijira when 2.0 mg/l of 2, 4-D was supplemented with 0.5 of NAA produce (97.22%) higher amount of callus but higher than single use of 2.0 mg/l of 2, 4-D (90.22%). However required

days of callus initiation were decreased. In combination of 2, 4-D with NAA treatment high amount of embryogenic callus produced (Xing. 1996). The result is conformity with similar findings reported by some researchers (Sripichitt and Cheewasestatham 1994; Islam *et al*., 2013; Islam *et al*., 2014).

Regeneration of plants via unorganized calli was the main objective of the experiment. Because regeneration is a necessary step when tissue culture methods are used for crop improvement (Yamada *et al*., 1984). It has been known as a major bottleneck on the successful application of genetic transformation of valuable genes into rice genome. Rice genotypes display a wide range in regeneration capacity depending on their genetic background and their interaction with the culture media (Lee *et al*., 2002, Lin and Zhang 2005). Many leading varieties in tropical country, show low regeneration capacities (Nishimura *et al.*, 2005), resulting in serious obstacle to efficient production of transgenic plants or improvement of crop. In the present study the results of different kinds and concentration of phytohormone for high frequency of plant regeneration from calli of the studied cultivars are presented through Table-3.

For regeneration via callus different combination of auxin and cytokinine were tried. The combinations were BAP + IBA, BAP+NAA, BAP+IAA, KIN+NAA and KIN+IAA. The results from these treatments varied upon with cultivars. The combination of BAP+IBA was best for regeneration efficiency. The combination of 0.5 mg/l of BAP + 0.1 of mg/l IBA showed the highest percentage of plant regeneration (Chinigura, 83.33%, Kalijira, 91.67%, Radhuni Pagal, 83.33%, Modhumala, 66.67%, Kataribog, 66.67% and Mohonbhog, 66.67%). In respect of all cultivars Kalijira gave best result, where remaining cultivars was comparatively low. This result was confirming the earlier results of Agrawal *et al*., (2005). This shows the selection of genotype is a key factor for *in vitro* study in rice. Variability of plant regeneration efficiency in rice genotypes are also reported by earlier workers (Khanna and Raina 1998; Lee *et al.*, 2002).

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