Study On Callus Initiation And Plantlet Regeneration Ability Of Some Rice Genotypes

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Abstract: An experiment was conducted with mature embryos of three HYD and resistant rice (varieties BRRIdhan-29, BR-14, BINA dhan-8) as explants to investigate the appropriate growth condition for callus induction as well as plant regeneration on MS basal medium supplemented with different concentration of auxin and cytokinin. Different concentrations of 2, 4-D (0.0, 1.5, 2, 2.5, 3 mgL-1) were used for callus induction. Among the concentrations 2.0mgL^{-1} 2, 4-D was best concentrations for callus induction (85%), callus size (4.533mm) and callus weight (81.847mg) in 10.66days and the highest maintaining ability was found in BR-14 at 2.0mgL^{-1} 2, 4-D (88%) in callus size (5.143a) and callus weight (87.50mg)) in 6.50days. Different concentrations of kinetin (0.0, 0.4, 0.6, 0.8, 10 mgL-1) with a constant concentration of NAA (0.5mgL-1) were used for plant regeneration from callus. Again, BRRI dhan-29 on T_3 (MS + 8 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) was found to be best in shoot length (6.00cm) with highest shoots initiation (97.5%) and the highest .number of shoots per callus (5.25) on T_2 (MS + 6 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) in 22.25days. In combination, BRRI dhan-29 the highest number of roots per explant (7.25), root length (2.31cm) was found in T_3 (MS + 0.6 mgL^{-1} IBA) and the highest root initiation (97.50%) was on T_2 (MS + 0.5 mgL^{-1} IBA).

Index Terms: Rice genotypes, MS, NAA, IBA, Different concentration, Callus initiation and Plantlet Regeneration

1 INTRODUCTION

Rice *Oryza sativa L* is a cereal crop belongs to the family *Graminae* and the genus *Oryza*. At present, genus *Oryza* consist of two cultivated species and twenty-one wild species. Bangladesh is predominantly a rice growing country where rice is the staple food for the people. Among the crops grown in Bangladesh, rice is covering an area 11.06 million hectare and producing 38.13 million tons of rice annually (FAO,2002). The area and production of rice in the country were 26018000 acres and 279000 metric tons, respectively with an average yield of 1020 kg acre-1 (BBS, 2006). Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama; Swings *et al.,* 1990) is one of the most destructive diseases of rice throughout the world (Mew, 1987). This disease is also serious rice problem in other parts of Asia (Alim, 1967, Ou, 1985) during the heavy rains of the monsoon season. The deployment of resistant cultivars through genetic engineering appears most effective and economical method of controlling BLB. A dominant gene designated as Xa 21, known to confer broad spectrum resistance to BLB, was transferred from a wild species, *Oryza longistaminata* to different rice cultivars (Tu *et al*., 2000).Bacterial leaf streak (BLS). Caused by the pathogen *Xanthomonas oryzae* pv. *oryzicola*, the disease is less invasive and less popular than its infamous cousin, the bacterial leaf blight (BLB). BLS is more prevalent when it rains non-stop, especially during the monsoon season. BLS damage ranges from 10 to 20% when most of the leaves are infected. Phil Rice has developed BLS-resistant rice varieties (PSB Rc82 and PSB Rc18). The potential for callus induction and regeneration in rice tissue culture depends on a number of factors, such as genotype of donor plant, the type and physiological status of the explant, the composition and concentration of salt and, organic components ad plant growth regulator in the culture medium. So suitable explants, medium composition and concentration are needed for large scale utilization in Biotechnology for improvement. From the above discussion, the present research work was undertaken to establish an efficient *in vitro* plant regeneration protocol using with BRRIdhan-29, BR-14, and BINAdhan-8. The objective of this research program was:

1. To evaluate the response of genotype on callus induction and plant regeneration
2. To observe the effect of different growth regulator at different concentrations on callus induction and plant regeneration

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2 CITATION

[1] Sikder *et al*. (2008) find out the *in vitro* callus induction and organogenesis potential of Chiniguri variety of aromatic rice *(Oryza sativa* L.) collected from farmers of Khulna, Bangladesh. MS media supplemented with different concentrations of 2, 4- D (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg L-1) were used for callus induction from the mature dehusked rice seeds. Callus derived from the 2.0 mg L-1 2, 4-D showed the best results for plantlet regeneration.

