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In vitro regeneration protocol for *indica* rice genotypes

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ABSTRACT

Embryogenic calli from mature seeds of four indica rice genotypes were used to observe their regeneration potentiality and establish a suitable in vitro plantlet regeneration protocol. MS medium supplemented with different phytohormone combinations were used to observe the callus induction ability of the explant. The highest callus induction (73.19%), biggest size of callus (3.133mm) and higher callus weight (0.7167g) were observed in Binadhan-6 in MS medium supplemented with 1.0 mg L^{-1} 2,4-D over all the genotypes and MS medium supplemented with 1.5 mg L^{-1} 2,4-D was the best over all the treatments (66.83%). Among the phytohormone combinations, MS + 8 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA showed the highest shoot regeneration (50.67%) and shoot length (13.7cm). Among the genotypes, Binadhan-6 was highly responsive to shoot regeneration (55.83%). The best root formation from regenerants (87.889%), maximum number of roots per plant (20) and the highest length (4.467 cm) of roots were in MS media supplemented with 0.5 mg L^{-1} IAA in Binadhan-6. In pot and soil, Binadhan-6 showed the highest survival rate of the plantlet 91.30% and 85%, respectively. This callus induction and in vitro regeneration protocol will be widely applicable for the tissue culture of indica rice.

Key Words: *Rice, In vitro callus induction, Protocol development and plant regeneration*

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I. Introduction

Rice *(Oryza sativa* L.) a cereal grain, is the most widely consumed staple food for a large part of the world's human population. Rice is the agricultural commodity with the third-the highest worldwide production, after sugarcane and maize (FAOSTAT, 2012). In Bangladesh, rice is occupying 11.0 million ha (BBS, 2013) almost 13 million farm families of the country grow rice which has remained almost stable over the past three decades (BRRI, 2013).

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Global population is increasing very rapidly. Loss in crop production could lead to hunger and famine, especially in the developing county like Bangladesh. For this, it requires research to produce more food efficiently. This improvement can possible be achieved by creating genetic variability. To bring genetic variability, conventional breeding method is not sufficient to elevate rice productivity and yields (Bhuiyan and Karim, 2002). Although conventional breeding will continue to play a major role in increasing crop yield, laboratory based techniques, such as genetic transformation to introduce novel genes into crop plants, will be essential in complementing existing technologies (Ingram et al., 2001). Plant tissue culture technique had made possible to produce genetic variation that are raise crop yield and quality, and that can help to substantially food security (Vasil, 1998). The objective of the experiment was to develop suitable protocol for callus induction and *in vitro* plant regeneration.

II. Materials and Methods

Binadhan-5, Binadhan-6, BRRI dhan32 and Basmati 370 were used as plant material to study different parameters associated with in vitro regeneration of plant. The experiments were carried in 2010 at the Tissue Culture Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh and Biotech Laboratory, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Culture technique: For callus induction, callus proliferation, shoot regeneration and rooting MS medium (Murashige and Skoog, 1962) was used. The culture techniques were explants culture, partial desiccation, subculture or transfer into regeneration media and rooting employed. Mature embryos attached to endosperm were the main source of explants for embryo culture. The hormones and their respective number of combinations of embryo culture were as follows treatment:

a) For callus induction

MS medium + 0.50 mg L^{-1} 2,4-D (T_1)
MS medium + with 1.00 mg L^{-1} 2,4-D (T_2)
MS medium + with 1.50 mg L^{-1} 2,4-D (T_3)
MS medium + 2.00 mg L^{-1} 2,4-D (T_4)
MS medium + 2.50 mg L^{-1} 2,4-D (T_5)
MS medium + 3.00 mg L^{-1} 2,4-D (T_6)

b) For shoot differentiation

MS medium + 2 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_1)
MS medium + 4 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_2)
MS medium + 6 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_3)
MS medium + 8 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_4)
MS medium + 10 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_5)
MS medium +12 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA (T_6)

c) For root initiation

MS medium + 0.4 mg L^{-1} IBA (T_1)
MS medium + 0.5 mg L^{-1} IBA (T_2)
MS medium + 0.6 mg L^{-1} IBA (T_3)

Explants culture: Sterilized mature seeds were cultured directly in MS medium supplemented with different concentrations of hormones and sucrose required as per treatment. The culture plates containing explants were placed under fluorescent light in a room with controlled temperature (22±2°C) using 16 hrs photoperiod.

