Response on callusing and regeneration of two quality rice (*Oryza sativa* L.) varieties

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ABSTRACT

The present investigation was carried out to study the response of two quality rice varieties *viz.*, White Ponni and BPT 5204. This study focused to evaluate the genotypes for their callus induction frequency, relative growth rate of callus and regenaration capacity using Murashige and Skoog medium. Among the two varieties under investigation, the mean callus induction percentage, embryogenic callus induction percentage and relative growth rate of callus was higher in BPT 5204 when compared to White Ponni. BPT 5204 was more responsive for tissue culture than White Ponni.

Key words: Rice, Somaclone, callus, White Ponni and BPT 5204

Rice (*Oryza sativa* L.) is one of the most important staple food crops and its production plays a significant role in the strategy to overcome food shortage and improvement of self sufficiency for local consumption and export. Tissue culture induced phenotypic and genotypic variations are collectively termed as 'Somaclonal variation' (Larkin and Scowcroft, 1981). It is defined as genetic and phenotypic variation among clonally propagated plants of a single donor clone (Olhoft and Phillips, 1999). Somaclonal variation can be manifested as either somatically or meiotically stable events. Somatically stable variation includes phenotypes such as habituation of cultures and physiologically induced variation observed among primary regenerants. This form of variation has been extensively reported in a variety of plants, including many self-pollinated plants such as rice, wheat and barley (Ryan et al., 1987; Breiman et al., 1987), besides providing an additional source of novel variation for plant breeding and genetics. India is facing stiff competition in the world market for export of rice. A decade ago, India used to export only Basmati rice. Non-Basmati rice has also become a major item for export, registering a steady upward trend in recent years. The rice varieties White Ponni and BPT 5204 are considered as the best suitable varieties with high quality rice which fetches very high price for the farmers due to its slender grain. The main drawbacks of these varieties are poor fertilizer responsiveness. The main objective of this study was to isolate somaclonal variants in these varieties for agronomically important traits of plant architecture and yield improvement.

MATERIALS AND METHODS

Two promising varieties of rice *viz.*, White Ponni and BPT 5204 constituted the biological materials of the present study. The experimental materials, White Ponni and BPT 5204 were obtained from the Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

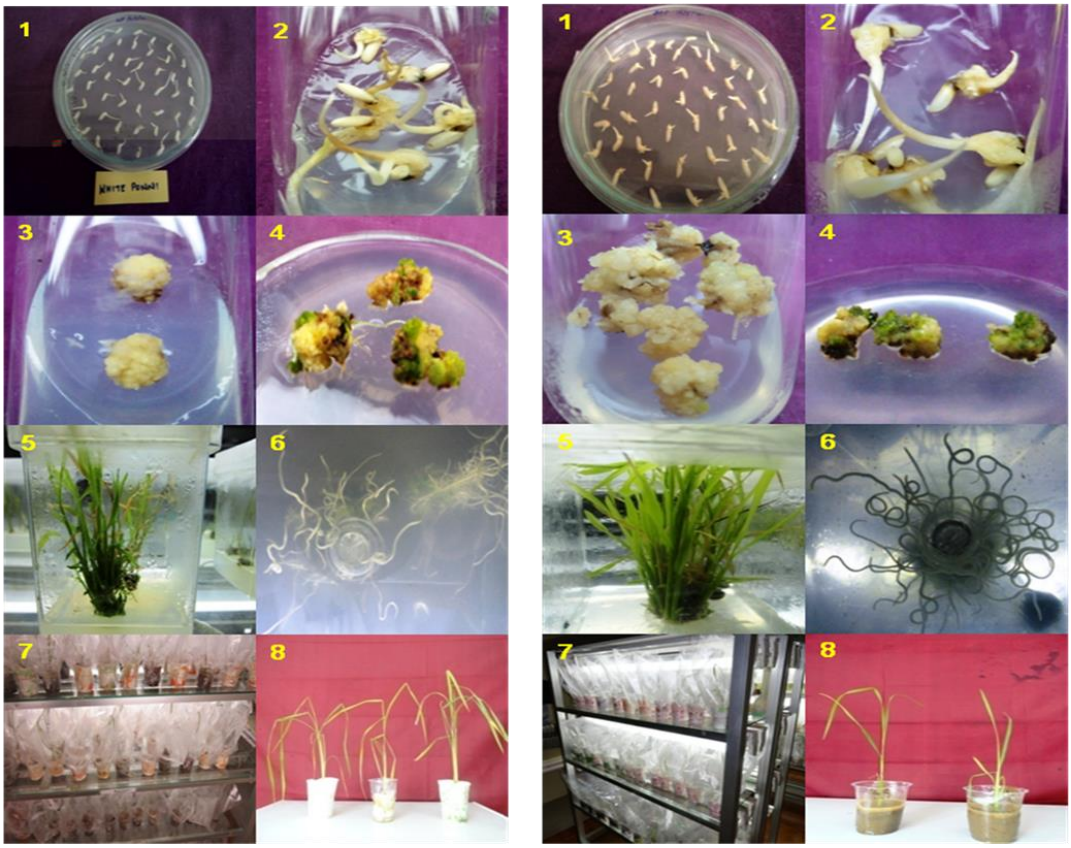
Callus induction of seeds without EMS treatment