[2] Shaziz *et al.* (2005) inoculated seeds of rice (Oryza sativa cv.Swat-II) onto Ms medium containing various concentrations of auxin s and cytokynins. Excellent callus resulted on MS containing 2, 4-D and Kn .Plantlet regeneration occurred on MS containing BAP at 0.5mg/l in combination with IAA at a concentration of 0.2mg/L.

[3] Amin *et al.(*2004) reported that *indica* rice varieties were cultured for callus induction, shoot regeneration and root induction and plantlet s were transferred to rooting media (MS supplemented with 0.2mg/L: IBA , pH 5.8) and kept under 10-14 light / dark regime until sufficient roots were formed.

[4] Wang *et al*. (2003) reported that a comparative effect was observed of indole 3- acetic acid (IAA) and indole 3-butyric acid (IBA) on root formation in IRA rice. Result showed that IBA at all concentration s (0.5-0.8) increased the number of root induction.

[5] Asaduzzaman *et al.*(2003) reported that five rice varieties (viz.BR-5, BR-31,BR-34, BR-37 and BR-38)were cultured for callus induction, plant regeneration and had MS medium supplemented with 2.0mg/L BAP +0.5mg/L Kn+ 1.0mg/L NAA had highest percentage (33.32%) of green plantlet regeneration from calli.

[6] Lee *et al* (2002) concluded on the effect of Kinetin was for shoot regeneration and found that the highest shoot regeneration frequencies when cytokinin was combine with concentration (2.0mg/L) of NAA.

[7] Rasid *et al.(*2001) studied callus induction and plant regeneration of rice *Oryza sativa cv.* super Basmati) and reported that in MS medium 2mg/L 2,4-D is best for callus induction and combination with 1mg/L NAA+0.55 mg/L BAP showed best plant regeneration frequency.

3 MATERIALS AND METHOD

To achieve the objectives, the experiments were conducted during the period from January, 2011 to June at the Laboratory of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh.

3.1 Source of the experimental materials

The rice (*Oryza sativa L*.) genotypes BRRI dhan-29, BR-14, BINA dhan-8 were obtained from Rice Research Institute (BRRI), Gazipur and Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh respectively. Here BRRI dhan-29, BR-14 which is partially resistant to bacterial laef blight diseases and BINA dhan-8 is salt resistant varieties.

Experimental explant

The healthy and dehusked seeds of three rice varieties were used as explants for the study.

Culture method

The following methods were applied during the course of culturing of different explants. Mature rice embryos were used as the main source of explants for callus induction and subsequently plants regeneration.

Mature embryos attached to endosperm were the main source of explants for embryo culture.

Sterilized matured seeds contain endosperm pert were cultured directly in MS medium supplemented with different combination of hormones and sucrose required as per treatment. The hormones and their respective number of combinations of embryo culture were as follows:

A For callus induction:

1. MS medium Supplemented with 1.5.0 mgl^{-1} 2, 4-D(T_1), 2.0 mgl^{-1} 2, 4-D (T_2), 2.5 mgl^{-1} 2, 4-D (T_3) and 3.0 mgl^{-1} 2, 4-D (T_4)

B. Regeneration media

a) for shoot differentiation: MS medium Supplemented with 0.5 mgl-1 NAA 4.0 mgl^{-1} Kn (T_1),0.5 mgl-1 NAA 6.0 mgl^{-1} Kn (T_2),0.5 mgl-1 NAA 8.0 mgl^{-1} Kn (T_3) and 0.5 mgl-1 NAA 10.0 mgl^{-1} Kn (T_4)

b) For root initiation: MS medium supplemented with 0.4mgl^{-1} IBA (T_1),0.5 mgl^{-1} IBA (T_2),0.6 mgl^{-1} IBA (T_3) and 0.7 mgl^{-1} IBA (T_4)

3.2 Methods

Preparation of culture media

A nutrient medium for callus induction and regeneration of transformed plantlet of rice usually consist of organic salts, irons, a carbon source, some vitamins and growth regulators. Based on the nature of culture, the composition of medium and the type of media differ considerably. In this study MS as basal medium for both callus induction and regeneration of plants from the callus was used. The composition of MS medium is given as below in table 1

Table 1. *Composition and concentration of MS (Murashige and Skoog, 1962) medium*