Subculture in regeneration media: Three weeks after inoculation, the calli attained convenient size. Then they placed again on freshly prepared sterilized medium containing appropriate hormonal supplements for shoot induction from the calli. The subculture media were MS medium containing different combinations and concentrations of NAA and Kinetin. The subcultured petridishes were again incubated at 22±2°C with 16 hrs photoperiod and mentioned for calli and organogenesis.

Rooting: The subcultured calli continued to proliferate and differentiated into shoots. When these shoots grew about 2-3 cm in length, they were separated from each other and again cultured individually on vials or petridishes with freshly prepared root induction medium to induce root. The vials or conical flasks containing plantlets were incubated at 22±2˚C with 16 hrs photoperiod.

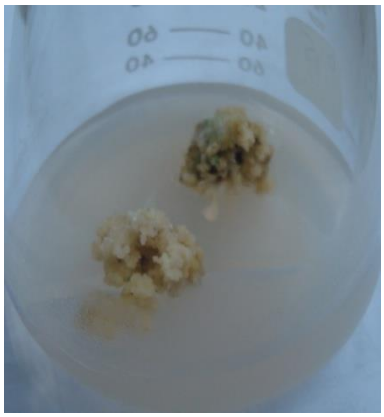
Data recorded on Callus induction (days of callus initiation, number of explants with callus, size of callus) and plantlet regeneration (days to shoot initiation, number of callus with shoot, number of shoots per callus, average number of roots per plants, percent plant establishment). Complete Randomized Design (CRD) was used in growth room Tissue Culture Laboratory. The analyses of variances for different parameters were performed and means were compared by the Duncan's Multiple Range Test (DMRT) in MSTATC program.

III. Results and Discussion

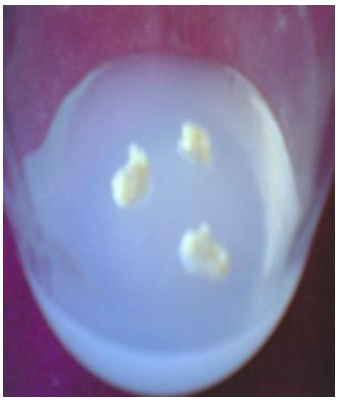
In vitro regeneration protocol of four *indica* rice genotypes was investigated. Genotypes were accomplished with callus induction, shoot-root development and finally plantlet regeneration.

Callus induction

Callus initiation started from 05 days of incubation and took about 14 days for the completion followed by Binadhan-6, Binadhan-5, BRRI dhan32 and Basmati 370 respectably (Plate 01). The average percentage of callus induction was the highest in Binadhan-6 (72%) and the lowest in Basmati 370 (22%). The highest (94%) percentage of callus induction was observed in Binadhan-6, T_2 (MS + 1.0 mgL^{-1} 2,4-D) and the lowest (12%) percentage of callus induction was observed in T_6 (MS + 3.0 mgL^{-1} 2,4-D) (Table 01). Callusing was also the highest (66.83%) in T_3 (MS + 1.5 mgL^{-1} 2,4-D) and the lowest (20.29%) in T_6 (MS + 3.0 mgL^{-1} 2,4-D). Hidayat *et. al.,* (2007) reported that maximum callus formation (62.5%) was recorded for Basmati-385, followed by Basmati-370 (55.55%) when seeds were cultured on MS medium supplemented with 2,4-D@ 2.0 mgL^{-1}.



