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IN VITRO MORPHOGENESIS IN TWO INDIGENOUS RICE (*Oryza sativa* L.) CULTIVARS THROUGH DEHUSKED SEED CULTURE

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Abstract: The local cultivars of rice are the important reservoirs of many valuable traits that need special attention for their conservation and improvement through breeding. To improve the potentiality of using these cultivars into breeding program, standardized protocol is required for their regeneration. The present investigation was carried out to find out the potentiality of two indigenous rice cultivars for callus induction, plant regeneration and to determine the most suitable concentration and combination of growth regulators for the purpose. Mature dehusked seeds of two local rice cultivars- *Chamak* and *Shama* were cultured on MS media supplemented with different concentrations and combinations of 2,4-D(2,4-dichlorophenoxy acetic acid) and NAA(∝-naphthalene acetic acid) for callus culture. For plant regeneration MS medium fortified with 0.5 mgL^{-1}NAA and 3.0mgL^{-1} BAP was applied. Between these two cultivars, *Shama* and MS media supplemented with 2,4-D 1.5 mgL^{-1} and NAA 1.0 mgL^{-1} showed promising performance in frequency of callus induction (100%), weight of callus (1.90 g), diameter of callus (9.04 mm), color (white and cream) and texture of callus (compact). The cultivar *Shama* and MS media supplemented with 2,4-D 1.5 mgL^{-1} and NAA 1.0 mgL^{-1}alsoshowed better performance in plant regeneration (75%caulogenesis, 13.84%calulo-rhizogenesis, 94.99%total morphognesis and 92%root regeneration frequency in root inducing medium)and average survival percentage(45.62%)of the regenerated plants. In this respect, the cultivar *Shama* and MS media supplemented with 2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} may be recommended for the study of somaclonal variation and for genetic engineering studies.This study will serve as a base line for *in vitro* somaclonal variation of local rice cultivars.

Keywords: Local *Aus* rice, dehusked seeds, callus induction, *in vitro* regeneration, survival

Introduction

The greater Khulna region of Bangladesh is known for the cultivation of many indigenous rice cultivars. These indigenous cultivars have special adaptations at varying levels for resistance to biotic and abiotic stresses. In spite of the introduction of many high yielding rice cultivars, some local cultivars are still popular in the farmers' field for their unique qualities such as better taste, aroma, protein content, adaptation to local climatic conditions, resistance to pest and diseases, requirement of lesser quantities of fertilizer and water. Among these the *Chamak* and *Shama* are unique in grain quality, seed color and texture of leaves, and plant height as compared to other cultivars. Presently these cultivars are on the verge of extinction due to their long life span and invasion of hybrid races but they form an

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important reservoir of valuable trait of genetic diversity and thus need special attention for future conservation. Another reason for selecting the local rice cultivar is that they are low grain yielder. Very little work had been done with these unimproved local rice cultivars. The conventional breeding techniques are time consuming and self in-compatibility acts as barrier for distant hybridization. The local cultivars can be improved through tissue culture techniques *viz.* somaclonal variation or genetic manipulation like protoplast fusion (hybrid and cybrid) and through gene transfer (Chawla, 2004).

Crop improvement through tissue culture technique offers possibilities of introducing variability among the regeneration (Ganeshan*et al.*, 2003). The frequency of callus induction and plant regeneration in tissue culture of rice is influenced by many factors: media composition, explants sources, genotypes, and optimum concentration and combination of growth regulators (Kabir and Basu, 2006). Among them, genotype and nutrient composition are regarded to be the major sources for inducing variation in *in vitro* culture (Khanna and Raina, 1998). In contrast, there are some studies that found on micropropagation of local rice cultivars of Bangladesh. But the major problem in *in vitro* culture of rice is the low rate of callus production, somatic embryogenesis and subsequent plantlet regeneration (Chu and Croughan, 1990). Very few studies have been found on unimproved local rice cultivars of greater Khulna region such as *Bashfulbalam, Kalizira, Lucky, Pajam*. However, no study result has still been found about *in vitro* regeneration of the cultivar- *Shama*and *Chamak*, which may be promising. After all the production of callus, its subsequent regeneration and finally acclimatization are the prime steps in crop plant to be manipulated by biotechnological means and to exploit somaclonal variation.