Components	Concentration (mg l^{-1})
Inorganic nutrients	
Macro-nutrients	
KNO_3	1900.00
NH_4NO_3	1650.00
KH_2PO_4	170.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
Micro nutrients	
$MnSO_4.4H_2O$	22.30
H_3BO_3	6.20
$ZnSO_4.H_2O$	8.60
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$COCl_2.6H_2O$	0.025
Iron	
$FeSO_4.7H_2O$	27.80
Na_2-EDTA	37.30
Organic nutrients	
Glycine	2.0
Nicotinic acid	0.50
Pyridoxine HCl	0.50
Thiamine HCl	0.10
Myo-Inositol	100.300
Sucrose	30000.00
Agar	8000.00

Different steps of media preparation are described below:

Preparation of stock solutions

The first requisite for media preparation was the preparation of stock solutions Stock solution of growth regulators were prepared separately by dissolving the desired quality of ingredient in appropriate solvent and the required final volume was made with distilled water for ready use to expedite the preparation of the medium wherever needed. Separate stock solution for macronutrient, micronutrient, iron, vitamin, growth regulators etc. were prepared and stored appropriately for use.

Stock solution A (macronutrients)

The stock solution of macronutrients was made up to 10 times (10x) the final strength of the medium in 1000 ml of distilled water. Ten timed the weight of salts required per liter of the medium were weight ed accurately and dissolved by using a magnetic stirrer in about 750 ml of distilled water and than final volume was made up to 1000 ml by further addition of distilled water. This stock solution was filtered and poured into a clean brown bole, labeled with marker and stored in a refrigerator at 4 ºC for later use.

Stock solution B (micro-nutrients)

This was made up to 100 folds (100x) the final strength of the medium in 1000 ml of distilled water (DW). The stock solution was filtered, labeled and stored in refrigerator at 4oc for later use.

Stock solution C (Iron source)

This was prepared at 10 folds (10x) the final strength of FeSO4 and Na2-EDTA in 100 ml of distilled water and chelted by heating on hater cum magnetic stirre.Then the volume was made up to 1000 ml by further addition of distilled water. Finally, the stock solution was filtered and stored in a refrigerator at 4 ºC for later use.

Stock solution D (Vitamins)

Each of the desired ingredients except myo- inositol were taken at 100 folds (100x) of their final strength in a measuring cylinder and dissolved in 750 ml distilled water. Their the final volume was made up to 1000ml by further addition of distilled water. The solution was dispensed into 10 ml aliquots and stored at -2 ºC. Myo-inositol was used directly at the time of media preparation.

Hormonal stock solution

Stock solution of hormones was prepare separately at 100 ppm dissolving the quantity of ingredient in appropriate solvent and the required volume was made with distilled water and stored in a refrigerator at 4 ºC for later use.
The following growth regulators (phytohormone supplements) were used in the present investigation.

A .Auxine

NAA (α- napthaleneacetic acid) and IBA (indolebuterric acid)

B. Cytokinins

BAP (6-benzyleamino purine) , 2,4-D (dichlrophenoxyacetic acid) (as cytokynin) and 6-Furfurryl amino purine (Kinetin)
The growth regulators were dissolved in appropriate solvent as shown below For the preparation of stock solution of the above mentioned growth regulators, 10 mg (concentrated) of each of the growth regulators was taken on a watch glass and then dissolved in 1 ml of the particular solvent. The mixture was then collected in a 100 ml measuring cylinder and the volume was made up to 100 ml by further addition of distilled water. The solution was then stored 0^0C for use up to 2 weeks.

Steps followed for the preparation of culture media

After the preparation of the stock solution the later step was the preparation of culture media. To prepare one liter of the above-mentioned media the following steps were followed:

Preparation of MS medium

To prepare one liter (1000ml) of MS medium, the following steps were followed: 100ml of macronutrients, 10ml of micronutrients, 100ml of irons and 10ml of vitamins were taken from each of this stock solution into a 2 liter Erlenmeyer flask on a heater cum magnetic stirrer.500ml distilled water was added in the flask to dissolve all the ingredients. 100mg of myo –inositol was added directly to the solution and dissolve well. 30gm of sucrose was added to this solution and agitated gently to dissolve completely. Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well. The whole mixture was then poured into a L measuring cylinder and made the volume up to 1000 ml with further addition of distilled water. pH of the medium was adjusted to 5.8 with a digital pH meter with the help of 0.1N NaOH or 0.1 N HCL, which ever was necessary. After adjustment the pH, 9 g agar was added to the solidify the medium. The mixture was then heated gently with continuous stirring till complete dissolution of agar. Required volume of hot medium was dispensed into culture vessels or conical flasks. After dispensing the medium the culture vessels were plugged with cork and/or non-absorbent cotton and marked with different codes with help of a glass marker to indicate specific hormonal combinations.

