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Effect of Culture Media on Seed Dormancy and Callus Induction Ability of Some Wild and Cultivated Rice Genotypes

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Abstract: Seed dormancy status was evaluated using agar, MS media and DKW media with or without sugar (3%) in two wild (Jhora 4325 and Wild 4855) and three cultivated (Hab. Aman 2, Jagliboro and Dular) rice genotypes. Jhora 4325 showed strong seed dormancy than the wild 4855 and cultivated rice genotypes. Embryo germination percentage in the studied rice genotypes was higher in agar and MS media than DKW media. Number and percent explants with induced callus was comparatively higher in MS media in combination with 2, 4-D (1.5 mg L^{-1} 2, 4-D) and BAP (0.5 mg L^{-1}) than MS media supplemented with 2, 4-D alone. Embryogenic callus initiation capability of wild rice genotypes was higher than the cultivated genotypes. Embryo germination and callus induction were influenced by concentration of plant growth regulators, culture media and rice genotypes.

Key words: Rice (*Oryza sativa* L.), seed dormancy, plant growth regulators, culture media, callus induction

INTRODUCTION

In crop improvement program of cereal like rice, the production of hybrid generation is obvious importance to investigate the inheritance pattern of different qualitative and quantitative characters for selection in advance generation. Hybridization program involving wild and cultivated rice result interspecific barriers like dormancy of seed, embryo abortion, failure of complete plant production and low seed production (Mariam *et al*., 1996). Tissue culture techniques have become necessary for germplasm recovery and overcoming the interspecific barrier. Rice plants have been regenerated through somatic embryogenesis from mature seeds (Rueb *et al*., 1994), but Indica type of rice is less amenable to culture among the cereals. Information on somatic embryogenesis and callus production ability of wild and cultivated rice genotypes of Bangladesh has not reported much. *In vitro* approaches to rice improvement, germplasm recovery and phylogenetic study require an efficient regenerable protocol particularly in wild and cultivated rice genotypes. More over mature seeds may not germinate always in culture media because wild and some cultivated (traditional) rice possess seed dormancy. It is important to develop efficient protocol for breaking seed dormancy and plant regeneration from mature seed cultures in rice. In the present investigation the effect of media and growth regulators on breaking seed dormancy and callus induction ability in some selected rice genotypes representing wild and cultivated (Traditional) were studied.

MATERIALS AND METHODS

Seeds of five rice genotypes viz., Jhora 4325, Wild 4855, Dular, Jagliboro and Hab. Aman 2 were collected from GRS-BRRI. The experiment was carried out at the tissue culture laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh in 2002. Dehusked seeds were surface sterilized using 0.1% $HgCl_2$ solution for 10-20 min and washed 4-5 times repeatedly. Agar (no sucrose), MS medium (Murashige and Skoog, 1962) (with 3% sucrose) and DKW medium (Driver and Kuniyuki, 1984) (with 3% sucrose) were used for embryo germination. Five seeds per petridish were placed with 4 replications for embryo germination and call us induction. MS medium was supplemented with 1, 1.5 and 2 mg L^{-1} of 2, 4-D alone and with BAP 0.5 mg L^{-1} and used for callus induction. Medium contained 0.6% Agar, 3.0 % sucrose and pH was adjusted to 5.8. Cultures were incubated at 25±2°C, 65-70% RH and 16/8 h L/D cycle with approx. 2000-3000 lux of light intensity. Germination was observed after 5 days. Data were collected on number of embryo germinated and No. of explants showing callus induction. Embryo germination percent and percent explants

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showing callus induction were calculated. Analyses were made using the model CRD factorial experiments with various factor and interaction effects.