Therefore, this research work was conducted to find out the potentiality of selected local *Aus*rice (*Oryza sativa* L.) cultivars for callus induction and subsequent plant regeneration from mature dehusked seed and to determine the most suitable concentrations and combinations of growth regulators for maximum callus induction and plantlet regeneration

Materials and Methods

Explant: Mature dehusked seeds from two local *Aus*rice cultivars *viz.* *Chamak* and *Shama* were collected during October 2012 to July 2013 as explants to conduct the experiment. Prior to culture establishment, seed germination test was done by petridish method. The germination percentage of *Chamak* was 90% and that of *Shama* was 95%.

Design of experiment: The experiment was laid out in the laboratory following Completely Randomized Design with two factors and three replications. Each culture bottle containing 25 ml of nutrient medium with 10 seeds was considered as one replication for callus induction.

Factor A: i) *Chamak* and ii) *Shama*

Factor B: Hormonal concentrations (2,4-D @ of 1.0, 1.5, 2.0 mgL^{-1} and NAA at 0.0, 0.5, 1.0 mgL^{-1})

Transfer of calli onto the regeneration media: After 4 weeks of inoculation of seeds, the calli were plated in test tube containing 5ml regeneration medium (MS medium supplemented with 0.5 mgL^{-1} NAA and 3.0 mgL^{-1} BAP). After 3 weeks of transference of

calli, a single subculture was done on the same regeneration medium. The test tubes were placed under fluorescent light in a growth room with controlled temperature (25±1 ^{0}C) and under 16 hours photoperiod with light intensity of 3000 lux.

Transfer of rootless plantlets onto the liquid media: The plantlets which had only shoot were transferred to the liquid media supplemented with 1.5 mgL^{-1} IBA and 0.5 mgL^{-1} NAA. Two subcultures were done at 3 weeks interval.

Hardening: Regenerated plants with profuse roots were transferred in sand supplemented with MS liquid medium and were kept in hardening room for pot culture. Before transferring the plants were treated with Bavistin solution @ 0.1% (w/v).

Established plants from small pots were transferred to larger pots containing puddle field soil and were kept in net house. They were frequently watered and kept under observation for 3 weeks. Data on survival were then recorded by using the followingformula:

$$\text{Survival}(\%) = \frac{\text{No of plants survived after 3 weeks}}{\text{Total no. of plants transferred to larger pots}} \times 100.$$

Collection, calculation and analysis of data: Data were collected by counting the calli induced and plants regenerated in the test tubes. After four weeks of inoculation of rice seeds, callus induction frequency was calculated. Data on plant regeneration were collected after 3 weeks of transference of calli onto regeneration medium. All the calli originated from a single seed and all the plants regenerated from single plated callus was considered as one. The weight and diameter of calli were measured by digital balance and slide calipers, respectively. Callus induction frequency (%), weight of callus (g), diameter of callus (mm), response of calli to caulogenesis (%), response of calli to rhizogenesis (%), response of calli to caulo-rhizogenesis (%), total regeneration (%), frequency of root regeneration in root inducing media (%) and *ex vitro* survival (%) were considered as parameters. The parameters were recorded by using the following formula:

$$\text{Callus induction frequency}(\%) = \frac{\text{No of seeds produced callus}}{\text{No of seeds inoculated}} \times 100.$$

$$\text{Caulogenesis}\ (\%) = \frac{\text{No. ofcalli regeneratd into shoot}}{\text{No. of calli plated No. of calli regenerated}} \times 100.$$

$$\text{Rhizogenesis}(\%) = \frac{\text{intoroot}}{\text{No. of calli plated}}$$

$$\text{Caulo} - \text{rhizogenesis}(\%) = \frac{\begin{matrix}\text{No. ofcalli regenerated}\\ \text{intoshoot and root}\end{matrix}}{\begin{matrix}\text{No of calli}\\ \text{plated}\end{matrix}} \times 100$$

$$\text{Total morphogenesis (\%)} = \frac{\text{No. of calli responded tomorphogenesis}}{\text{No. of calli plated}} \times 100.$$

The recorded data on the frequency of callus induction and frequency of plants were statistically analyzed using ANOVA with the help of statistical package program MSTAT-C in computer. The mean differences were compared by Duncan's New Multiple Range Test (DMRT).