Dehusked seeds of White Ponni and BPT 5204 were surface sterilized with 70 per cent ethanol for two minutes and 0.1 per cent Mercuric chloride ($HgCl_2$) for three minutes. Then, the seeds were washed with sterile water for two to three times to remove ethanol and Mercuric chloride. The surface sterilized seeds were inoculated in the culture tubes and petriplates containing MS medium (Kinetin - 0.5 mg/l and 2, 4-D - 2 mg/l) under laminar air flow chamber. After that, the culture tubes were kept in dark for 15 to 20 days for callus induction. Twenty days after callus induction, callus

was transferred to new culture tubes containing the same medium and these were kept under light and dark for 16 and 8 hours respectively for callus proliferation. For shoot induction the friable calli of two rice varieties *viz*., White Ponni and BPT-5204 were transferred to the regeneration medium (Shooting media: MS+ 4 mg/l of BAP + 1 mg/l of NAA). Regenerated shoots were rooted by culturing on MS medium supplemented with 1-Naphthaleneacetic acid (NAA) at the concentration of 1 mg/l, 3 % sucrose and 0.8 % agar. The callus induction percentage was determined based on number of calli produced from seeds inoculated in the medium (Fig 1). Callus induction percentage and relative growth rate were determined as follows.

$$\textbf{Callus induction percentage} = \frac{\text{Number of seeds produced calli}}{\text{Number of seeds inoculated}} \times 100$$

$$\textbf{Relative growth rate} = \frac{\text{Final callus weight} - \text{Initial callus weight}}{\text{Initial callus weight}} \times 100$$



White Ponni **BPT 5204**

1. Seed inoculation, 2. Callus induction, 3. Callus proliferation, 4.Green islets formation, 5. Shoot regeneration, 6. Root regeneration, 7. Hardening, 8. Whole plant development

Fig 1. Development of plantlets in White Ponni and BPT 5204

RESULTS AND DISCUSSION

Callus was obtained four weeks after inoculation of seeds on callus induction medium. The callus induction was recorded and the per cent of callus induction of White Ponni and BPT 5204 in MS media is presented in Table 1. Among the two varieties under investigation, the mean callus induction percentage was maximum in BPT 5204 (88.00 %) with the range of 71.30 to 94.20 per cent whereas White Ponni had registered minimum callus induction percentage of 78.60 per cent with the range of 61.09 to 90.16 per cent (Fig 2).

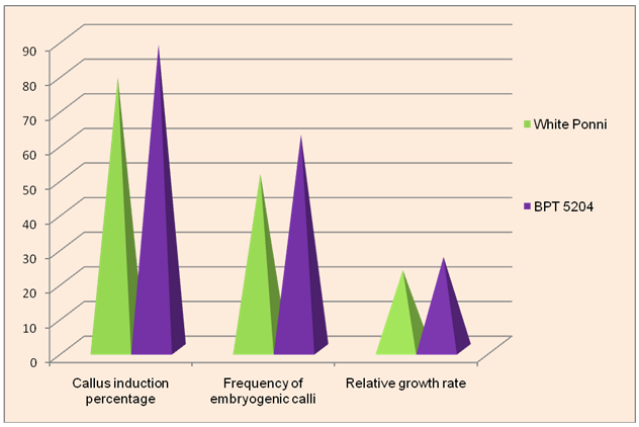


Fig 2. Callus induction percentage, frequency of embryogenic calli and relative growth rate of callus in White Ponni and BPT 5204

The embryogenic callus was essentially nodular and pale yellow which possess the regeneration capacity when transferred to regeneration media. When compared to White Ponni, frequency of embryogenic callus induction in terms of percentage was found to be higher in BPT 5204 (62.04 %) with the range of 43.41 to 78.49 per cent whereas it was found to be minimum (50.73 %) in White Ponni with the range of 32.33 to 71.95 per cent. Fresh weight of callus were recorded at the beginning and the end of the culture period. The relative growth was calculated on the basis of the initial and final growth of the callus. Among the two varieties, BPT 5204 had recorded higher relative growth rate (26.69 %) with the range of 24.47 to 28.69 per cent whereas White Ponni had recorded lower relative growth rate (22.90 %) with the range of 19.92 to 25.59 per cent.

Table 1. Callus induction percentage, Frequency of embryogenic calli and relative growth rate (%) of callus in White Ponni and BPT 5204

Variety	Mean	Range	SE(d)	CD (0.05)
Callus induction percentage				
White Ponni	78.60	61.09 – 90.16	7.06	14.17
BPT 5204	88.00	71.30 – 94.20	6.98	14.01
Frequency of embryogenic calli				
White Ponni	50.73	32.33 – 71.95	9.42	18.92
BPT 5204	62.04	43.41 – 78.49	8.59	17.25
Relative growth rate				
White Ponni	22.90	19.92 – 25.59	4.23	8.82
BPT 5204	26.69	24.47 – 28.69	3.24	6.76

In vitro culture of plant cells gives rise to genetic variation and somaclonal variation was widely tested as an alternative to traditional mutation breeding. Variations were found in plants regenerated through tissue culture for almost all traits. Somaclones provide a novel and valuable source of genetic variability, which can be exploited for crop improvement particularly, for the development of stress tolerance rice cultivars (Lutts et al., 1999). The present investigation on *in vitro* culture of seeds of White Ponni and BPT 5204 on MS medium revealed that, callus induction percentage, frequency of embryogenic calli and relative growth rate of callus was higher in BPT 5204 when compared to White Ponni (Fig 2). These results clearly emphasized that both the genotypes were well response to *in vitro* culturing under artificial aseptic condition. A similar kind of findings were reported by Summart (2008) and Pandey et al. (1994) at 2 mg L^{-1} 2, 4-D. Asad et al. (2001) also observed that N6 medium containing 2 mg L^{-1} of 2, 4-D was optimum for callus induction of four rice genotypes *viz*., Swat I, Swat II, Dilrosh 97 and Pakhal. Decrease in growth value and

percentage of adapted callus through consecutive phases, is the result of media composition and growing condition. The highest growth rate was recorded in BPT 5204 when compared White Ponni growth rate in MS media. Similar results were disclosed by other authors (Cheema et al., 2002; Lee et al., 2003).

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