RESULTS AND DISCUSSION

Seed dormancy status and callus induction ability of 2 wild and 3 cultivated rice genotypes (*Oryza sativa* L.) was studied in tissue culture media to facilitate the protocol which can be used to recover germplasm and hybrid plants after hybridization. Embryo germination test was carried out using dehulled mature seeds of five rice genotypes viz., Jhora 4325, Wild 4855, Hab. Aman 2, Jagliboro and Dular in 3 kind of germination medium (Table 1). Embryo germination percentage ranged from 10 to 55% in agar, 50 to 95% in MS medium and 20 to 70% in DKW medium. In sugar free agar medium highest germination percentage was found in Jagliboro (55%) followed by Dular (50%), Hab. Aman 2 (25%), Wild 4855 (20%) and lowest in Jhora 4325 (10%). On the other hand in MS medium (3% sugar) highest germination percentage was observed in Jagliboro (95%) followed by Dular (90%), Hab. Aman 2 (80%), Wild 4855 (60%) and lowest was observed in Jhora 4325 (50%). In DKW media with 3% sugar embryo germination percentage was also found highest in Jagliboro (70%) followed by Hab. Aman 2 (75%), Dular (90%), Wild 4855 (35%) and lowest in Jhora 4325 (20%). No dormancy was observed for Hab. Aman 2, Jagliboro and Dular. Jhora 4325 was more dormant than Wild 4855. The results of embryo germination percentage indicated that rice genotype with dormancy in seed showed low response to culture media. The trend of germination percentage in Jhora 4325 remains same in all medium followed by Wild 4855. Comparatively the germination percentage was low in DKW medium. In agar medium (0% sugar) the germination percentage was 10 % and did not exceed 60% whereas the lowest germination percentage was almost 50% and seed dormancy was low in MS medium when 3% sugar was added, which agreed with the earlier reports by Takahashi (1963).

Table 1: Embryo germination percent of seeds of 2 wild and 3 cultivated rice genotypes in different culture media

Genotypes	Medium (Sucrose concentration %)	No. of Embryo germinated	Embryo germination (%)
Jhora 4325	Agar (0)	02	10
Wild 4855	Agar (0)	04	20
Dular	Agar (0)	10	50
Hab. Aman 2	Agar (0)	05	25
Jagliboro	Agar (0)	11	55
Jhora 4325	MS (3)	10	50
Wild 4855	MS (3)	13	65
Dular	MS (3)	18	90
Hab. Aman 2	MS (3)	16	80
Jagliboro	MS (3)	19	95
Jhora 4325	DKW (3)	04	20
Wild 4855	DKW (3)	07	35
Dular	DKW (3)	10	50
Hab. Aman 2	DKW (3)	13	65
Jagliboro	DKW (3)	14	70

The influence of different concentration of 2, 4-D (1.0, 1.5 and 2.0 mg L^{-1}) alone and in combination with 0.5 mg L^{-1} BAP were recorded (Table 3). Mean square due to genotype, different concentrations of 2, 4-D alone and in combination with BAP were significant indicated the presence of variation in the treatments (Table 2). Genotypic ability for production of percent explants with callus was found highest in Wild 4855 (38.33) that closely followed by Jhora 4325, Dular, Hab. Aman 2. Lowest percent of explants with callus was produced in Jagliboro (18.33). Higher percentage of explants showed callus induction in 2.0 mg L^{-1} 2, 4-D and lower in 1.0 mg L^{-1}. After 3-4 weeks of explants placement, increasing level of 2, 4-D was found to influential for higher callus induction. 2, 4-D concentration 2.0 mg L^{-1} and Wild 4855 interaction produced highest percent of explants with callus. On the other hand lowest percent of explants with callus was produced in 1.0 mg L^{-1} 2, 4-D concentration in interaction with Dular and Jagliboro. In most of the earlier reports, it was observed that for callus induction in rice different concentrations of 2, 4-D was used in MS medium. In the present study 2.0 mg L^{-1} 2, 4-D was found suitable for callus induction from mature embryo in all the genotypes except Hab. Aman 2. The present study showed similarity with the findings of Yamada *et al.* (1986), Brisibe *et al.* (1990) and Medina *et al.* (2004).