Binadhan-6 (the highest callus)



Basmati 370 (the lowest callus)

Plate 01. Callus of four indica rice genotypes from mature embryos with desiccation treatment (subculture).

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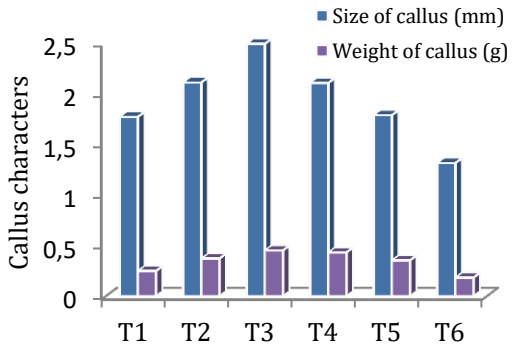
Table 01. Effect of different treatment combinations for callus induction from mature embryos of four *indica* rice variety

Treatment	Genotype	No. of explants inoculated	Explants no. producing callus	% callus induction	Days required for callus induction
MS + 0.5 mgL^{-1} 2,4-D (T_1)	Binadhan-5	50	28	56	9-14
	Binadhan-6	50	42	84	6-8
	Basmati 370	50	8	16	11-15
	BRRI dhan 32	50	27	54	6-9
MS + 1.0 mgL^{-1} 2,4-D (T_2)	Binadhan-5	50	31	62	11-12
	Binadhan-6	50	47	94	7-8
	Basmati 370	50	13	26	13-15
	BRRI dhan 32	50	30	60	5-7
MS + 1.5 mgL^{-1} 2,4-D (T_3)	Binadhan-5	50	36	72	10-11
	Binadhan-6	50	42	84	8-9
	Basmati 370	50	13	26	11-14
	BRRI dhan 32	50	37	74	5-6
MS + 2.0 mgL^{-1} 2,4-D (T_4)	Binadhan-5	50	35	70	11-14
	Binadhan-6	50	37	74	8-9
	Basmati 370	50	19	38	14-16
	BRRI dhan 32	50	29	58	7-10
MS + 2.5 mgL^{-1} 2,4-D (T_5)	Binadhan-5	50	23	46	12-14
	Binadhan-6	50	29	58	8-10
	Basmati 370	50	7	14	14-15
	BRRI dhan 32	50	17	34	8-10
MS + 3.0 mgL^{-1} 2,4-D (T_6)	Binadhan-5	50	8	16	13-15
	Binadhan-6	50	19	38	8-11
	Basmati 370	50	6	12	13-15
	BRRI dhan 32	50	8	16	7-11
Average	Binadhan-5	50	26.83	53.67	-
	Binadhan-6	50	36	72	-
	Basmati 370	50	11	22	-
	BRRI dhan	50	24.67	49.33	-

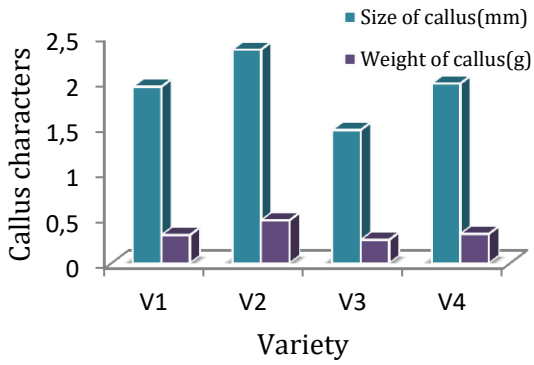
Effect of different treatments on callus characters