Results

Effect of genotypes on callus induction: The effect of genotypes on callus induction from dehusked rice seeds have been shown in Table 2. Callus induction in rice was found highly variable and genotype specific. Between the two cultivars, *Shama* produced 91.55% callus from the inoculated seeds, which was significantly higher than *Chamak*(80.50%). The weight of callus of *Chamak* and *Shama* was 0.472 g and 0.633 g, respectively, which also differed significantly. Callus diameter of cultivar *Shama* was (5.95 mm) which was statistically higher than the cultivar *Chamak*(5.46 mm).

Table 1: Effect of cultivars on callus induction

Genotype	Frequency of callus induction (%)	Weight of callus (g)	Diameter of callus (mm)
Chamak	80.50	0.47	5.46
Shama	91.55	0.63	5.95
Level of significance	0.01	0.01	0.01
CV (%)	4.43	6.63	8.73

Effect of growth regulators on callus induction: The responses of the explants to different hormonal concentration in terms of callus induction, weight and diameter of callus are shown in Table 3. Among the combinations, 2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} showed the highest percentage of callus induction (95%) which was statistically identical to 2,4-D 2.0 mgL^{-1}+NAA 0 mgL^{-1} (92.41%) and 2, 4-D 1.5 mgL^{-1}+NAA 0.5 mgL^{-1} (90.91%). Similar media was found better for callus weight (1.89 g) and diameter (8.04 mm). The lowest percentage of callus induction (77.83%) was found in 2,4-D 1.0 mgL^{-1}+NAA 0 mgL^{-1}. Similarly the lowest callus weight (0.04 g) was observed in 2,4-D 1.0 mgL^{-1}+NAA 1.0 mgL^{-1}. The lowest callus diameter(3.72 mm) was found in MS media supplemented with 2,4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1}.

Table 2: Effect of hormonal concentration on callus induction of two indigenous rice cultivars *Shama* and *Chamak*

Hormonal concentration (mgL^{-1})		Frequency of callus induction (%)	Weight of callus (g)	Diameter of callus (mm)
2,4-D	*NAA*			
1.0	0.0	77.83g	0.04e	4.58e
1.0	0.5	81.16efg	0.34cd	4.91de
1.0	1.0	79.00fg	0.07e	5.86bc
1.5	0.0	87.66bcd	0.37c	6.46b
1.5	0.5	90.91abc	0.96b	5.53cd
1.5	1.0	95.16a	1.89a	8.04a
2.0	0.0	92.41ab	0.96b	6.15bc
2.0	0.5	86.41cde	0.30d	5.90bc
2.0	1.0	83.66def	0.06e	3.72f
Level of Significance		0.01	0.01	0.01
CV (%)		4.43	6.63	8.73

Interaction effect of genotypes and media on callus induction: The results of interaction effect have been shown in Table 4. The rate of callus induction varied from 70% to 100% due to the interaction between the genotypes and hormonal concentrations (Fig.1-A). The rice seeds of the cultivar *Shama* inoculated on MS+2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} produced maximum calli (100%) which was statistically similar to MS+2, 4-D 1.5 mgL^{-1}+NAA 0.5 mgL^{-1} (98.16%), MS +2,4-D 2.0 mgL^{-1}+NAA 0.0 mgL^{-1} (96.83%) and MS +2,4-D 1.5 mgL^{-1} +NAA 0.0 mgL^{-1} (96.16%). Minimun calli (70%) were obtained from the seeds of *Chamak*, inoculated on MS +2,4-D 1.0 mgL^{-1}+NAA 1.0 mgL^{-1} .

In case of callus weight the maximum weight was found in *Shama* inoculated on MS +2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} (1.90 g) and that of minimum was found in *Chamak* on MS+2, 4-D 1.0 mgL^{-1}+NAA 0.0 mgL^{-1} (0.04 g), MS+2, 4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1} (0.04 g) and *Shama* on MS+2, 4-D 1.0 mgL^{-1}+NAA 0.0 mgL^{-1} (0.04 g).

The maximum diameter was recorded for Shamainoculated on MS+2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} (9.04 mm) and that of minimum in *Chamak* on MS+2, 4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1} (3.33 mm).

