



IN VITRO PLANT REGENERATION IN LOCAL AUS RICE THROUGH MATURED SEED CULTURE

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Abstract: An investigation was carried out to decipher the effect of genotypes and concentrations of growth regulators for callus induction and subsequent plant regeneration from mature seeds of rice. Two local rice cultivars *viz.* *Nayanmony* and *Kalomona* and two growth regulators *viz.* 2, 4-D and NAA were used to evaluate their performance. The seeds were cultured under aseptic condition onto MS basal medium at varying concentrations and combinations of 2, 4-D (1.0, 1.5 and 2.0 mgL⁻¹) and NAA (0.0, 0.5 and 1.0 mgL⁻¹) for callus induction. For plant regeneration MS medium fortified with 0.5 mgL⁻¹ NAA and 3.0 mgL⁻¹ BAP was applied. Between the two cultivars *Nayanmony* showed better responses in callus induction frequency (91.38%), weight of callus (0.90 g) as well as diameter of callus (7.48 mm). Among the combinations 2, 4-D (1.5 mgL⁻¹) alone produced the best callus (95.16%) and the highest weight of callus (1.88 g) but diameter of callus (8.27 mm) was the highest from 2, 4-D 1.0 mgL⁻¹ + 0.5 mgL⁻¹ NAA. It was observed that both genotype and growth regulator significantly affected callus induction and plant regeneration. The medium supplemented with 2, 4-D 1.5 mgL⁻¹ + NAA 0.0 mgL⁻¹ exhibited better performance in callus induction (99.33%) and weight of callus (1.92 g) in cultivar *Nayanmony* whereas *Kalomona* was better for diameter of callus (8.38 mm) at 2, 4-D 1.0 mgL⁻¹ + NAA 0.5 mgL⁻¹. The cultivar *Nayanmony* performed better regarding plant regeneration (88.15%) compared to *Kalomona* (86.67%). Plantlets derived from the 2, 4-D 1.5 mgL⁻¹ + NAA 0.0 mgL⁻¹ showed the best results for survival. Therefore, it is suggested that application of MS medium supplemented with 2,4-D 1.5 mgL⁻¹ and NAA 0.0 mgL⁻¹ is advantageous to accomplish overall efficiency of callus induction, subsequent plant regeneration and survival from seeds of *Nayanmony* cultivars. Thus, selection of better responsive cultivar like *Nayanmony* and MS medium fortified with 1.5 to 2.0 mgL⁻¹ 2, 4-D would be beneficial in transformation attempts to improve the crop.

Keywords: Local *aus* rice, mature seeds, *in vitro*, callus induction, plant regeneration

Introduction

Rice is one of the most important cereal crops providing food for more than half of the world's population next to wheat and maize (Tyagi *et al.*, 2004). The importance of rice as staple food emphasizes its improvement undoubtedly. A considerable improvement has been done through traditional rice breeding. Traditional rice breeding has made significant progress towards higher yield, improved quality, greater disease resistance and other

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important characters of agricultural importance in the past and even in future, it will still play an important role (Sun *et al.*, 1990).

Albeit the success made in the last century, traditional breeding efforts alone cannot meet the increasing demand of rice consumers in the 21st century. Therefore, at present various tissue culture techniques are being used for the genetic improvement of rice plant throughout the world (Raina, 1989). It has been demonstrated in several cases that when higher plant tissues undergo a process of dedifferentiation and cell proliferation *in vitro*, wide range of mutations occur at a frequency much higher than expected (Brar and Khush, 1994). Somaclones provide a novel and valuable source of genetic variability, which can be used for crop improvement, particularly for the development of stress tolerant rice cultivars (Lutts *et al.*, 1999). Dehusked grain culture (embryo) being the most popular and easily accessible materials among wide ranges of tissue is used as explants for rice tissue culture (Raina, 1989). Many experiments have been conducted to optimize the techniques and composition of culture medium for callus induction from dehusked rice seed for various purposes (Islam *et al.*, 2005; Khatun *et al.*, 2003; Wang *et al.*, 1987). However, its application is still limited by many factors among them the genotype and nutrient composition are regarded to be the major sources of variation in *in vitro* culture (Khanna and Raina, 1998).