Sterilization

To ensure aseptic condition *in vitro*, all instruments, glassware and culture media were sterilized properly by autoclaving.

Sterilization of culture media

The culture vessels containing prepared media were autoclave at 1.16 $kgcm^{-2}$ pressure and 121 º C temperatures for 22 minutes. After autoclaving the testtubes containing the medium were allowed to cool as slants or vertical position whichever necessary.

Sterilization of glassware and instruments

Beakers, test tubes, conical flasks, pipettes, metal instruments viz, forceps, scalpels, needles, spatulas and aluminum foils were sterilized in an autoclaved at temperature of 12 ºC 1 for 20 minutes at 1.16 kg/ cm^2 pressure.

Sterilization of culture room and transfer area

The culture room was initially cleaned by gently washing all floors and walls with a detergent followed by wiping with 70% ethyl alcohol. The pressure of sterilization was repeated at regular intervals. Generally, switching on the cabinet with UV light and wiping the working with 70%ethyl alcohol sterilized laminar airflow cabinate.

Preparations to ensure aseptic condition

All inoculation and aseptic manipulations were carried out in a laminar air flow cabinate. The cabinet was switched on for at least half an hour before use and cleaned with absolute ethyl alcohol to overcome the surface contaminations. During the entire period of inoculation the autoclaved scalpels, forceps and inoculation loop were kept immerse ed into absolute alcohol contained in a glass jar inside the cabinate. At the time of inoculation these were again sterilized by flaming method inside the cabinet. Both the hands were rinsed with 70% alcohol. All measures were taken to obtain maximum contamination free condition during the surgical operation of the explants.

Sterilization of experimental materials (seed)
Mature seeds of three rice varieties like BRRI dhan-29, BR-14, BINAdhan-8 were dehusked manually with special care so that, the embryo remains intact. The surface sterilization of the dehusked seed was carried out in the aseptic conditions of the laminar air flow cabinate. The dehusked seeds were then washed thoroughly in distilled water. The floating seeds were discarded later the seeds were dipped in 70% ethyl alcohol for three minutes with vigorous shaking followed by washing with sterile distilled water. Surface disinfection's was done by the use of the 70% ethanol for three minutes, rinsed with sterile water then sterilized again with 0.1% HgCL2 with one drop of Tween-20 for fifteen minutes. During this period, the sterilant was occasionally agitated, and subsequently would be toxic to the seed materials if kept for long duration. The surface sterilized seeds were then kept on a sterilized Petridish containing sterile filter paper to soak the excess water droplets.

Culture techniques were employed in the present investigation-
i) Explant culture ii) Subculture or transfer into regeneration medium iii) Rooting

Explant culture
Mature rice grain embryos attached to endosperm were the main source of explants for embryo culture. Sterilized mature seeds were cultured directly in MS medium supplemented with different concentrations of hormone and sucrose required as per treatment. The culture plates containing explants were placed under fluorescent light in a room with controlled temperature 22 $\pm^0$C using 16 hrs photoperiod. The test tubes were checked daily to note the response and the development of contamination. Callus were initiated three days after three days after inoculation and after two weeks inoculated explants were transferred onto fresh medium.

Subculture or transfer in regeneration media
Three weeks after inoculation of explants, the calli attained convenient size. The sub culturing media used in present investigation were MS containing different combinations of NAA and kinetin. The subcultures Petridis's were again incubating d at 22$\pm$ ^{0}C with 16 hrs photoperiod. After shooting initiation, more light intensity was used for shoot elongation. The culture vessels showing signs of contamination were discarded. Repeated subcultures were done at and interval of 15 days and incubated under the same temperature as mentioned previously for maintenance of calli and organogenesis.

Rooting
The sub cultured calli continued to proliferated and differentiated into shoots. When these shoots grew about 2-3 cm in length. The test tube containing plantlets were incubated at 22 $\pm$ ^{0}C with 16 hrs photoperiod. Day to day observation s was carried out to note the responses.