The callus color and texture was observed visually. From the present study it was found that compact white or cream color calli responded better in regeneration media. The rice seeds of the variety *Chamak* and *Shama* inoculated on MS+2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1}, MS +2, 4-D 1.0 mgL^{-1}+NAA 0.5 mgL^{-1}, MS+2,4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1},

Table 3: Interaction effect of cultivars and hormone concentration on callus induction

Genotype	Hormonal concentration (mgL^{-1})		Frequency of Callus induction (%)	Weight of callus (g)	Diameter of callus (mm)	Color of callus	Texture of callus
	2, 4-D	NAA					
Chamak	1.0	0.0	74.66hi	0.04k	3.67jk	Light yellow	Compact
	1.0	0.5	78.66gh	0.27i	4.53ij	Light yellow	Compact
	1.0	1.0	70.00i	0.07jk	6.29bcdef	Light yellow	Compact
	1.5	0.0	79.16gh	0.12j	6.76bc	Dark Brown	Compact
	1.5	0.5	83.66efg	0.84e	5.96cdefh	Dark Brown	Compact
	1.5	1.0	90.33cde	1.79b	7.03b	White or Cream	Compact
	2.0	0.0	88.00def	0.95d	5.86cdefgh	Dark brown	Compact
	2.0	0.5	81.00fgh	0.08jk	5.30fghi	Light yellow	Compact
	2.0	1.0	79.00gh	0.04k	3.33k	Light yellow	Compact
Shama	1.0	0.0	81.00fgh	0.04k	5.50defghi	Light yellow	Friable
	1.0	0.5	83.66efg	0.40h	5.24ghi	Light yellow	Compact
	1.0	1.0	88.00def	0.07jk	5.44efghi	Light yellow	Compact
	1.5	0.0	96.16abc	0.61f	6.15bcdefg	Brown	Compact
	1.5	0.5	98.16ab	1.07c	5.11hi	Brown	Compact
	1.5	1.0	100.00a	1.90a	9.04a	White	Compact
	2.0	0.0	96.83abc	0.97d	6.44bcdef	Dark brown	Compact
	2.0	0.5	91.83bcd	0.52g	6.49bcd	Brown	Compact
	2.0	1.0	88.33def	0.08jk	4.11jk	Light yellow	Friable
Level of Significance			0.05	0.01	0.01		
CV (%)			4.43	6.63	8.73		

Mean values in a column having the same letter do not differ significantly whereas values with dissimilar letters differ significantly as per DMRT

MS+2,4-D 1.0 mgL^{-1}+NAA 0.0 mgL^{-1}, MS+2, 4-D 1.0 mgL^{-1}+NAA 1.0 mgL^{-1} produced white and cream colour callus while MS+2, 4-D 1.5 mgL^{-1}+NAA 0.0 mgL^{-1}, MS+2, 4-D 1.5 mgL^{-1}+NAA 0.5 mgL^{-1}, MS+2, 4-D 2.0 mgL^{-1}+NAA 0.0 mgL^{-1}, MS+2, 4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1} produced brown colour calli. Maximum callus were compact and only *Chamak* and *Shama* on MS+2,4-D 1.0 mgL^{-1}+NAA 0.0 mgL^{-1} and *Shama* at MS+2, 4-D 2 mgL^{-1}+NAA 1.0 mgL^{-1}were friable.

Performance of genotypes in morphogenesis from inoculated calli: Data on morphogenesis presented in Table 5 showed that the calli of two cultivars responded well in organogenesis. Between these two cultivars, highest organogenic potentiality (66.94%), caulo-rhizogenic potentiality (11.55%) and total regeneration (88.81%) was found in calli of *Shama*. In contrast, the calli of *Chamak* showed better performance only in rhizogenesis (11.09%).