Nayanmony and *Kalomona* are two popular local *Aus* rice cultivars in the southern coastal districts of Bangladesh. These two cultivars have both salt and flood or water logging potentiality to some extent. They are the important grain crop of the farmers in southern part of this country whose life is built on the expectations of good yields that is rarely achieved. The local rice cultivars used in this study were selected on the basis of their local importance and salt tolerance potentiality. The development of a reproducible tissue culture system for efficient plant regeneration via callus induction from mature seed scutella of local *Indica* rice cultivars will be a base line to develop a universal approach for rice transformation. Therefore, this research intended to determine the potentiality of two local *Aus* rice (*Oryza sativa* L.) cultivars for callus induction and plant regeneration from mature dehusked seeds and to find out the suitable concentrations and combinations of growth regulators.

Materials and Methods

The experiment was carried out at Plant Breeding and Biotechnology Laboratory, Agrotechnology Discipline, Khulna University, Khulna during the period of October 2012 to April 2013. Mature seeds of two local *Aus* rice *viz.* *Nayanmony* and *Kalomona*, collected from Agrotechnology Discipline, Khulna University, were used in the present investigation and were manually dehusked and surface sterilized by immersing in 70% ethanol for 1 minute, then in 0.2% HgCl₂ for 15 minutes followed by 4-5 rinsing with sterile water.

The sterilized seeds were cultured onto the MS medium fortified with 2,4-D and NAA in various concentrations for callus induction. The cultures were kept in dark at 25±1 °C and 65% RH. The produced calli were then transferred to the MS medium supplemented with 0.5 mgL⁻¹ NAA and 3 mgL⁻¹ BAP for regeneration under 16 hours photoperiod at 2000 lux. The pH of the media was adjusted to 5.8. The regenerated plants were subjected for two successive subcultures.

The regenerated plants (8-10 cm) were transferred to a small pot containing sterilized sand supplemented with liquid MS media after removal of agar attached with roots and treated with Bavistin @ 0.1%. Each pot was covered with polythene after spraying water inside it and closed to check evapotranspiration. Established plants were then transferred to large pots containing puddle field soil and were kept in net house.

Results and Discussion

Effect of cultivars on callus induction, and weight and diameter of callus: The rice genotypes exerted a remarkable effect on callus induction, and weight and diameter of callus (Table 1). Frequency of callus induction and their diameter were significantly affected by the cultivars. But no significant influence of cultivars on weight of callus was recorded. Between the two cultivars *Nayanmony* performed better in respect of callus induction frequency (91.38%), weight of callus (0.90 g) as well as diameter of callus (7.47 mm). Chen *et al.* (1982) observed variation in callus induction frequency with genotype. Differences in the frequency and weight of callus in different genotypes were also reported by Tiwari *et al.* (2012).

Table 1: Effect of rice cultivars on callus induction, and weight and diameter of callus

<i>Cultivar</i>	<i>Frequency of callus induction (%)</i>	<i>Weight of callus (g)</i>	<i>Diameter of callus (mm)</i>
<i>Nayanmony</i>	91.38	0.90	7.47
<i>Kalomona</i>	82.98	0.88	7.04
CV (%)	4.59	9.68	8.31
Level of Significance	0.01	NS	0.05

NS = Not significant

Effect of growth regulators on callus induction, and weight and diameter of callus

