

Response for Callus Induction in Popular Indica Rice Varieties and Its Mutant Lines Using Different Media Combinations

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The callus induction potential of a genotype is a prerequisite for rice improvement through biotechnological approach. The present study aims to identify the response of popular indica rice varieties and its mutants for different traits indicating callus induction ability of a genotype. Five different concentrations of media were fixed for the study based on the response of an elite indica cultivar, BPT 2231. The variety CO 52 produced best quality callus in most of the traits studied viz., number of days for callus induction (14.08), callus induction percent (83.68), dry weight of callus (0.0288) and percent of white/yellow callus (78.40). Among different media combinations used, high quality callus was observed in MS + 2 mg/l-1 2,4-D + 2 mg/l-1 KN. Different mutant lines included in the study were found to be both on par as well as exhibited significant differences between its wild type, essentially due to somaclonal variation. Mutant lines exhibiting significant differences can further be used for identification of candidate genes responsible for callus induction ability by biotechnological approaches.

Keywords: 2,4-D; Callus induction; Kinetin; Mutants; Varieties.

Rice is one of the prime food crops across the globe. It serves as the source of carbohydrate for more than half of the world's population and it is cultivated under variety of agro-climatic conditions¹.

Rice improvement through biotechnological approaches depend on an efficient protocols through, in vitro callus induction as well as a suitable regeneration protocols²⁻⁴. In vitro

rice regeneration can be accomplished through somatic embryogenesis and organogenesis. Somatic embryogenesis of rice is one of the most promising approaches for rapid propagation due to production of large numbers of plantlets and for the application of genetic transformation technology especially against biotic stresses⁵. The success of somatic embryogenesis is highly influenced by a suitable genotype, growth medium, plant growth

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regulators (PGRs), carbon sources and gelling agent affect the somatic embryogenesis as well as plant regeneration of rice⁶.

But, most of the indica rice genotypes are recalcitrant for tissue culture studies, i.e., they exhibit poor response for callus induction and plant regeneration⁷. Callus induction potential of a particular genotype is fundamental requirement for tissue culture based crop improvement, especially for genetic transformation⁸. Hence, a study was conducted in order to optimise the callus induction protocol of popular indica genotypes for its utilization in tissue culture based crop improvement programmes.

MATERIALS AND METHODS

In Vitro Response of Different Genotypes to Callus Induction Ability

The response of popular rice genotypes for their difference in callus induction ability was studied under controlled in vitro condition. An elite indica cultivar, BPT 2231 (Akshaya) was used for optimisation of callus induction media. Then the appropriate five different concentrations of media were used for other indica rice genotypes that were used in this study. The protocol for callus induction and various genotypes used in the study were described in this section and in Table 1.

Well-filled, mature and dehulled seeds were taken for callus induction studies. They were

washed thoroughly with running tap water for ten minutes to wash off external dust or contaminants. Later the explant materials were washed with Tween@20 detergent solution (Sigma-Aldrich chemicals Pvt. Ltd.) for 15 minutes. To remove the detergent the explant materials are thoroughly washed several times with sterile water. The explants are then surface sterilized in Laminar Airflow Chamber (Cleanair Flow Systems, India).

Surface Sterilization of Explants

After preliminary washings, the explants were taken to laminar air flow chamber and surface sterilization was done by keeping the explants for three minutes in 70% ethyl alcohol (Changshu Hangsheng Fine Chemicals Co., Ltd. China) followed by treatment with 0.1% mercuric chloride (HiMedia Laboratories, LLC) for eight to ten minutes⁹ and finally washed thoroughly with sterile distilled water for 3-5 times to remove any traces of mercuric chloride.

Two to three seeds per tube were inoculated in the media for callus induction and kept in darkness at 25°C with a lightly intensity of 2000-3000 lux alternatively maintained at a cycle of 16 hours light and 8 hours dark for three weeks.