Table 4: The effect of cultivars on morphogenesis

Genotype	Total regeneration (%)	Organogenesis (%)	Rhizogenesis (%)	Caulo- rhizogenesis (%)	Rooting (%)
Chamak	83.97	66.30	11.09	08.78	87.06
Shama	88.81	66.94	10.04	11.55	88.87

Performance of genotypes and auxin concentrations in morphogenesis from inoculated calli:

Effects of genotypes and auxin concentrations on morphogenesis of indigenous rice are represented in Table 6. Calli of genotype *Shama* on MS media supplemented with 2, 4-D 1.5mgL^{-1}+NAA 1.0 mgL^{-1}showed high caulogenic potentiality (75%), followed by 70% for *Chamak* in MS supplemented with 2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} whereas the lowest organogenic potentiality (59.09%) was found in calli of *Chamak* on MS media supplemented with 2, 4-D 1.0mgL^{-1}+NAA 0 mgL^{-1} (Fig. 1-B). The calli of genotype *Chamak* produced on MS media supplemented with 2,4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{1} showed the highest rhizogenesis (13.84%), followed by 12.30% in MS supplemented with 2,4-D 1.0 mgL^{-1}+NAA 0.5 mgL^{-1}; 12.12% in 2,4-D 1.0 mgL^{-1}+NAA 0.0 mgL^{-1}.

The caulo-rhizogenic potentiality varied from 6.15 to 13.84%. The calli of cultivar *Shama* derived from MS media containing 2,4-D 2.0 mgL^{-1}+NAA 0.0 mgL^{-1} showed the highest caulo-rhizogenesis (13.84%), followed by (13.33%) in 2,4-D 1.0 mgL^{-1}+NAA 1.0 mgL^{-1} and 2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1}. The lowest caulo-rhizogenic potentiality (6.15%) was observed in *Chamak* in 2,4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1}.

Table 5: Performance of two rice cultivars in morphogenesis from inoculated calli

Genotype	Hormonal concentration (mgL^{-1})		Total morphogenesis (%)	Calli responded to caulogenesis (%)	Calli responded to rhizogenesis (%)	Calli responded to caulo-rhizogenesis (%)	Rooting of regenerated plants (%)
	2,4-D	NAA					
Chamak	1.0	0.0	80.30	59.09	12.12	09.09	84.90
	1.0	0.5	81.53	60.00	12.30	09.23	86.00
	1.0	1.0	81.35	61.01	11.86	08.47	86.53
	1.5	0.0	84.74	64.40	11.86	08.47	86.79
	1.5	0.5	84.99	66.66	10.00	08.33	88.67
	1.5	1.0	89.21	70.76	07.69	10.76	90.19
	2.0	0.0	87.87	68.18	09.09	10.60	88.46
	2.0	0.5	85.71	66.66	11.11	07.93	88.23
	2.0	1.0	80.00	60.00	13.84	06.15	86.79
Shama	1.0	0	84.47	63.79	12.06	08.62	86

Genotype	Hormonal concentration (mgL^{-1})		Total morphogenesis (%)	Calli responded to caulogenesis (%)	Calli responded to rhizogenesis (%)	Calli responded to caulo-rhizogenesis (%)	Rooting of regenerated plants (%)
	2,4-D	NAA					
	1.0	0.5	88.12	64.40	11.86	11.86	86.27
	1.0	1.0	89.99	65.00	11.66	13.33	87.75
	1.5	0.0	90.15	65.57	11.47	11.47	88.00
	1.5	0.5	90.37	66.12	11.29	12.90	90.00
	1.5	1.0	94.99	75.00	06.66	13.33	92.00
	2.0	0.0	90.76	69.23	07.69	13.84	91.83
	2.0	0.5	86.15	67.69	09.23	09.23	89.79
	2.0	1.0	84.37	65.62	09.37	09.37	88.23

Among the calli, the highest total plant regeneration (94.99%) was found in the calli of cultivar *Shama* derived from MS media supplemented with 2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} followed by 90.76% in 2,4-D 2.0 mgL^{-1}+NAA 0.0 $mgL^{-1}$of the same cultivar (Fig.1-C). The lowest total plant regeneration percentage (80%) was found in the calli of cultivar *Chamak* derived from MS media supplemented with 2,4-D 2.0 mgL^{-1}+NAA 1.0 mgL^{-1}.The highest root induction was found (92%) in the calli of *Shama* produced on MS media supplemented with 2,4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1}, and the lowest root induction was found in the calli of *Chamak*(84.90%)produced on MS media supplemented with 2,4-D 1.0 mgL^{-1} devoid of NAA.