All the parameters studied varied significantly by different concentrations and combinations of growth regulators. Results on callus induction, and weight and diameter of callus are presented in Table 2. Among the combinations 2, 4-D 1.5 mgL⁻¹ without NAA was found to be the best for callus induction (95.16%). The lowest callus induction frequency (79.16%) was recorded in the medium with 2, 4-D 2.0 mgL⁻¹+ NAA 1.0 mgL⁻¹. The maximum weight of callus was noticed in medium contained only 2, 4-D 1.5 mgL⁻¹ (1.88 g). The lowest callus weight (0.10 g) was obtained in the medium fortified with 2, 4-D 2.0 mgL⁻¹ + NAA 1.0 mgL⁻¹. Maximum callus diameter (8.27 mm) was recorded in the medium supplemented with 2, 4-D 1.0 mgL⁻¹+ NAA 0.5 mgL⁻¹ and the lowest parameter was (6.10 mm) with 2, 4-D 1.0 mgL⁻¹+ NAA 0.5 mgL⁻¹. Similar trend in frequency of callus induction from rice seed culture was reported by Pandey *et al.* (1994) who observed that seed response for callusing was the best when 2, 4-D was applied at a concentration of 2.0 mgL⁻¹. However, the optimum concentration of 2, 4-D varies depending on the explants source and genotype of rice (Raina, 1989).

Table 2: Effect of growth regulators on callus induction, and weight and diameter of callus of two rice cultivars

Growth regulators (mgL^{-1})		Frequency of callus induction (%)	Weight of callus (g)	Diameter of callus (mm)
2,4-D	NAA			
1.0	0.0	82.83de	0.58e	7.95ab
1.0	0.5	84.00cde	0.43f	8.27a
1.0	1.0	88.75bc	1.27c	6.82cde
1.5	0.0	95.16a	1.88a	6.10e
1.5	0.5	91.00ab	1.44b	7.19bcd
1.5	1.0	90.25ab	0.95d	7.65abc
2.0	0.0	85.83bcd	0.85d	7.23bcd
2.0	0.5	87.66bcd	0.50ef	7.42abcd
2.0	1.0	79.16e	0.10g	6.66de
CV (%)		4.59	9.68	8.31
Level of Significance		0.01	0.01	0.01

Mean values in a column followed by same letter(s) are not significantly different as per DMRT

Interaction effect of growth regulators and cultivars on callus induction, and weight and diameter of callus: Interaction effect of cultivars and growth regulators on callus induction, and weight and diameter of callus was significant (Table 3). Among the combinations, maximum callus induction frequency (99.33%) and weight of callus (1.92g) were recorded in cultivar *Nayanmony* in MS medium supplemented with 1.5 mgL^{-1} 2, 4-D only. The lowest frequency of callus induction (72.66%) and callus weight (0.06 g) were found in Kalomona with the treatment combination of 1.0 mgL^{-1} 2, 4-D and 0.5 mgL^{-1} NAA.

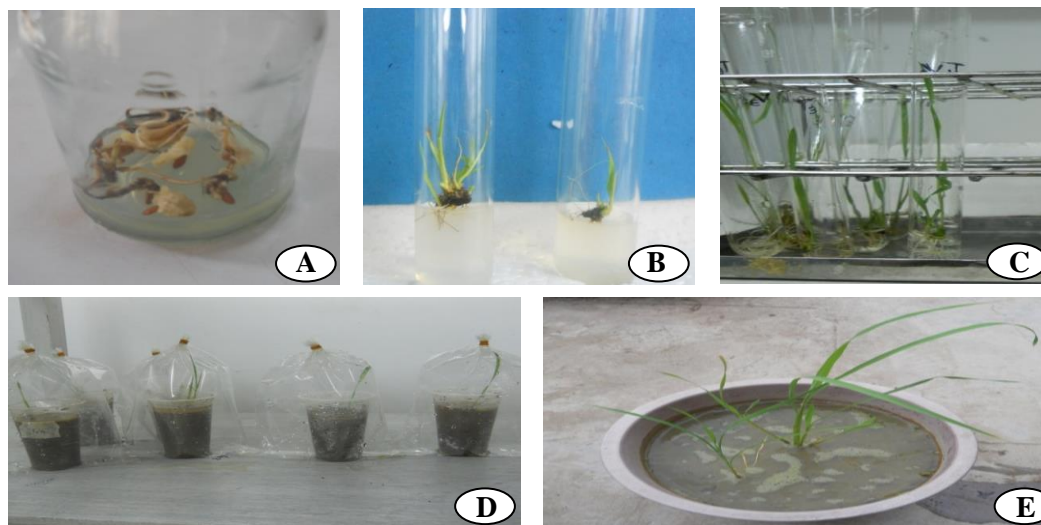


Fig 1: Different stages of *in vitro* regeneration of local rice on growth regulators supplemented MS. A. Calli appeared from the mature seeds. B. Regeneration of shoots from calli. C. Root initiation. D. Plantlets growing in the green house. E. Hardened plants.

