

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Studies on Genotype Response to Callus Induction from Three Basmati Cultivars of Rice (*Oryza sativa* L.)

Hamid Rsahid, ¹Syeda Nazrat Rahim Bokhari, Zubeda Chaudhry and ¹Syed Muhammad Saqlan Naqvi
Agricultural Biotechnology Programme,
National Agricultural Research Center, Park Road, Islamabad, Pakistan
¹Department of Biological Science, University of Arid Agriculture, Rawalpindi, Pakistan

Abstract: The Present study was carried out to optimize an efficient protocol for callogensis in rice. Three rice varieties Rachna Basmati, Basmati 2000 and 370 were tested. Rice seeds were cultured on N₆ medium with three different concentrations of 2, 4-Dichlorophenoxy acetic acid i.e. 1, 2 and 3 mg⁻¹ of 2, 4-D while Basmati 370 had maximum callogensis at 2 mg⁻¹ of the same hormones. Rachna Basmati and Basmati 2000 did not exhibit any significant difference in callogensis. However, Rachna Basmati and Basmati 2000 had more callus induction frequency than Basmati 370. It was concluded that the callus growth of Rachna Basmati was more then other two varieties.

Key words: Basmati rice, callogensis, 2, 4- Dichlorophenoxy acetic acid, growth, varieties

Introduction

Rice is the second chief commodity of Pakistan. Due to its high nutritional value and increasing importance in economy, now it is needed to develop new varieties. Tissue culture techniques proved to be an easy way to develop new varieties having good agronomic characters, disease tolerance and adaptability traits (Rashid *et al.*, 1996). Rice occupies about eleven percent of crop area in Pakistan. The main groups of rice is aromatic (Basmati) and coarse varieties. Aroma adds to its cash value of Pakistani Basmati rice.

Tissue culture is the foundation on which all biotechnology rests. The beginning of research in this area can be traced to Haberlandt, which observed that plant cells are totipotent. One of the important technique by which new varieties can be produced is somaclonal variations. Oono (1978) reported that production of somaclonal variations are the variations necessarily involving a callus stage. The calli can be induced by using high concentrations of auxins and cytokinins (Yamada *et al.*, 1967). Effect of growth regulators particularly 2, 4-D on callus growth were studied (Gonzalez, 2000). Basal media was one of the important factor which affected callus growth (Yamada *et al.*, 1967; Al-Khayri *et al.*, 1996). Genotype specificity was also found (Abbasi *et al.*, 2000). It was also reported that callus induction frequency of Basmati cultivars is genotype specific (Rashid *et al.*, 1996).

The present study was conducted to study effect of 2, 4-D on callus induction and growth of three varieties of rice, i.e. Rachna Basmti 370 and 2000.

Materials and Methods

The present study was carried out at Agricultural Biotechnology Programme, National Agricultural Research Centre, Islamabad during 2001-2002. Seeds of rice varieties i.e. Rachna Basmati, Basmati 2000 and 370 were used as explant source, obtained from Crop Science Institute of the same Centre.

Seed disinfection: Clean healthy and nearly equal sized seeds were sorted out and then washed thoroughly with distilled water and a dilute detergent solution. For disinfection, seeds were dipped in 70% Clorox for 20 min. After that, seeds were washed three times under aseptic conditions with autoclaved distilled water.

Media preparation: N₆ basal media (Chu *et al.*, 1975) was used for disinfection experiments. N₆ basal medium was also used for callus induction with different concentrations of 2, 4-D (1, 2 and 3 mg l⁻¹) was used. After addition of all components, pH of the media was adjusted at 5.75 Media was then salified with 0.6% agar and poured into clean and dried test tubes followed by plugging with sterilized cotton. For sterilization, media was autoclaved at 121°C and 15 psi for 20 min.

Inoculation: After sterilization procedure, seed were cultured on N₆ medium for determining callus induction frequency. All cultures were incubated at 25±3°C for 16 hrs photoperiod (2000 lux). Seeds were monitored for callus frequency After 3-4 weeks callus appearance and growth were also noted.

Statistical analysis: The data so collected was statistically analyzed by applying ANOVA.

Results and Discussion

Various levels of Clorox were applied for sterilization to achieve maximum callus induction frequency. Fifty percent Clorox treatment to seed used as explant source showed 90% contamination although growth of calli was better. Clorox treatment 70% was successful for sterilization of rice seeds. During this treatment, only 10-15% contamination was observed with as for above treatment. Clorox treatment 100% showed no contamination but browning was observed.

Three varieties of rice Rachna Basmati, Basmati 2000 and 370 were cultured on N_6 medium containing various levels of 2, 4-D. The effect of concentrations of 2,4-D on these varieties was investigated. The callus induction frequency range was 60-81, 63-88 and 35-98% for Basmati 370 and 2000 and Rachna Basmati, respectively. Callus induction frequency of Rachna Basmati was increased with the increase of concentration of 2, 4-D (Fig. 1). In case of Basmati 370, the callus induction frequency was increased by increasing the concentration of 2, 4-D from 1 to 2 $mg\ l^{-1}$ and was maximum at 3 $mg\ l^{-1}$. Callogenesis frequency was affected by raising concentration of 2, 4-D for Basmati 370. This was in accordance with results shown by other researchers (Dode *et al.*, 2000). The changes in callus induction frequency was probably due to fact that a certain concentration of 2, 4-D required for callus induction. As 2, 4-D is an auxin which affects on cell elongation and stimulation of cell division by increasing the permeability of cells and also have effects on mRNA and protein synthesis. So, a concentration less than the required may not be enough to cause sufficient permeability, required effect on mRNA and protein synthesis while a concentration higher than the required may disturb the balance. (Malik and Srivastava, 1985).