3.3 Recording of data
To investigate the effect of different treatments of the experiment, data were collected on the following parameters.

Callus Induction

Days of callus initiation
Generally callus initiation started after six days of induction of explants. The number of callus proliferated over a number of days was recorded. The mean value of the data provided the days required for callus initiation.

3.4 Percent callus induction
Percentages of callus induction were noted after 14to 21 days of inoculation by using following formula:

$$\text{Percent callus regeneration} = \frac{\text{Number of explants induced calli}}{\text{Number of explant inoculatex plant}} \times 100$$

Size of callus
After three weeks of incubation, the size was measured in millimeters (mm)

Weight of callus
After three weeks of incubation, the weight was measured in millimeters (mg)

3.5 Percent shoot regeneration
The percent of plants which were regenerated from embryo derived calli was calculated as follows:

$$\text{Percent plantlet regeneration} = \frac{\text{Number of calli with plantlets}}{\text{Number of inoculated calli}} \times 100$$

Days to shoot initiation
Shoot initiation started after 15-29 days of incubation of explants. The number of shoots proliferated over a number of days were recorded. The mean value of the data provided the days required for shoot initiation.

Average number of shoots per callus
Number of shoots/plant was recorded at weekly interval and the mean was calculated the following formula:

$$\bar{x} = \frac{\sum xi}{n}$$

where

$\bar{X}$ = Mean number of shoots / plant
$\sum$= Summation
Xi= Number of shoots / plant
N= Number of observations

Shoot length
After three weeks of callus incubation, in shoot medium the shoot length was measured in centimeters (cm)

Percent root initiation
Percent of root initiation was noted after 10-21 days of inoculation by using the following formula:

$$\text{Percent root regeneration} = \frac{\text{Number of roots initiation}}{\text{Number of explants inoculated}} \times 100$$

Average number of root per callus

Number of root/plant was recorded at weekly interval and the mean was calculated the following form

$$\bar{x} = \frac{\sum xi}{n}$$

where

X = Mean number of root / plant
∑= Summation
Xi= Number of root / plant
N= Number of observations

Average number of shoots per callus

Number of root/plant was recorded at weekly interval and the mean was calculated the following formula:

Root length

After two weeks of plantlet incubation, in root medium the root length was measured in centimeters (cm)

Statistical analysis of data

The data for the parameters under present experiments were statistically analyzed wherever applicable. The experiments were conducted with Completely Randomized Design in growth room (Tissue Culture Laboratory).The analysis of variances for different parameters were performed and means were compared by the Duncan's multiple range test (DMRT).

4 RESULTS AND DISCUSSION

In the present investigation, the technique for the plantlet regeneration in vitro through callus induction, shoot regeneration and rooting of the shoots have been established by using mature embryos as explant of two BRRI rice genotypes namely BRRI dhan-29, BR-14 and one BINA rice genotype namely BINA dhan-8.

4.1 Callus induction

To observed the callus forming ability of mature embryos of three (BRRI dhan-29, BR-14 and BINA dhan-8) rice varieties, the mature embryo explants were cultured on MS medium supplemented with 0.0, 1.5, 2.0, 2.5,3 mgl^{-1} 2, 4-D. Calli were formed in the plated embryos both the BRRI and BINA rice varieties on media containing different concentrations of 2, 4-D . Callus induction performances of theses varieties were evaluated and compared. The results of the statistical analysis are presented in the tables and Figures.

Analysis of variance for different parameters of calli

Among the three cultivars studied, the efficiency of callus induction was investigated through four qualitative trait such as percent callus induction ,size of callus, Weight of callus and days to callus initiation .The results of the analysis of variance (mean square) for percent callus induction, size of callus, weight of callus, days to initiation, effect of varieties, effect of different concentration and combination of phyto hormone on those characters are summarized appendix-1.