Mean square values of six different combinations of treatments were found statistically significant for the callus characters such as size and weight of callus. T_3 (MS + 1.5 mgL^{-1} 2,4-D) showed the highest percentage (66.83%) of callus induction and the lowest (20.29%) T_6 (MS + 3.0 mgL^{-1} 2,4-D). Size of callus was biggest in T_3 (MS + 1.5 mgL^{-1} 2,4-D) 2.494, closely followed T_2 (MS + 1.0mgL^{-1} 2,4-D) 2.112 and T_4 (MS + 2.0 mgL^{-1} 2,4-D) 2.108 which was not statistically significant, but T_5 (MS + 2.5 mgL^{-1} 2,4-D) 1.788 (Figure 01). Smallest (1.315) size of callus was observed in T_6 (MS + 3.0 mgL^{-1} 2,4-D). Such type of results was obtained by Saharan *et al.* (2004); Suresh *et al.* (2001); Chand and Sahrawat (2001). Weight of callus was the highest (0.4508) in T_3 (MS + 1.5 mgL^{-1} 2,4-D) and the lowest (0.1817) in T_6 (MS + 3.0 mgL^{-1} 2,4-D) which were statistically significant (Figure 01). Similar size and weight of callus was reported by Azira and Bhalla (2000); Dode *et al.* (2000); Lin and Zhang (2005); Lee *et al.* (2002).



Treatment: T1= MS + 0.5 mgL^{-1} 2,4-D, T2= MS + 1.0 mgL^{-1} 2,4-D, T3= MS +1.5 mgL^{-1} 2,4-D, T4= MS+2.0 mgL^{-1} 2,4-D, T5= MS+2.5 mgL^{-1} 2,4-D, T6= MS+3.0 mgL^{-1} 2,4-D

Figure 01. Response of different treatments on callus characters.



Genotype: V1= Binadhan-5, V2= Binadhan-6, V3= Basmati 370, V4= BRRI dhan32

Figure 02. Response of different genotypes on callus characters.

Effect of different genotypes on callus characters

The genotypic mean square values of rice were found statistically significant for all the characters of callus induction. The highest (73.19%) percentage of callus induction was observed in Binadhan-6 followed by Binadhan-5 (54.83%), BRRI dhan32 (51.67%) and the lowest (23.44%) in Basmati 370 which were statistically significant (Figure 02). Size of callus was biggest (2.347) in Binadhan-6, closely followed BRRI dhan32 (1.975), Binadhan-5 (1.939) which was not statistically significant, Basmati 370 showed smallest (1.464) size of callus. Binadhan-6 showed much 0.4708 weight of callus followed by BRRI dhan32 (0.3217), Binadhan-5 (0.3081) and Basmati 370 showed the lowest (0.2558) weight of callus which were statistically significant. YuMei *et al.* (2006) also found this type of result.

Effects of Genotype x Treatment interaction on callus characters

Among the four genotypes Binadhan-6 showed the highest (95.0%) percentage of callus induction in T_2 (MS + 1.0mgL^{-1} 2,4-D). The lowest percent of callus induction was observed in Basmati 370, BRRI dhan32, Binadhan-5 and Binadhan-6 11, 15, 21.33, and 33.83% respectively, in T_6 (MS + 3.0 mgL^{-1} 2,4-D). Biggest size of callus (3.133) was observed in Binadhan-6 on the interaction of T_2 (MS + 1.0 mgL^{-1} 2,4-D), followed by BRRI dhan32, Binadhan-5 and Basmati 370 2.867, 2.583 and 1.758 respectively on the interaction of T_3 (MS + 1.5 mgL^{-1} 2,4-D). Smallest size of callus (1.650, 1.342, 1.267 and 1.000) was found in Binadhan-6, BRRI dhan32, Binadhan-5 and Basmati 370 respectively on the interaction of T_6 (MS + 3.0 mgL^{-1} 2,4-D). Higher weight of callus (0.7167) was observed in Binadhan-6 on the interaction of T_2 (MS + 1.0 mgL^{-1} 2,4-D). These findings compared the findings of Azira and Bhalla (2000), Dode *et al.* (2000) in callus induction and Lin and Zhang (2005) and Lee *et al.* (2002) in size of callus.