Nayanmony produced maximum callus diameter (8.38 mm) in the treatment combination of 1.0 mgL⁻¹ 2, 4-D and 0.5 mgL⁻¹ NAA and minimum diameter (5.71 mm) was recorded in *Kalomona* in the medium with 1.5 mgL⁻¹ 2, 4-D with no NAA. Pandey *et al.* (1994) and Islam (2005) reported that the success of *in vitro* dehusked rice culture largely depends on nutritional media, hormone concentration, genotype and on the interaction of genotype and medium. Similar reports were also found elsewhere (Rahim *et al.* 1991; Wu and Chen, 1987; Abe and Futsfara, 1984 and Guo and Cao, 1982).

Table 3: Interaction effect of cultivars and growth regulators on callus induction, and weight and diameter of callus

Cultivar	Concentration of growth regulators (mgL ⁻¹)		Callus induction (%)	Weight of callus (g)	Diameter of callus (mm)	Colour of callus	Texture of callus
	2,4-D	NAA					
<i>Nayanmony</i>	1.0	0.0	79.00hij	1.06c	7.56abc	Creamish	Friable
	1.0	0.5	95.33abc	0.80d	8.38a	Creamish	Friable
	1.0	1.0	98.33ab	1.79ab	7.69abc	Whitish	Friable
	1.5	0.0	99.33a	1.92a	6.49cdef	Whitish	Compact
	1.5	0.5	92.33abcde	1.76ab	7.46abc	Brown	Compact
	1.5	1.0	95.50abc	0.15f	7.92ab	Whitish	Friable
	2.0	0.0	88.33cdefg	0.09f	7.05bcde	Whitish	Compact
	2.0	0.5	94.33abcd	0.42e	7.47abc	Brown	Compact
	2.0	1.0	80.00hij	0.10f	7.22abcd	Dark Brown	Compact
<i>Kalomona</i>	1.0	0.0	86.66defgh	0.10f	8.34a	Creamish	Friable
	1.0	0.5	72.66j	0.06f	8.15ab	Whitish	Friable
	1.0	1.0	79.16hij	0.75d	5.96ef	Creamish	Compact
	1.5	0.0	91.00bcdef	1.84a	5.71f	Whitish	Compact
	1.5	0.5	89.66cdef	1.11c	6.92bcdef	Brown	Compact
	1.5	1.0	85.00efghi	1.76ab	7.39abc	Whitish	Friable
	2.0	0.0	83.33fghi	1.61b	7.41abc	Whitish	Friable
	2.0	0.5	81.00ghi	0.57e	7.36abc	Brown	Compact
	2.0	1.0	78.33ij	0.09f	6.10def	Dark Brown	Compact
CV (%)		4.59	9.68	8.31			
Level of Significance		0.01	0.01	0.05			

Mean values in a column followed by same letter(s) are not significantly different as per DMRT

Performance of two local rice cultivars and growth regulators in morphogenesis:

Under morphogenetic study, the colour and texture of callus were considered. The produced calli were whitish, creamish, brown and dark brown in colour and the texture varied from friable to compact. Most of the calli were whitish in colour. Compact calli weighed more than friable calli but friable calli had more diameter compared to compact calli.

Effect of cultivars and growth regulators on morphogenesis of plated calli is represented in Table 4. Calli of the cultivar *Nayanmony* performed better in all parameters considered for morphogenesis except caulo-rhizogenesis. The cultivar *Nayanmony* showed the highest caulogenesis (76.30%), rhizogenesis (4.15%) and total plant regeneration (88.15%). Variations in plantlet regeneration due to varietal differences were also reported by Islam *et al.* (2005). Naqvi *et al.* (2005) reported that regeneration frequency depended on genotype and on compositions as well as concentrations of regeneration medium.