Table 2 . *Combined effect of variety and treatment on callus characters*

Variety	2,4-D (mg/l)	Callus Size (mm)	Callus Weight (mg)	Days to Callus
BRRI dhan-29	0	0.000f	0.000m	0.000i
	1.5	3.975bcd	80.525d	16.000de
	2	4.487abc	76.743g	13.750 a
	2.5	4.087bcd	83.025b	14.750 cd
	3	3.150c	79.013e	18.500 e
BR-14	0	0.000f	0.000M	0.000i
	1.5	3.710cde	82.005c	16.500 f
	2	5.143a	58.522L	6.500 cd
	2.5	4.028bcd	87.507A	11.750 bc
	3	3.577de	78.495f	17.750 f
BINA dhan-8	0	0.000f	0.000M	0.000i
	1.5	3.877bcde	71.225K	14.000g
	2	4.620ab	75.007i	10.750 ab
	2.5	4.430abc	75.507h	9.250 e
	3	3.413de	74.003j	15.750 h
LSD		0.723	0.045	1.412

Figures followed by same letter (s) in column did not statistically significant.

4.2 Shoot regeneration from embryonic callus

For shoot induction calli of three genotypes of *indica* rice were cultured on MS medium supplemented with different concentrations of Kinetin (4/6/8/10 mg L^{-1}) with constant concentration of NAA (0.5 mg L^{-}) (plate.3). Number of explants incubated was 4 per treatment. The responses of calli to different concentrations of Kinetin towards shoot induction are presented in table-3

Table 3. *Combined effect of variety and Treatment on shoot character*

Variety	NAA (mgL^{-1}) + Kn (mgL^{-1})	Shoot Regeneration (%)	No. of Shoot	Days to Shoot	Shoot Length (cm)
BRRI dhan-29	0	0.000e	0.000f	0.000f	0.000h
	0.5+4	25.100d	3.750c	30.250a	3.550g
	0.5+6	73.750b	5.250a	25.250d	5.100c
	0.5+8	97.500a	4.750ab	22.250e	6.000a
	0.5+10	48.750c	3.250cd	27.000c	4.150e
BR-14	0	0.000e	0.000f	0.000f	0.000h
	0.5+4	25.200d	3.750c	29.000ab	3.525g
	0.5+6	51.250c	4.750ab	23.250e	4.625d
	0.5+8	75.000b	3.750c	25.000d	5.050c
	0.5+10	25.000d	2.750de	29.250ab	3.825f
BINA dhan-8	0	0.000e	0.000f	0.000f	0.000h
	0.5+4	25.000d	3.750c	30.250a	3.538g
	0.5+6	48.750c	4.500b	25.000d	5.000c
	0.5+8	75.000b	2.750de	28.250b	5.575b
	0.5+10	73.750b	2.500e	29.250ab	4.500d
LSD					

Figures followed by same letter (s) in column did not statistically significant.

4.4 Root induction

Next to shoot regeneration, the formation of root is also important for *in vitro* development of free living plantlets. In the present study, the shoots regenerated from these experiments were transferred to the root induction medium (plate.4). In order to induce root the differentiated shoots were transferred to MS medium supplemented with different concentration of IBA. The shoot produced roots on these media but vary for percent root induction from shoot, number of roots $plant^{-1}$, and root length. The result was summarized in the Table-4.

Table 4. *Combined effect of variety and Treatment on shoot character.*

Variety	IBA (mgL^{-1})	Root (%)	No. of Root	Root Length (cm)
BRRI dhan-29	0	0.000f	0.000g	0.000k
	0.4	25.000e	3.750de	1.312i
	0.5	75.00.750b	5.500b	1.912e
	0.6	48.750cd	7.250*	2.215c
	0.7	23.750e	3.500e	1.713g
BR-14	0	0.000f	0.000g	0.000k
	0.4	0.000f	3.750d	1.512h
	0.5	97.500a	4.250cd	1.905e
	0.6	45.000d	4.500c	2.112d
	0.7	23.850e	2.500f	1.810f
BINA dhan-8	0	0.000f	0.000g	0.000k
	0.4	26.250e	3.500de	1.810f
	0.5	75.000b	4.750c	2.317b
	0.6	51.250c	5.500b	2.510a
	0.7	26.250e	3.250e	1.910j
LSD				

Figures followed by same letter (s) in column did not statistically significant

Analysis of variance for root induction

The results of analysis of variance (mean square value) for number shoot producing root and number of root $plant^{-1}$ and effects of different varieties and different concentration of IBA on this characters were summarized in Appendix-4. It was observed that there were significant variations among the cultivars for number of shoots producing roots. The media supplemented with IBA different concentrations significantly affected by the cultivars. There were significant variations for the interaction of cultivars and media composition for percent root formation. From the analysis of variance it is revealed that, both the cultivars and media composition conspicuously affected the root formation and their interaction also affected the root formation and the parameter under study of root formation.

