

STUDY ON EFFECT OF GENOTYPE AND CULTURE MEDIUM ON CALLUS FORMATION AND PLANT REGENERATION IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

A study on the effect of genotype and culture medium on callus induced in seed explants of ten rice cultivars indicated significant variances due to genotypes, media and genotype x medium interactions (except callus induction frequency) for eight characters related to callusing and plant regeneration. Among the genotypes, Pusa Basmati 1 gave the best overall callusing response. For plantlet regeneration, variety Pant Dhan 4 recorded the highest number of green spots and shoots while Sarju 52 had the highest potential for the length of longest shoot and rooting. Likewise the MS medium supplemented with 2.0 mg l⁻¹, 2,4-D for callusing and 2.0 mg l⁻¹ IAA plus 3.0 mg l⁻¹ KN (MS (3)) for shooting while 3.0 mg l⁻¹ IAA plus 4.0 mg l⁻¹ KN (MS (4)) for rooting gave the best results. Some of the selected combinations like Pant Dhan 4 on MS medium supplemented with 2.0 mg l⁻¹ 2,4-D plus 1.0 mg l⁻¹ KN displayed excellent callusing capacity while Pant Dhan 4 on MS (3) and MS (4), Sarju 52 on MS (4) and Pusa Basmati 1 4.0 mg l⁻¹ IAA plus 4.0 mg l⁻¹ KN (MS (5)) exhibited best regeneration capacity, and therefore, offer good scope for high totipotency using appropriate genotype and media with proper combination of hormones.

Key words: Rice, *Oryza sativa* L., callus growth, plant regeneration, tissue culture.

Somaclonal variation is an important source of variability in well adapted genotypes without resorting to hybridization. Useful somaclonal variants have been isolated in few instances but they have not been used efficiently by breeders due to low frequency of plant regeneration from long term cultures of various genotypes of interest to them. Therefore, an attempt has been made in the present investigation to study the effects of both genotype and media on callus growth and plant regeneration in ten genotypes of rice. All the genotypes used in the present study are well adapted cultivars.

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MATERIALS AND METHODS

Ten genotypes of rice comprising four basmati and six nonbasmati types (Table 2) were used. Healthy mature seeds were dehusked and used as experimental material. The seeds were sterilized in 70% alcohol for 30 sec., followed by treatments with 0.05% HgCl_2 for 1.5–2.0 min. Seeds were then washed and treated with 1% KCl to remove Hg^{++} ions from the surface. The sterilized seeds were treated with 1% NaOCl for 5–7 min. to ensure complete sterilisation and finally washed 3–4 times in sterilised distilled water.

CALLUS INDUCTION

The calli were induced from explants (dehusked seed) and cultured on MS medium supplemented with five concentrations of 2,4-D: 1.0 (MS(A)), 2.0 (MS(B)), 3.0 (MS(C)), 4.0 (MS(D)) and 5.0 mg l^{-1} (MS(E)). They were kept at 26°C and 70% RH in the culture room. To maintain growth and viability of callus for longer periods, the calli formed from seed explants were isolated from the original and further subcultured at 26°C for 40 days on similar medium. Observations were recorded 40 days after subculturing.

Callus induction frequency (CIF). The CIF was measured as the percentage of explants showing callus formation to the number of explants inoculated on the medium.

Callus diameter. Cross-wise breadth of each callus was measured and averaged for individual genotype/medium.

Apparent callus volume. It was obtained from multiplication of length, breadth and height of the callus measured from outside the flask with the help of a measuring scale.

Callus weight. Recorded as the difference between the weight of the flask with callus plus medium before and after subculturing.

PLANT REGENERATION

Healthy looking calli initiated from seed explants were put on regeneration medium which was the MS medium with IAA and KN added at the concentrations of 0.0 and 1.0 (MS(1)), 1.0 and 2.0 (MS(2)), 2.0 and 3.0 (MS(3)), 3.0 and 4.0 (MS(4)) and 4.0 and 5.0 mg l^{-1} (MS(5)), respectively. Observations on all the characters except number of green spots were recorded 40 days after the calli was transferred to the regeneration medium. Observations on number of green spots were recorded at 15 days after transfer.

STATISTICAL ANALYSIS

The data on all the measurable characters were statistically analysed for a 2 factorial experiment in randomized complete block design [1]. Critical difference for comparison among genotypes, media and interaction effects and the coefficient of variation were calculated by the standard formulae [1].