Effects of different treatments on shoot regeneration

The various levels of Kinetin showed significant variations for percent of shoot regeneration from callus presented on. It was observed that among the treatments tested, number of shoots per explants showed maximum (17.67) on T_4 (MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) and minimum (3.083) on T_6 (MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) (Figure 03). Similar trends of result were found by Saharan *et al.* (2004) and Chand and Sahrawat (2001). The length of shoots was the highest (13.70) on T_4 (MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) and the lowest (4.358) on T_6 (MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA).

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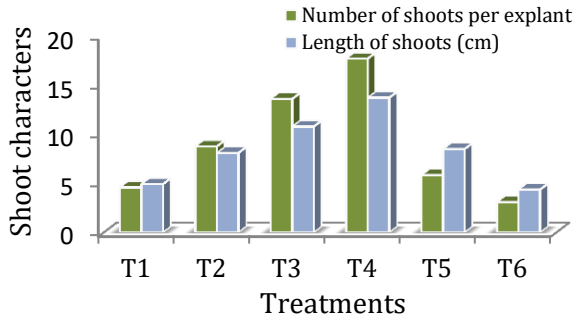

T1= MS + 2 mgL^{-1} Kn + 0.5 mgL^{-1} NAA,
T2= MS + 4 mgL^{-1} Kn + 0.5 mgL^{-1} NAA
T3= MS + 6 mgL^{-1} Kn + 0.5 mgL^{-1} NAA,
T4= MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA
T5= MS + 10 mgL^{-1} Kn + 0.5 mgL^{-1} NAA and T6= MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA

Figure 03. Response of different treatment on

Effects of genotype on shoot regeneration

Out of four genotypes tested Binadhan-6 showed maximum (16.89) number of shoot callus^{-1} and minimum (2.778) by Basmati 370 (Table 02). Length of shoot was observed the highest in Binadhan-6 (11.64) and the lowest (5.078) by Basmati 370. Hoque and Mansfield (2004) reported that plant regeneration was influenced by genotype.

Effects of treatment x genotype interactions on shoot regeneration

The highest percentage of shoot regeneration was obtained by Binadhan-6 (79.33) on T_4 (MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) and the lowest by Basmati 370 (8%) on T_1 (MS + 2 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) and T_6 (MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) (Table 03). Out of four genotypes Binadhan-6 showed the highest (34.00) number of shoots per explants. It was observed that Basmati 370 showed the lowest number of shoot callus (0.667) followed by Binadhan-5 (2.333), BRRI dhan32 (4.333) and Binadhan-6 (5.000) on T_6 (MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA). This finding is similar to Visarada *et al.* (2002) result.

Effect of genotypes on root induction

Mean square values of four genotypes were found statistically significant for per cent root induction, number of root/plant and length of roots (Figure 04). The highest (87.88%) percentage of root induction was observed by Binadhan-6 and the lowest (40.00%) by Basmati 370. Binadhan-6 showed the highest (4.000) length of roots and Basmati 370 showed the lowest (1.778).

Effects of treatment on root induction

The various levels of IAA tested on root regeneration showed significant variation for percentage of root induction, number of roots per plant and length (Figure 05). The highest (84.17) percentage of root induction was found on T_2 (MS + 0.5 mgL^{-1} IAA) followed by T_1 (MS + 0.3 mgL^{-1} IAA) 64.75% and T_3 (MS + 0.7 mgL^{-1} IAA) 61.42% but both were statistically similar. T_2 (MS + 0.5 mgL^{-1} IAA) showed the highest length (3.408) of roots closely followed by T_3 (MS + 0.7 mgL^{-1} IAA) 2.842, T_1 (MS + 0.3 mgL^{-1} IAA) 2.583.