Among the 3 combinations, 0.5 mg L^{-1} BAP with 1.5 mg L^{-1} 2, 4-D produced highest percent of explants with callus. Other two combinations produced lowest percent of explants with callus (Table 3). Highest percent of explants showed callus induction in Dular (68.33%) and

Table 2: Analysis of variance for callus induction from mature embryos of 2 wild and 3 cultivated rice genotypes in MS medium with different concentration of 2,4-D (1,1.5 and 2 mg L^{-1}) and BAP (0.5 mg L^{-1})

	No. of explants showed callus induction		(%) Explants showed callus induction	
Sources of variation	2, 4-D	2, 4-D and BAP	2, 4-D	2, 4-D and BAP
Genotype (With 4 df)	25.856**	90.300**	667.50**	2257.50**
Treatment (With 2 df)	20.822**	50.40**	555.00**	1260.00**
Genotype × Treatment (With 8 df)	13.906**	15.90**	348.75**	397.50**
Error	0.889	1.20	21.66	30.00

**Significant at 1% level of probability

Table 3: Effect of different concentrations of 2, 4-D (1, 1.5 and 2 mg L^{-1}) and BAP (0.5 m gL^{-1}) in MS medium on callus induction from mature embryos of 2 wild and 3 cultivated rice genotypes

	No. of Explants showed callus induction		(%) Explants showed callus induction	
Effects	2, 4-D	2, 4-D and BAP	2, 4-D	2, 4-D and BAP
Genotypes				
Wild 4855	7.66	11.67	38.33	58.33
Jhora 4325	6.66	7	33.33	35.0
Dular	5.0	13.67	25.0	68.33
Hab. Aman 2	4.0	12	20.0	60.0
Jagliboro	3.77	6.67	18.33	33.33
Treatment (2, 4-D)				
1.0	4.067	9.80	20	49
1.5	6	12.20	30	61
2.0	6.20	8.60	31	43
Genotype×Treatment				
Wild 4855×1.0	5.0	11	25	55
Wild 4855×1.5	8.0	13	40	65
Wild 4855×2.0	10.0	11	50	55
Jhora 4325×1.0	7.0	6.0	35	30
Jhora 4325×1.5	4.0	8.0	20	40
Jhora 4325×2.0	9.0	7.0	45	35
Dular×1.0	2.0	16	10	80
Dular×1.5	7.0	15	35	75
Dular×2.0	6.0	10	30	50
Hab. Aman 2×1.0	4.0	13	20	65
Hab. Aman 2×1.5	6.0	15	30	75
Hab. Aman 2×2.0	2.0	8.0	10	40
Jagliboro×1.0	2.33	3.0	10	15
Jagliboro×1.5	5.0	10	25	50
Jagliboro×2.0	4.0	7.0	20	35

lowest in Jagliboro (33.33%). Genotype, auxin and cytokinin interaction viz., Dular×1.0 mg L^{-1} 2, 4-D and BAP (0.5 mg L^{-1}), Dular×1.5 mg L^{-1} 2, 4-D and BAP (0.5 mg L^{-1}) and Hab. Aman 2×1.5 mg L^{-1} and BAP (0.5 mg L^{-1}) were found to be highest respectively for percent explants with callus. Jagliboro×1.0 mg L^{-1} 2, 4-D and BAP (0.5 mg L^{-1}) gave lowest percent of explant with induced callus. From these results it is evident that 1.0 and 2.0 mg L^{-1} 2, 4-D in combination with BAP (0.5 mg L^{-1}) was not suitable for good callus induction. Percent explants with induced callus was higher after addition of BAP than the percent explants found with callus in 2, 4-D alone in MS media. These findings showed similarities with the findings of Abe and Futsuhara (1984) and Shamsul *et al*. (2001). In earlier report addition of one or more growth regulators was found to stimulate callus induction. Genotypes in the present study also showed variation in callus induction. This indicates the genotypes possess variability in their capability of regeneration. Guo and Cao (1982), Yoshida *et al*. (1983) and Abe and Futsuhara (1984) also reported the varying role of genotype in callus induction.

In the present study, after adding 3% sugar in the MS media for breaking seed dormancy in rice gave better result than the culture media without sugar. Two wild rice genotypes showed better callus production ability than the cultivated genotypes. MS media supplemented with 2, 4-D in combination with BAP induced more callus. In conclusion, it is possible to break seed dormancy using tissue culture media and MS media supplemented with 0.5 mg L^{-1} BAP and 1.5 mg L^{-1} 2, 4-D suitable for propagation of wild and cultivated rice genotypes from mature seed derived callus.

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