As far as the varietal response for callus induction is concerned, Basmati 2000 and Rachna Basmati did not exhibit any significant difference. The optimal dose of 2, 4-D for callus induction was 3 $mg\ l^{-1}$ for Rachna Basmati and Basmati 2000 and 2 $mg\ l^{-1}$ for Basmati 370. The callus induction frequency is also genotype specific (Katiyar *et al.*, 1999). The results for callogenesis revealed that Basmati 370 was significantly different from Rachna Basmati 2000 while both of these varieties behaved in a similar way for callogenesis. This can be attributed to less genotypic difference between two varieties as different genotypes had different responses on the same media

(Naqvi *et al.*, 1989). One of the parents of Basmati 2000 is Basmati 385. Basmati 385 was produced by crossing Basmati 370 with Tai Chung Native 1. The variety obtained was four time back crossed. So, the Basmati 385 has more characters of Basmati 370. Rachna Basmati is also a variant of Basmati 370. Both varieties Rachna Basmati and Basmati 2000 were developed form Basmati 370 so it can be said that they have less genotypic difference. Also, many characters of Rachna Basmati are similar to Basmati 385. (Rashid *et al.*, 1996).

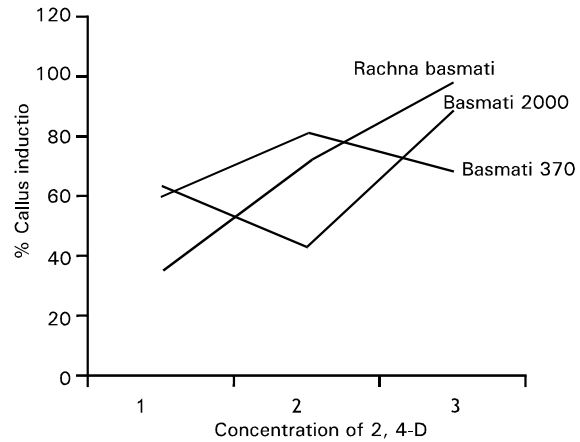


Fig. 1: Effect of 2, 4-D on Callus induction in three varieties of rice

The co-efficient of variation for effect of concentration of 2, 4-D was 5.07%, indicating that 94% changes were due to effect of concentration and 5.07 were by chance.

Calli in all the three varieties were produced in a period of 3-4 weeks. The calli obtained were compact, granular and off-white in colour. Callus size was different in three varieties. This difference in callus size is may be due to different proliferation capacities of these varieties. In appearance, the callus of Rachna Basmati was excellent then other two varieties while of Basmati 2000 was better than Basmati 370.

Rachna Basmati had more callus induction frequency as well as more callus growth. So was concluding that Rachna Basmati should also perform more regeneration ability and was more suitable for transformation experiments.

Acknowledgment

The authors acknowledge the grant from Pakistan Science Foundation, Islamabad with project No. PSF/F&D/CDYST/C-NARC/Bio(11).

References

- Abbasi, F.M., H. Rashid and A. Quraishi, 2000. Regeneration efficiency and embryogenic callus production of three production of three cultivars of rice. *Pak. J. Agric. Res.*, 16: 97-99.
- Al-khayri, J.M., C.E. Shambin, R.W. McNew and E.J. Anderson, 1996. Callus induction and plant regeneration of U.S. rice genotypes as affected by medium content. *Theor. Appl. Genet.*, 58: 87-90.
- Chu, C.C., C.S. Wang, C.S. Sun, V. Hsu, K.C. Yin, C.Y. Chu and F.Y. Bi, 1975. Establishment of an efficient medium for anther culture of rice through experiments on the nitrogen source. *Scient. Sin. I.*, 18: 659-668.
- Dode, L.B., I.L. Vighi, J.A. Peters and F.S. Goncalves, 2000. Effect of culture media hormonal balance in the induction and proliferation of mature seed derived calli. *Revistaa-Ciantifica-Rural*, 5: 27-31.
- Gonzalez, M.C., 2000. Effect of different growth regulators on *in vitro* culture of rice. *Cultivars-Tropicales*, 21: 27-28.
- Katiyar, S.K., G. Chandel, P. Singh and R. Pratibha, 1999. Genetic variations and effect of 2, 4-D on *in vitro* plant regeneration in indica cultivars. *Oryza*, 36: 254-256.
- Malik, C.P. and A.K. Srivastava, 1985. *Phytohormones. Textbook of Plant Physiology*. Kalyani Publishers New Delhi, India, pp: 447-491.
- Naqvi, S.M.S., S.T. Abbas and A. Quraishi, 1989. Effect of sucrose, phytohormones and some amino acids on callus culture and subsequent regeneration in basmati 385. *Pak. J. Agric. Res.*, 10: 224-230.
- Oono, K., 1978. *In vitro* methods applied to rice. *Colloq. Int. CNRS.*, pp: 273-298.
- Rashid, H., S. Yokoi, K. Toriyama and K. Hinata, 1996. Transgenic plant production mediated by *Agrobacterium* in Indica rice (Basmati). *Pak. J. Biol. Sci.*, 3: 2229-2231.
- Yamada, Y., T. Nishi, T. Yasuda. and E. Takahasi, 1967. The sterile culture of rice callus, *Oryza sativa* L. and its application. In: M. Miyakawa and T.D. Luckey, (Eds). *Advances in Germfree Research and Gnotobiology*. CRC Press, Cleveland., pp: 377-386.