RESULTS AND DISCUSSION

Analysis of variance for all the characters (Table 1) indicated that the mean squares due to treatments were highly significant. Further, mean squares due to its components, viz. genotypes media and genotype x medium were also significant for all the characters except the callus induction frequency. This indicates significant differences between genotypes, media and genotype x medium interactions. For callus induction frequency, mean squares due to media and genotype were significant at 1 and 5% levels of significance, respectively, while interaction mean squares were nonsignificant.

Table 1. Analysis of variance (MSS) for callus, shoot and root characteristics in rice

Source	d.f.	Callus induction frequency	Callus diameter	Callus volume	Callus weight	Number of green spots	Number of shoots	Length of longest shoot	Number of root fibrils
Replications	4	1652.4	0.19**	0.05**	10042.0*	0.4	1.8	0.3	1.1
Treatments	49	2545.05	0.68	0.28	74476.0	153.3	123.4	34.0	421.8
Genotypes	9	1976.4*	0.23**	0.10**	53702.7*	61.9**	39.4**	8.4**	218.0**
Media	4	18905.4**	6.38**	2.38**	664148.2**	1143.8**	918.6**	275.9**	3622.5**
Genotypes x media	36	869.4	0.16**	0.09**	14150.2**	66.1**	56.0**	13.5**	117.0**
Error	196	1007.7	0.04	0.01	3916.7	0.7	1.6	0.4	3.1
Total	249								
CV (%)		39.7	26.1	18.6	19.7	9.4	20.3	17.3	18.5

*Significant at 5% and 1% levels, respectively.

The mean effects of genotypes, media and genotype x medium interaction for various characters related to callus and plantlet regeneration (Table 2) revealed large genotypic variation in callus formation and plant regeneration among the genotypes. Relative performance of genotypes indicated that most of the Basmati genotypes like Kasturi, Pusa Basmati 1, and Basmati 370 exhibited very high potential for all the callusing characters with the respective values of 96%, 94% and 84% for callus induction frequency, 265.8 mg, 248.2 mg and 223.8 mg for callus weight, 0.65 cm, 0.77 cm and 0.70 cm for the diameter of callus and 0.33 cm³, 0.37 cm³ and 0.35 cm³ for callus volume. These genotypes were usually, at par with each other but significantly superior to IR 36 and Haryana Basmati 1 genotypes. Pant Dhan 4 recorded moderate values for all these characters. For plantlet regeneration characters, generally the nonbasmati indicas give the best response. Pant Dhan 4 showed

Table 2. Effect of genotype and medium on callus growth and plant regeneration characteristics in rice

Medium	Bas- mati 370	Pusa Bas- mati 1	Haryana Bas- mati 1	Kas- turi	Sarju 52	IR 36	IR 72	Gov- ind	Pant Dhan 4	Pant Dhan 10	Mean (\bar{X})
1. Callusing frequency (%)											
MS(A)	100	100	60	100	80	100	100	80	60	80	86
MS(B)	100	100	80	100	100	100	100	100	100	100	98
MS(C)	100	100	100	100	100	60	80	100	100	100	94
MS(D)	100	199	60	100	60	40	80	60	100	60	76
MS(E)	20	70	40	80	40	20	40	40	60	40	44
\bar{X}	84	94	68	96	76	64	80	76	84	76	
CD (genotype) at 5%						12.4					
CD (medium) at 5%						17.6					
CD (genotype x medium) at 5%						39.4					
2. Diameter of callus											
MS(A)	0.72	0.60	0.52	0.66	0.66	0.60	0.66	0.46	0.44	0.48	0.58
MS(B)	0.98	0.82	0.66	0.84	1.18	1.02	1.16	1.16	1.34	1.24	1.04
MS(C)	1.16	1.26	1.02	0.94	0.76	0.32	0.64	0.88	0.86	0.84	0.87
MS(D)	0.60	0.88	0.30	0.64	0.36	0.10	0.50	0.38	0.40	0.46	0.46
MS(E)	0.06	0.28	0.08	0.18	0.10	0.04	0.10	0.16	0.10	0.16	0.13
\bar{X}	0.70	0.77	0.52	0.65	0.61	0.42	0.61	0.61	0.63	0.64	
CD (genotype) at 5%						0.10					
CD (medium) at 5%						0.07					
CD (genotype x medium) at 5%						0.21					
3. Callus volume (cm³)											
MS(A)	0.32	0.25	0.27	0.27	0.32	0.22	0.18	0.16	0.20	0.17	0.24
MS(B)	0.52	0.41	0.23	0.43	0.80	0.43	0.71	0.66	0.83	0.64	0.57
MS(C)	0.66	0.66	0.62	0.65	0.35	0.08	0.31	0.34	0.45	0.42	0.45
MS(D)	0.22	0.44	0.10	0.25	0.14	0.01	0.19	0.10	0.10	0.21	0.18
MS(E)	0.01	0.08	0.06	0.06	0.01	0.01	0.01	0.04	0.01	0.03	0.02
\bar{X}	0.35	0.37	0.25	0.33	0.32	0.15	0.28	0.26	0.32	0.29	
CD (genotype) at 5%						0.06					
CD (medium) at 5%						0.04					
CD (genotype x medium) at 5%						0.12					
4. Callus weight (mg)											
MS(A)	218.8	222.2	108.8	262.4	174.4	164.8	189.0	117.2	178.6	167.2	180.3
MS(B)	259.8	322.6	250.7	359.4	342.0	252.8	338.6	360.2	420.0	361.2	326.7
MS(C)	353.4	397.0	343.6	416.2	159.8	69.6	242.6	376.6	358.8	316.8	303.4
MS(D)	266.0	197.8	93.2	213.6	123.0	36.6	209.0	120.2	118.8	143.2	152.5
MS(E)	20.8	101.4	25.0	77.0	40.6	6.0	40.6	27.6	41.8	25.8	40.6
\bar{X}	223.8	248.2	164.3	265.8	184.0	106.0	204.0	200.4	223.6	202.9	
CD (genotype) at 5%						34.7					
CD (medium) at 5%						24.5					
CD (genotype x medium) at 5%						77.6					