Table 4: Performance of two local rice cultivars and growth regulators in morphogenesis

Genotype	Callus inducing media(mgL ⁻¹)		Morphogenesis			
	2,4-D	NAA	Caulogen.	Rhizogen.	Caulo-rhizogen.	Total reg.
			(%)	(%)	(%)	(%)
<i>Nayanmony</i>	1.0	0.0	75.09	2.22	9.36	86.67
	1.0	0.5	89.13	0.00	4.23	93.33
	1.0	1.0	79.06	1.52	6.09	86.67
	1.5	0.0	76.93	6.89	9.52	93.34
	1.5	0.5	66.20	6.67	7.15	80.02
	1.5	1.0	82.56	3.08	7.69	93.33
	2.0	0.0	77.69	6.67	8.98	93.34
	2.0	0.5	84.16	3.61	5.56	93.33
	2.0	1.0	55.85	6.67	10.81	73.33
Mean			76.30	4.15	7.71	88.15
<i>Kalomona</i>	1.0	0.0	76.42	1.05	9.20	86.67
	1.0	0.5	81.81	2.06	6.55	90.42
	1.0	1.0	78.80	0.00	7.86	86.67
	1.5	0.0	77.45	6.67	9.21	93.33
	1.5	0.5	67.72	2.11	6.58	76.41
	1.5	1.0	72.96	6.67	7.04	86.67
	2.0	0.0	61.75	3.16	8.43	73.34
	2.0	0.5	73.59	0.00	6.41	80.00
	2.0	1.0	74.05	3.31	9.30	86.66
Mean			73.84	2.78	7.84	84.46

Survival of regenerated plants: The survival of the regenerated plants was variable and ranged from 31.03% to 58.33% (Figure 2). Between the two cultivars, survival of *Nayanmony* (44.59%) was greater than *Kalomona* (36.95%). Gnanesh *et al.* (2012) reported genotypic differences in plantlet survival.

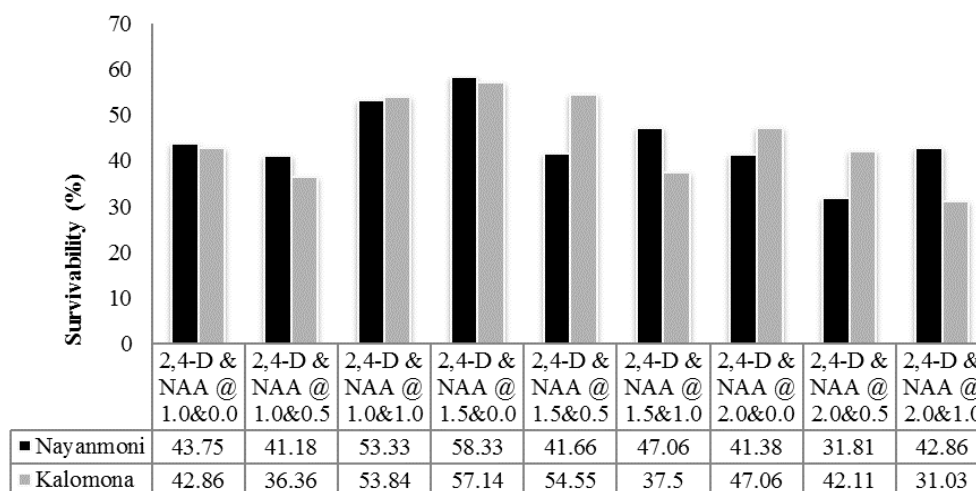


Fig 2: Graphical representation of survival rate of regenerated plant of two rice cultivars

Conclusion

Selection of better responsive cultivar like *Nayanmony* and MS medium fortified with 1.5 to 2.0 mgL⁻¹ 2, 4-D would offer great promise for the induction of better quality callus and subsequent plant regeneration for various means of genetic transformation for improving the world's most important cereal crop.

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