Effects of genotype x treatment interactions on root induction

Percent of root induction was found the highest (97.67%) by Binadhan-6 onT_2 (MS + 0.5 mgL^{-1} IAA) and the lowest (29.00%) by Basmati 370 onT_1 (MS + 0.3 mgL^{-1} IAA. There is no significant difference between the interaction on genotypes and treatment (Table 04). Maximum number of roots per plant (20) was observed on T_2 (MS + 0.5 mgL^{-1} IAA) in genotype of Binadhan-6 and the lowest (4.333) on T_3 (MS + 0.7 mgL^{-1}) in Basmati 370.

Table 02. Effect of genotypes on shoot regeneration

Genotypes	No. of explants Showing shoot regeneration	Percent of shoot regeneration	Days required for shoot regeneration
Binadhan-5	12.61 b	41.83 b	16.500
Binadhan-6	16.89 a	55.83 a	13.500
Basmati 370	4.833 d	16.11 d	19.500
BRRI dhan32	10.39 c	34.22 c	14.000
CV (%)	*9.07*	*9.45*	*5.59*
$LSD_{(0.05)}$	*0.6795*	*2.343*	*0.6132*

In the column figures followed by same letter(s) do not differ significantly

Table 03. Effects of treatment x genotype interactions on shoot regeneration of four *indica* rice Genotypes

Genotypes	Treatment	Explants Showing shoot regeneration	Percent of shoot regeneration	Days required shoot regeneration	Number of shoot per explant	Length of shoot (cm)
MS+2 mgL^{-1} Kn + 0.5 mgL^{-1} NAA (T_1)	Binadhan-5	9.000 hi	29.67 hi	19.33	4.333 hij	5.000 jkl
	Binadhan-6	12.33 ef	41.00 ef	11.67	8.333 fg	6.833 gh
	Basmati 370	2.333 m	8.000 m	24.00	1.667 jk	3.167 no
	BRRI dhan32	7.667 ij	25.00 ij	23.00	4.000 ijk	4.667 klm
MS+4 mgL^{-1} Kn + 0.5 mgL^{-1} NAA(T_2)	Binadhan-5	14.00 de	46.33 de	16.00	4.667 hij	7.833 fg
	Binadhan-6	15.00 cd	49.67 cd	9.333	20.00 c	11.23 d
	Basmati 370	5.000 kl	16.67 kl	20.67	2.667 jk	5.600 ijk
	BRRI dhan32	11.33 fg	37.33 fg	20.00	7.667 fgh	7.667 fg
MS+6 mgL^{-1} Kn + 0.5 mgL^{-1} NAA (T_3)	Binadhan-5	16.00 c	53.00 c	11.33	15.33 d	13.53 c
	Binadhan-6	18.00 b	59.33 b	8.333	27.67 b	15.43 b
	Basmati 370	6.667 jk	22.00 jk	17.00	4.667 hij	5.833 ij
	BRRI dhan32	12.33 ef	40.67 ef	17.33	6.667 fghi	8.300 f
MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA (T_4)	Binadhan-5	14.00 de	46.33 de	16.33	11.67 e	11.90 d
	Binadhan-6	24.00 a	79.33 a	6.667	34.00 a	19.83 a
	Basmati 370	8.000 ij	26.33 ij	19.67	3.667 ijk	7.533 fg
	BRRI dhan32	15.33 cd	50.67 cd	13.33	21.33 c	15.53 b
MS + 10 mgL^{-1} Kn + 0.5 mgL^{-1} NAA (T_5)	Binadhan-5	12.00 f	40.67 ef	20.00	3.667 ijk	9.767 e
	Binadhan-6	18.00 b	59.33 b	11.00	6.333 ghi	10.17 e
	Basmati 370	4.667 l	15.67 l	23.33	3.333 ijk	5.700 ij
	BRRI dhan32	10.00 gh	33.00 gh	21.33	10.00 ef	8.300 f
MS+12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA (T_6)	Binadhan-5	10.67 fgh	35.00 fgh	23.33	2.333 jk	3.867 mn
	Binadhan-6	14.00 de	46.33 de	13.00	5.000 ghij	6.367 hi
	Basmati 370	2.333 m	8.000 m	24.67	0.667 k	2.633 o
	BRRI dhan32	5.667 kl	18.67 kl	25.67	4.333 hij	4.567 lm
	CV (%)	*9.07*	*9.45*	*5.59*	*21.02*	*6.71*
	$LSD_{(0.05)}$	*1.665*	*5.739*	*1.876*	*3.077*	*0.9228*