(Contd.)

Table 2. (contd.)

Medium	Bas- mati 370	Pusa Bas- mati 1	Haryana Bas- mati 1	Kas- turi	Sarju 52	IR 36	IR 72	Gov- ind	Pant Dhan 4	Pant Dhan 10	Mean (\bar{X})
5. Number of green spots											
MS(1)	1.2	1.6	2.8	1.4	2.4	2.0	2.6	3.8	11.8	2.0	3.16
MS(2)	5.4	6.2	5.0	5.4	7.8	5.2	6.2	8.0	17.0	5.6	7.18
MS(3)	9.2	10.4	15.2	14.4	16.2	11.4	17.2	17.8	20.8	18.8	15.14
MS(4)	16.6	13.6	11.4	11.0	11.8	16.4	11.6	12.6	8.8	12.0	12.58
MS(5)	6.2	18.2	3.4	4.0	5.8	5.0	5.2	4.6	4.8	5.8	6.30
\bar{X}	7.72	10.00	7.56	7.24	8.80	8.00	8.56	9.36	12.64	8.84	
CD (genotype) at 5%						0.46					
CD (medium) at 5%						0.32					
CD (genotype x medium) at 5%						1.02					
6. Number of shoots											
MS(1)	0.2	0.6	0.2	0.4	1.2	0.2	1.6	1.8	8.0	1.0	1.52
MS(2)	3.2	3.0	3.0	1.4	4.4	1.2	3.6	4.8	11.0	3.6	3.92
MS(3)	6.6	6.18	11.6	11.8	12.6	7.2	13.2	14.8	17.2	15.2	11.70
MS(4)	13.4	9.8	8.2	7.0	8.6	12.4	8.8	9.4	4.2	8.4	9.02
MS(5)	2.0	14.8	0.4	1.4	2.4	1.0	2.2	1.8	1.2	3.0	3.02
\bar{X}	5.08	7.00	4.68	4.40	5.84	4.40	5.88	6.52	8.32	6.24	
CD (genotype) at 5%						0.69					
CD (medium) at 5%						0.47					
CD (genotype x medium) at 5%						1.54					
7. Length of longest shoot											
MS(1)	0.16	0.64	0.24	0.32	0.64	0.36	1.10	1.44	2.80	1.18	0.89
MS(2)	1.88	2.42	1.84	1.38	0.76	2.10	2.78	3.32	4.74	2.84	2.50
MS(3)	3.52	4.94	7.84	7.24	6.28	5.96	7.84	7.84	8.30	7.96	6.92
MS(4)	6.88	5.90	4.52	4.54	2.86	8.42	5.36	5.26	2.34	4.36	5.50
MS(5)	2.72	8.38	0.42	2.10	3.86	2.40	3.20	2.38	1.40	3.46	2.93
\bar{X}	3.03	4.46	2.97	3.12	6.60	3.85	4.06	4.05	3.91	3.93	
CD (genotype) at 5%						0.36					
CD (medium) at 5%						0.26					
CD (genotype x medium) at 5%						0.81					
8. Number of root fibrils											
MS(1)	0.2	1.6	0.4	0.2	6.6	0.2	2.0	0.8	3.0	2.6	1.76
MS(2)	0.8	10.8	4.2	5.2	15.0	4.0	6.6	5.8	7.8	8.0	7.43
MS(3)	23.4	29.8	22.6	19.0	25.6	18.2	10.6	10.0	14.8	7.4	18.14
MS(4)	18.4	13.8	9.2	8.0	34.4	28.4	18.0	16.2	24.0	19.4	18.98
MS(5)	2.2	0.4	0.4	1.4	1.8	1.4	2.0	1.6	0.6	4.2	1.66
\bar{X}	10.20	11.28	7.36	6.76	16.68	10.44	7.96	6.88	10.04	8.32	
CD (genotype) at 5%						0.98					
CD (medium) at 5%						0.69					
CD (genotype x medium) at 5%						2.19					