Different Media Composition for Callus Induction Studies

Murashige and Skoog (MS) medium¹⁰ was chosen for the present investigation, along with different hormonal combinations of 2,4-Dichlorophenoxy acetic acid (2,4-D) and

Table 1. List of genotypes used for Tissue culture Studies

S. No.	Genotypes	Special Features	Source
1	BPT 2231	Low glycemic index	Rice Research Unit, Bapatla
2	CR 1009	Resistant to BPH	Dept. of Farm management, AC&RI, Madurai
3	CR 1009 Sub1	Submergence tolerant	Dept. of Farm management, AC&RI, Madurai
4	CO 52	Medium slender grains	Dept. of Farm management, AC&RI, Madurai
5	ADT 45	Resistant to Gall midge	Dept. of Plant Breeding and Genetics, AC&RI, Madurai
6	ADT 37	Multiple resistance to many pests and diseases	
7	M6	Electron beam mutant of ADT 45	
8	M55	Electron beam mutant of ADT 45	
9	M66	Electron beam mutant of ADT 45	
10	M78	Electron beam mutant of ADT 37	
11	M79	Electron beam mutant of ADT 37	
12	M85	Gamma ray mutant of ADT 37	
13	M88	Electron beam mutant of ADT 37	

kinetin (KN). The different media combinations are described in Table 2.

Five replications were maintained in each media combinations and 50 explants were maintained in each of the replication. Series of events during callus induction is presented in Figure 1. The callus induction ability of a genotype is a complex trait that denotes both quantity and quality of the callus. Five different traits viz., Number of days for callus induction, callus induction percent, fresh weight of callus, dry weight of callus, percent of white/yellow callus and percent of callus browning were hence observed in this study for the precise estimation of the callus induction ability of a genotype.

Statistical Analysis

The data was analysed in Factorial Completely Randomised Design (FCRD) using AGRES statistical software and the treatmental means were grouped using Least Significant Difference (LSD) method.

RESULTS AND DISCUSSION

The effect of genotypes and different media combinations on various traits observed on callus was presented in Table 3.

Callus induction ability of different genotypes studied in different growth media exhibited significant variability for all the traits.

The variety CO 52 was found superior in callus induction ability among other genotypes. It out-performed other genotypes in most the traits studied viz., number of days for callus induction (14.08), callus induction percent (83.68), dry weight of callus (0.0288) and percent of white/yellow callus (78.40). The mutant M6 was found to be the poorest performing genotype and it produced poor quality callus in all the treatments. The traits,

number of days for callus induction (22.28), callus induction percent (45.60), fresh weight of callus (0.0299), dry weight of callus (0.0142) and percent of white/yellow callus (22.48) and percent of callus browning (23.12), were all found to be inferior compared to other genotypes.

MS medium is considered as the most suitable culture medium for callus induction of rice cultivars^{11,12}. Previous reports have also shown high callus induction on MS medium supplemented with 2,4-D¹³. Also, some reports suggest that the efficiency of callus induction is increased in a media containing proportionate quantity of both auxin and cytokinin¹⁴. Similarly, among the different media combinations, MS + 2 mg/l 2,4-D + 2 mg/l KN produced high quality callus in most of the traits studied, while MS + 2.5 mg/l 2,4-D + 2 mg/l KN was found to be poor media for callus induction of different genotypes. The difference in callus formation between different media combinations are presented in Figure 2.

Differences in Callus Induction Ability among Mutants

Varietal differences in callus induction potential of rice genotypes have already been reported by several authors^{15,16}. The treatmental means were grouped by LSD method using AGRES statistical software and the differences among the mutants were studied and presented herewith.

