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 2,4-D + 2 mg/l 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 protocols²⁴. 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...
regulators (PGRs), carbon sources and gelling agent affect the somatic embryogenesis as well as plant regeneration of rice.

But, most of the indica rice genotypes are recalcitrant for tissue culture studies, i.e., they exhibit poor response for callus induction and plant regeneration. Callus induction potential of a particular genotype is fundamental requirement for tissue culture based crop improvement, especially for genetic transformation. 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 minutes 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) medium was chosen for the present investigation, along with different hormonal combinations of 2,4-Dichlorophenoxy acetic acid (2,4-D) and

| 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 | Rice Research Unit, Bapatla |
| 7     | M6         | Electron beam mutant of ADT 45 | Rice Research Unit, Bapatla |
| 8     | M55        | Electron beam mutant of ADT 45 | Rice Research Unit, Bapatla |
| 9     | M66        | Electron beam mutant of ADT 45 | Rice Research Unit, Bapatla |
| 10    | M78        | Electron beam mutant of ADT 37 | Rice Research Unit, Bapatla |
| 11    | M79        | Electron beam mutant of ADT 37 | Rice Research Unit, Bapatla |
| 12    | M85        | Gamma ray mutant of ADT 37 | Rice Research Unit, Bapatla |
| 13    | M88        | Electron beam mutant of ADT 37 | Rice Research Unit, Bapatla |
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\textsuperscript{11,12}. Previous reports have also shown high callus induction on MS medium supplemented with 2,4-D\textsuperscript{13}. Also, some reports suggest that the efficiency of callus induction is increased in a media containing proportionate quantity of both auxin and cytokinin\textsuperscript{14}. Similarly, among the different media combinations, MS + 2 mgl-l 2,4-D + 2 mgl-l KN produced high quality callus in most of the traits studied, while MS + 2.5 mgl-l 2,4-D + 2 mgl-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\textsuperscript{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
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  | 0.0279ab | 71.76e  | 9.76d   |
| ADT 45    | 20.40d    | 61.52d   | 0.2440e  | 0.0296a  | 71.76e  | 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
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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