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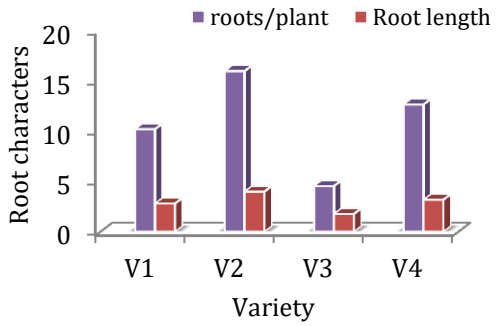
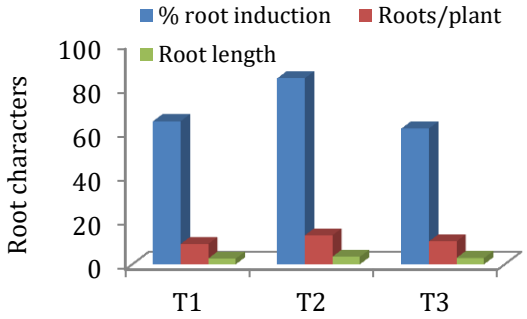


V1=Binadhan-5, V2= Binadhan-6
V3= Basmati 370 V4= BRRI dhan32

Figure 04. Performance of four *indica* rice genotypes on root induction.



T1= MS + 0.3 mgL^{-1} IAA, T2= MS + 0.5 mgL^{-1} IAA,
T3= MS + 0.7 mgL^{-1} IAA

Figure 05. Response of different treatments on root induction of four rice genotypes.

Table 04. Effects of genotype x treatment interactions on root induction of four *indica* rice Genotypes

Genotypes	Treatment	Percent of root induction	Number of roots per plant	Length of roots (cm)
Binadhan-5	MS + 0.3 mgL^{-1} IAA (T_1)	70.67 def	8.333 de	2.433 e
	MS + 0.5 mgL^{-1} IAA (T_2)	88.33 ab	12.67 bc	3.200 c
	MS + 0.7 mgL^{-1} IAA (T_3)	62.00 fg	9.667 cd	2.800 d
Binadhan-6	MS + 0.3 mgL^{-1} IAA (T_1)	84.00 bc	13.33 bc	3.700 b
	MS + 0.5 mgL^{-1} IAA (T_2)	97.67 a	20.00 a	4.467 a
	MS + 0.7 mgL^{-1} IAA (T_3)	82.00 bcd	14.67 b	3.833 b
Basmati 370	MS + 0.3 mgL^{-1} IAA (T_1)	29.00 h	4.000 f	1.433 f
	MS + 0.5 mgL^{-1} IAA (T_2)	57.67 g	5.333 ef	2.267 e
	MS + 0.7 mgL^{-1} IAA (T_3)	33.33 h	4.333 f	1.633 f
BRRI dhan32	MS + 0.3 mgL^{-1} IAA (T_1)	75.33 cde	11.00 bcd	2.767 d
	MS + 0.5 mgL^{-1} IAA(T_2)	93.00 ab	14.33 b	3.700 b
	MS + 0.7 mgL^{-1} IAA (T_3)	68.33 efg	12.67 bc	3.100 c
CV (%)		*6.96*	*6.51*	*6.45*
$LSD_{(0.05)}$		*11.840*	*3.556*	*0.2064*