Acclimatization of regenerated plant: Survivability of the *ex vitro* transferred plantlets are presented in Table 7. The average survival of plantlets was 45.62% in *Shama* and 39.56% in *Chamak*. The calli produced on MS media supplemented with 2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1} showed maximum survivalpercentage 73.33% in *Shama*and 69.23% in *Chamak*(Fig. 1-D,E,F).

Table 6: Survivability of the *ex vitro* transferred plantletsof two indigenous rice cultivars *Shama* and *Chamak*

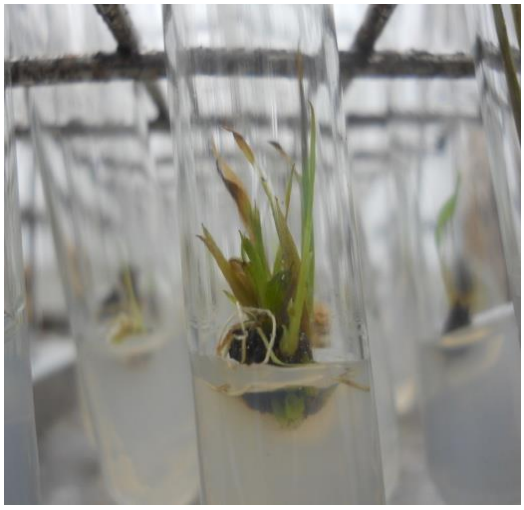
Genotype	Hormonal concentration (mgL^{-1})		Survival (%)	Average survival *per* cultivar (%)
	2, 4-D	NAA		
Chamak	1.0	0.0	26.67	39.56
	1.0	0.5	38.46	
	1.0	1.0	46.15	
	1.5	0.0	22.22	
	1.5	0.5	53.33	
	1.5	1.0	69.23	
	2.0	0.0	33.33	
	2.0	0.5	33.33	

Genotype	Hormonal concentration (mgL^{-1})		Survival (%)	Average survival *per* cultivar (%)
	2, 4-D	NAA		
Shama	2.0	1.0	33.33	45.62
	1.0	0.0	44.44	
	1.0	0.5	44.44	
	1.0	1.0	45.45	
	1.5	0.0	26.67	
	1.5	0.5	57.14	
	1.5	1.0	73.33	
	2.0	0.0	63.63	
	2.0	0.5	22.22	
	2.0	1.0	33.33	

Discussion

Callus induction: The present investigation revealed that rice genotypes, hormonal concentration and genotypes × hormonal concentration significantly affected callus induction. The callusing efficiency of *Shama* was better than *Chamak* i.e. callusing efficiency between these varieties is genotype dependent. The results are in agreement with the earlier reports (Guo and Cao, 1982; Abe and Futsufura, 1984; Rahim *et al.,* 1991; Islam *et al.*, 2005). It may be due to their difference in genetic makeup. The success of *in vitro* culture largely depends on the nutrition media and growth regulators. In the present study, MS media supplemented with 2, 4-D 1.5 mgL^{-1}+NAA 1.0 mgL^{-1}showedbetter callusing response in both varieties in all parameters. These results are in line with the work of other researchers (Islam *et al.,* 2004 and Zhang and Te-chato, 2012). Hoque and Mansfield (2004) reported that 2,4-D enhanced the callus induction and NAA influenced shoot regeneration.

A. Callus induction from dehusked seed



B. Morphogenesis

C. Root initiation

D. Plantlets transferred to sand

E. *Ex vitro* survival of plantlets

F. *Ex vitro* survival of plantlets

Fig. 1: Sequential steps of *in vitro* regeneration in rice from dehusked seed explant

Plant regeneration: It was observed that plant regeneration ability of plated calli depends on the genotypes and the callus induction media. This was in conformity with that of Hoque and Mansfield (2004) who found that genotypic differences strongly influenced plant regeneration potential. Different organogenic response was noticed in plated calli, some of the calli only regenerated into roots, but others regenerated into complete green plants. This phenomenon was also reported by Abe and Futsufura, (1984), Pandey *et al.* (1994) and

Abeyaratne *et al.*(2004). Liquid MS media supplemented with IBA and NAA showed promising performance on root initiation. Present investigation revealed that cultivar *Shama* produced 88.87 % root compared to *Chamak* (87.06%). Similar result was reported by Lai and Liu (1981), Sikder*et al.* (2006) and Chowdhury *et al.* (2012).