Table 2. Different media combinations used in the study

Treatment	Basal Media	PGR (mg/l)	
		2,4-D	KN
C1	MS	2	1.5
C2	MS	1.5	2
C3	MS	2	2
C4	MS	2.5	2
C5	MS	2	2.5

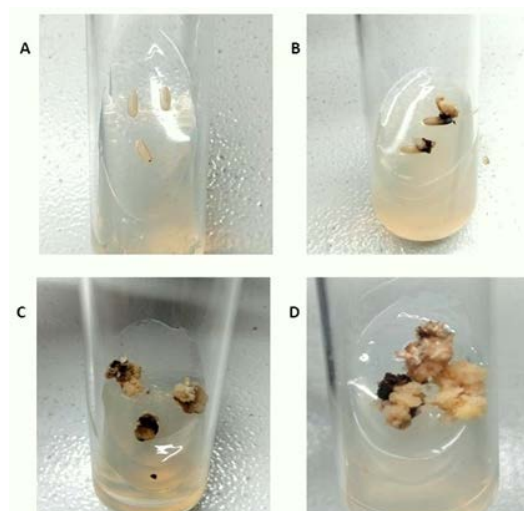


Fig. 1. Series of events during callus induction (A) Inoculation of explants in Tissue culture media; (B) Callus induction in rice explants; (C) Callus subculture in fresh medium; (D) Callus proliferation

Number of days for callus induction for M66 was found to be on par with its parent ADT 45 and M79 found to be on par with its parent ADT 37. Other studied mutants exhibited significant differences in mean values compared to parents and fall into other groups also.

Callus induction percent of all the mutants were statistically different compared to their parents. However, some of the mutants (M79 and M85) were grouped together or they were said to be on par, producing similar callus induction percent. Previous reports also showed the effect of rice genotypes on the variability of callus induction potential^{17,18}. In a similar study involving 510 indica and japonica accessions¹⁹, they found significantly different callus induction potential between the categorized populations.

Fresh weight of callus also showed significant differences in their mean value among the mutants to their parents. However, some of the mutants were grouped together (M6 and M55; M85 and M88) indicating absence of statistical significance between them. Similarly, dry weight of callus also exhibited significant differences in mean among the mutants, compared to their parents.

Percent of White/Yellow callus in M78 was found to be on par with its parent, while all other mutants exhibited significant difference in mean to their parents. However, in case of percent of callus browning, all the mutants studied exhibited significant difference in mean to their parents. However, they exhibit some amount of statistical similarity among them (M55 and M66; M78, M79 and M88). This result clearly indicated the genetic variability of rice cultivars with regard to callus browning which corresponds with the earlier reports²⁰.

CONCLUSION

The response of different genotypes for callus induction indicates their potential to use them in a genetic engineering programme for transformation. Further, the best callus induction medium, MS + 2 mg/l-1 2,4-D + 2 mg/l-1 KN, for most of the traits under study, was identified. The different mutants were also included in the study to estimate the differences in callus induction potential between its wild type. The significant differences found in callus induction potential

Table 3. Response of genotypes and media combinations on various traits observed on callus

Treatment	DCI(Days)	CIP (%)	CFW(g)	CDW(g)	WYC (%)	CBW (%)
BPT 2231	17.16c	70.56b	0.3530a	0.0170f	60.24c	10.32d
CR 1009	15.28b	81.44a	0.2886c	0.0220e	76.64ab	4.80a
CR 1009 Sub1	17.00c	81.92a	0.2613d	0.0222de	75.12b	6.80bc
CO 52	14.08a	83.68a	0.1949h	0.0288a	78.40a	5.28ab
ADT 37	20.28d	60.16d	0.2116g	0.0276b	50.80ef	9.36d
ADT 45	20.40d	61.52d	0.2440e	0.0279ab	51.76e	9.76d
M6	22.28g	45.60h	0.1299j	0.0142h	22.48i	23.12f
M55	21.92gf	57.28e	0.1398j	0.0156g	37.36g	19.92e
M66	20.28d	51.20g	0.1590i	0.0175f	31.36h	19.84e
M78	20.52de	54.32f	0.2288f	0.0222de	48.48f	5.84abc
M79	20.28d	66.00c	0.2844c	0.0247c	60.08c	5.92abc
M85	21.52efg	64.16c	0.3272b	0.0231d	56.88d	7.28c
M88	21.16def	59.60de	0.3357b	0.0179f	53.44e	6.16abc
C1	18.98c	65.26c	0.2299c	0.0222c	55.29c	9.97c
C2	13.63a	72.49b	0.2852b	0.0251b	63.75b	8.74b
C3	15.46b	77.78a	0.3666a	0.0296a	70.55a	7.23a
C4	25.97e	50.06e	0.1466e	0.0145e	36.95e	13.11d
C5	22.94d	56.49d	0.1865d	0.0166d	43.85d	12.65d