the maximum potential for the formation of green spots and shoots with the respective values of 12.64 and 8.32, respectively, and was significantly superior to all other genotypes studied. Similarly, Sarju 52 exhibited best rooting with the mean value of 6.60 cm for the length of longest root and 16.68 for the number of root fibrils. Pusa Basmati 1 had moderate expression for all the plantlet regeneration characters while other basmati genotypes like Haryana Basmati 1 and Kasturi exhibited significantly lowest potential for shooting (4.66 and 4.40) as well as rooting (7.36 and 6.76).

These variations for callus growth and regeneration characteristics among the various genotypes may be attributed to differences in the components and concentrations for endogenous phytohormones [2]. Further the genotypic variability in callusing ability may be due to difference in the susceptibility to these genotypes to 2,4-D as reported in relation to the growth in rice plant [3]. While basmati genotypes in general performed better for callusing characteristics, yet they trailed behind the nonbasmati types for the regeneration characteristics studied. These results suggest that the capacity for callus growth and regeneration is inherited from the progenitor and that at least two different genetic factors one each for callusing and regeneration may participate in the expression of these characters independently, as also suggested for barley anther culture [4].

The success of *in vitro* culture largely depends on the nutritional media and the growth regulators used. The MS medium supplemented with 2,4-D gave very good response for callus initiation although this response varied with the change in the levels of 2,4-D. On the overall, the MS medium supplemented with 2.0 mg l^{-1} 2,4-D (MS(B)) gave the best response for all the callusing characters studied. A plateau was reached at this level of 2,4-D and therefore, the response of 2,4-D on callus growth characteristics declined with every unit increase in its level. Media MS(B) was significantly superior for callus diameter (1.04 cm) and the callus volume (0.547 cm) and also the maximum but at par with MS(C) for callus induction frequency and callus weight. However, for shooting characteristics, the MS(3) medium gave the highest response with the mean of 15.14 green spots, 11.7 shoots and 6.92 cm length of longest shoot. It was followed by MS (4) and MS(2) media but the differences being statistically significant. For rooting, the MS(4) medium supplemented with 3.0 mg l^{-1} IAA plus 4.0 mg l^{-1} KN showed the best response of 18.98 roots for plantlet and was at par with MS(3) medium (18.14) for this character.

The results on genotype \times medium interaction revealed significantly superior and at par combinations for callus diameter (Pant Dhan 4 on MS(B), Pusa Basmati 1 on MS(C) and Pant Dhan 10 on MS(C)), callus volume (Pant Dhan 4, Sarju 52 and IR 72 on MS(B)) and callus weight (Pant Dhan 4 on MS(B), Kasturi, Pusa Basmati 1 and Govind on MS(C)). These combinations were also superior for the remaining callus growth related characters. Similarly, for plantlet regeneration the best and significantly superior combination of genotype and medium was Pant Dhan 4 on MS(B) for number of green spots (20.8) and

number of shoots (17.2). For length of longest shoot, the best combination was IR 36 on MS(4) which was at par with Pusa Basmati 1 on MS (5) and Pant Dhan 4, Pant Dhan 10, Haryana Basmati 1, IR 72 and Govind on MS(3)). For rooting, Sarju 52 on MS(3) medium gave the best response. On overall basis, the MS medium supplemented with 2.0 mg l^{-1} IAA plus 3.0 mg l^{-1} KN (MS(3)) provided the best results for shooting while 3.0 mg l^{-1} IAA plus 4.0 mg l^{-1} KN (MS(4)) combination was best for rooting. Some of the selected combinations like Pant Dhan 4 on MS(3) and MS(4), Sarju 52 on MS(4) and Pusa Basmati 1 on MS(5) displayed excellent regeneration capacity and therefore, offer a definite scope for further improvement of these well adapted genotypes through somaclonal variations.

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