References

Abe, T., & Futsufura, Y. (1984). Varietal differences of plant regeneration from root derived callus tissue in rice. *Japanese Journal of Breeding, 34* (2), 147-155

Abeyaratne, W. M., De Silva, U. N., Kumari, H. M. P. S., & Abeysiriwardena, D. S. De Z. (2004). Callus Induction, Plant Regeneration and Occurrence of Somaclonal Variation in Somatic Tissue of Some Indica Rice Varieties. *Annals of the Sri Lanka Department of Agriculture, 6*, 1-11

Chawla, H.S. (2004). *Introduction to Plant Biotechnology* (2nd ed.). Oxford& IBH Publishing Co. Pvt. Ltd.,New Delhi

Chowdhury, G.G.M., Ashrafuzzaman, M., Raihan, F., & Prodhan, S. H. (2012). Comparative Study on Regeneration Potentiality of Aromatic Indica Rice (*Oryza sativa* L.) of Bangaldesh. *Research Journal of Biology, 2*(3), 98-103

Chu, Q.R.,&Croughan, T.P.(1990). Genetics of plant regeneration in immature panicle culture of rice. *Crop Science, 30*, 1194

Ganeshan, S., Baga, M., Harwey, B.L., Rossnagel, B.G., Scoles, G.J., & Chibbar, R.N. (2003). Production of multiple shoots from thiadiazuron-treated mature embryos and leaf-base/apical meristems of barley (*Hordeumvulgare* L.). *Pant Cell Tissue Organ Culture, 73,* 57–64

Guo, C.Y.,& Cao, Z.Y. (1982). Effect of different genotypes on induction frequency in anther and scutellum culture of maize *in vitro*. *Hereditess, 4*, 8-10

Hoque, E.H.,& Mansfield, J.W. (2004). Effect of genotype and explants age on callus induction and subsequent plant regeneration from root-derived callus of Indica rice genotypes. *Plant Cell Tissue and Organ Culture, 78*, 217-223

Islam, M.M., Ahmed, M., & Mahaldar, D. (2005). *In Vitro* Callus Induction and Plant Regeneration in Seed Explants of Rice (*Oryzasativa* L.). *Research Journal of Agriculture and Biological Sciences, 1*(1), 72-75

Islam, M.M., Wahed, S.A.,& Khan, S.A.K.U. (2004). Studies on Callus Induction and Regeneration from Dehusked Rice (*Oryza sativa* L.) Seeds. *Plant Tissue Culture, 14*(2), 155-160

Kabir, N.,&Basu, S. (2006). Effect of Varieties and Calcium Silicate on Callus Induction and Morphogenesis in Dehusked Rice Seed Culture. *B.Sc. Thesis, Khulna University, Khulna,* pp. 42.

Khanna, H.K.,& Raina, S.K. (1998). Genotype and culture media interaction effects on regeneration response of three indica rice cultivar. *Plant Cell Tissue and Organ Culture, 52*, 145

Lai, K.L.,& Liu, L. F. (1981). Induction and plant regeneration of callus from immature embryos of rice plants (*Oryza sativa* L). *Japan Journal of Crop Science, 51* (1), 70-74

Pandey, S.K., Ramesh, B., & Gupta, P.K. (1994). Study of Effect on Genotype and Culture Medium on Callus Formation and Plant Regeneration in Rice (*Oryza sativa* L.). *Indian Journal of Genetics, 54*, 293-299

Rahim, M.A., Hakim, L.,& Miah, A.J. (1991). Induction of callus and plant regeneration. *Plant Tissue Culture, 1*(1), 27-32

Sikder, M.B.H., Sen, P.K., Mamun, M.A.A., Ali, M.R., & Rahman, S.M. (2006). *In Vitro* Regeneration of Aromatic Rice (*Oryza sativa* L.). *International Journal of Agriculture &Biology, 4*(4), 451-458

Zhang, X., & Te-chato, S. (2012). Callus induction and plantlet regeneration from mature embryos of indica rice (*Oryza sativa* L.) cultivar Kra Dang Ngah, *Journal of Agricultural Technology, 8*(7), 2423-2433