In the column figures followed by same letter(s) do not differ significantly

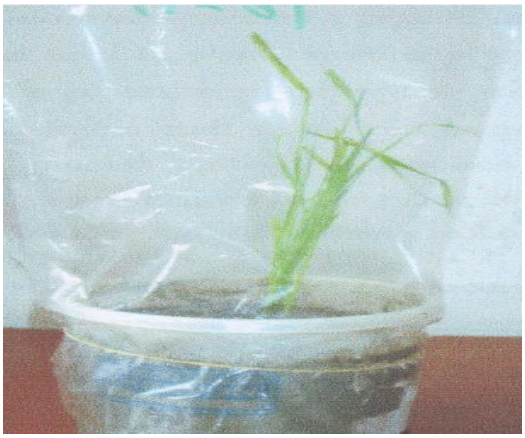


Figure 06. Acclimatized plantlets of Binadhan-6 in plastic pot covered with polythene bag.

Figure 07. Established plantlets of Binadhan-6 in earthen pot.

Establishment of plantlets

The small plantlets, after sufficient development in root system, were taken out from the culture vessels without causing any damage to roots. The plantlets of Binadhan-6, BRRI dhan32, Binadhan-5, and Basmati 370 was 91.30, 85, 80 and 53.33%, respectively in pot and Binadhan-6, Binadhan-5, BRRI dhan32 and Basmati 370 was 85, 75, 72.72 and 40%, respectively in soil.

IV. Conclusion

This experiment was carried out to develop a protocol for callus induction and plant regeneration from four *indica* rice genotypes. The percent callus induction was the highest (72%) in Binadhan-6 which required minimum (6-7) days for callus initiation and the lowest (22%) on Basmati 370. Biggest size (3.133) and the highest weight (0.7167) of callus were observed in Binadhan-6 in T_2 (MS + 1.0 mgL^{-1} 2,4-D). After callus formation kinetin and NAA on MS medium were used to observe the shoot regeneration capacity of different calli. Among four genotypes, the maximum (16.89) number of shoot per callus and the highest length of shoot (11.64) were observed in Binadhan-6, while minimum (2.778) number of shoots per callus and the lowest length (5.078) of shoot were obtained in Basmati 370. Among the four treatments, the highest (50.67) percentage of shoot regeneration, maximum (17.67) number of shoots per explants and the highest (13.70) length of shoots were observed on T_4 (MS + 8 mg/L Kn + 0.5 mg/L NAA), while the lowest (25.92%) percentage of shoot regeneration was observed in T_1 (MS + 2 mgL^{-1} Kn + 0.5 mgL^{-1} NAA). Minimum (3.083) number of shoots per explants and the lowest (4.358) length of shoots were observed on T_6 (MS + 12 mgL^{-1} Kn + 0.5 mgL^{-1} NAA).

MS medium with different concentrations of IAA (0.3, 0.5 and 0.7 mgL^{-1}) were used to observe the rooting responses of regenerated shoots. Among the four genotypes, Binadhan-6 showed the highest percentage (87.889%) of root initiation whereas Basmati 370 showed the lowest (40%). The percentage of root initiation was the highest 84.17% in T_2 (MS + 0.5 mgL^{-1} IAA) and the lowest 61.42% on T_3 (MS + 0.7 mgL^{-1} IAA). Maximum number (20.0) of roots per plant and the highest length (4.467) of roots were observed in Binadhan-6 on T_2 (MS + 0.5 mgL^{-1} IAA) and minimum number (4.333) of roots per plant and the lowest length (1.633) of roots were observed in Basmati 370 on T_3 (MS + 0.7 mgL^{-1} IAA). The survival rate of the plantlet was the highest in Binadhan-6 in the pot (91.30%) and (85%) in soil. but BRRI dhan32 showed the highest survival rate (Both in pot and soil). Basmati 370 showed the lowest survival rate of the plantlet 53.33% and 40% respectively. This protocol will to help to further study

V. References

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