4.5 Effects of Variety X treatment interactions on root induction

An effect of variety ^{x} treatment interaction on root induction was found significant for number of root/plant and number of shoot producing root. The results are presented in Table-4.

Highest percent root induction (97.50%) on T_2 (MS + 0.5 mgL^{-1} IBA) with varieties BR-14 followed by (75.00%) on T_2 (MS + 0.5 mgL^{-1}IBA) with variety BINA dhan-8 and lowest percent root induction (23.75%) on T_4 (MS + 0.7 mgL^{-1} IBA) with varieties BRRI dhan-29. Highest number of root $plant^{-1}$ (7.25) was observed on T_3 (MS + 0.6 mgL^{-1} IBA) in variety of BRRIdhan-29 and lowest (2.50) on T_4 (MS + 0.7 mgL^{-1} IBA) in BINAdhan-8. Highest root length (2.31cm) was observed on T_3 (MS + 0.6 mgL^{-1} IBA) in variety of BINA dhan -8 and lowest (1.51cm) on T_1 (MS + 0.4 mgL^{-1} IBA) in BRRI dhan-29.

4.3 Effects of variety x treatment interactions on shoot regeneration

Variety *x* treatment interactions on shoot regeneration were found statistically significant for percent shoot,

number of shoot days to shoot regeneration and shoot length from callus' indicating significant differences among the interactions. The results are presented in (Table-3).
Out of three varieties BRRI dhan-29 was showed the highest (97.5%) shoots regeneration in 22.25 days on T_3 (MS + 8 mgL⁻' Kn + 0.5 mgL⁻' NAA) followed by BR-14 and BINA dhan-8 (75%) in 25.00 days on T_2 (MS + 6 mgL" Kn + 0.5 mgL⁻' NAA). It was observed that BINA dhan-8 showed lowest shoot regeneration (25.00) in 25 days on T_1 (MS + 4mgL⁻' Kn + 0.5 mgL⁻' NAA) This finding was similar to obtained by Visarada and Sharma (2002) result. In three varieties BRRIdhan-29, BR-14 and BINAdhan-8 showed maximum number of shoot callus⁻' 5.25, 4.70, 4.50 respectively in T_2 (MS + 6 mgL⁻' Kn + 0.5 mgL⁻' NAA). The minimum number of shoot callus-' was found in variety BINAdhan-8 in T_4 (MS + 10 mgL^{-1} Kn + 0.5 mgL⁻'NAA) 2.50. Maximum shoot length was recorded BRRIdhan-29, BR-14 and BINAdhan-8 showed maximum number of shoot callus⁻' 6.00, 5.05and 5.00 cm respectively in T_3 (MS + 8 mgL⁻' Kn + 0.5 mgL⁻' NAA). The minimum number of shoot callus-' was found in variety BR-14 in T_1 (MS + 4 mgL^{-1} Kn + 0.5 mgL^{-1} NAA) 3.52 cm.

5 CONCLUSION

Experiment revealed a wide range of variation in callus induction ability as influenced by the genotypes in different hormonal concentration. The range of callus induction was 58.80% to 60.65%. The variety BRRI dhan-29 shoed maximum callus induction in 12.5 days from explant. The lowest callus induction was observed in BINA dhan-6 in 9.10 days. Concentrations 2.0mgL^{-1} 2, 4-D was best concentrations for callus induction (85%), callus size (4.533mm) and callus weight (81.847mg) in 10.66days and the highest maintaining ability was found in BR-14 at 2.0mgL^{-1} 2, 4-D (88%) in callus size (5.143a) and callus weight (87.50mg)) in 6.50days. BRRI dhan-29 on T_3 (MS + 8 mgL⁻' Kn + 0.5 mgL⁻' NAA) was found to be best in shoot length (6.00cm) with highest shoots initiation (97.5%) and the highest .number of shoots per callus (5.25) on T_2 (MS + 6 mgL⁻' Kn + 0.5 mgL⁻' NAA) in 22.25days. In combination, BRRI dhan-29 the highest number of roots per explant (7.25), root length (2.31cm) was found in T_3 (MS + 0.6 mgL^{-1} IBA) and the highest root initiation (97.50%) was on T_2 (MS + 0.5 mgL^{-1} IBA).

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