DCI- Days to callus induction; CIP- Callus induction percent; CFW- Callus fresh weight; CWD- Callus dry weight; WYC- Percent of White/ Yellow callus; CBW- Percent of callus browning
Values with same alphabets are not significantly different

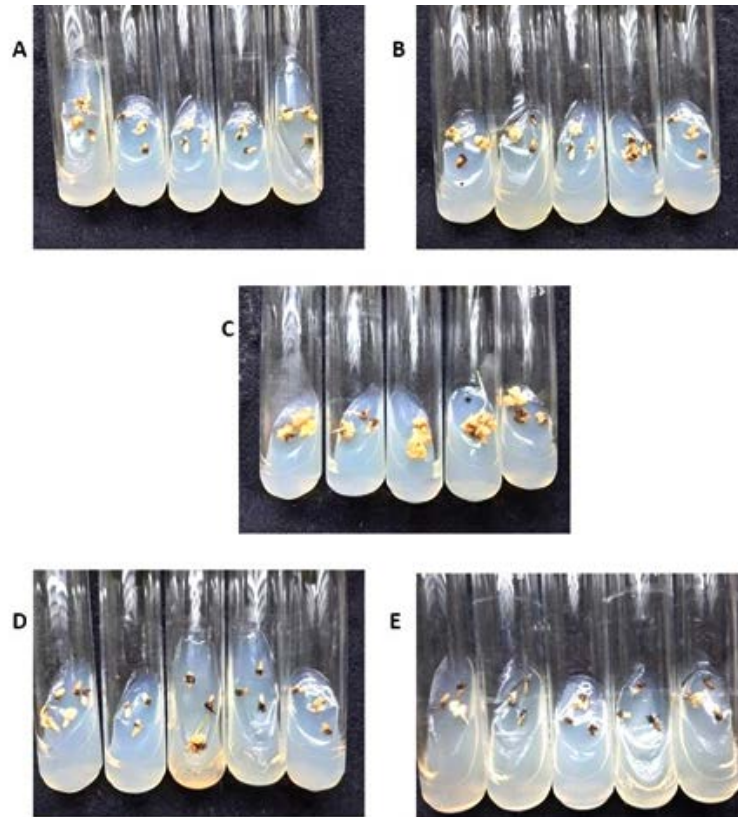


Fig. 2. Difference in callus induction potential in different media combinations. The sub-figures (A, B, C, D and E) in Figure 2 represent different media combinations in which callus was induced. (A) MS + 2 mg l⁻¹ 2,4-D + 1.5 mg l⁻¹ KN; (B) MS + 1.5 mg l⁻¹ 2,4-D + 2 mg l⁻¹ KN; (C) MS + 2 mg l⁻¹ 2,4-D + 2 mg l⁻¹ KN; (D) MS + 2.5 mg l⁻¹ 2,4-D + 2 mg l⁻¹ KN; (E) MS + 2 mg l⁻¹ 2,4-D + 2.5 mg l⁻¹ KN

may be due to somaclonal variation occurred in the particular gene. Hence, further studies in these mutants and its wild type can be used for identification of candidate genes controlling the traits studied.

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