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The role of growth hormones in rice (*Oryza sativa* L.) *in vitro* cultures

R. AMBIKA RAJENDRAN, DIJEE BASTIAN, ACHAMMA OOMMAN, V.V. RADHAKRISHNAN AND P. SRIDEVI

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SUMMARY

Mature, dehulled, surface sterilized seeds of rice variety Ptb26 were raised in MS medium with different combinations of 2,4-D and Kinetin. Callus induction studies revealed that MS + 2,4-D 2mg/l + Kinetin 0.5 mg/l was the best to induce callus in rice. The same medium was used for callus proliferation. Three weeks old sub cultured calli were transferred to regeneration shooting media MS + NAA 2mg/l + Kinetin 4mg/l +BAP 0.5 mg/l. The shoots obtained were transferred to rooting medium MS + NAA 2mg/l + Kinetin 0.5 mg/l. The success of hardening was 85.20 per cent.

Key words : Growth hormone, Rice, *In vitro* culture.

The techniques of cell, tissues and organ culture have made available a new range of unavailable variation for genetic manifestation. The application of advanced tissue culture techniques like protoplast fusion, gene transfer, inducts of somaclonal variation, *in vitro* mutagenesis, cell culture and subsequent plant regeneration have opened up new awareness in rice improvement (Predieri, 2001). The present investigation was envisaged to study the effect of growth regulators on callus induction and to find out optimum concentration required for better callus induction and regeneration in rice cultivar Ptb26.

MATERIALS AND METHODS

The experiment was conducted in Tissue Culture Lab. of College of Horticulture, Kerala Agricultural University, Thrissur. Rice cultivar Ptb 26 (Pattambi 26) obtained for the Regional Agricultural Research Station, Pattambi was used in this study. The explants used were mature and dehulled seeds of Ptb26. As suggested by Murashige and Skoog (1962) the basal medium was used. Medium was prepared by following the standard procedure by Gamborg and Shyluk (1981). pH of the medium was adjusted to 5.8 using 0.1N NaOH / HCl. Agar was added and the media was heated to melt. About 15ml medium was poured to the culture tube and then plugged by non absorbent cotton. Tubes were autoclaved at 121^{0}C for 20 minutes. It was allowed to at cool room temperature and stored at 10^{0}C.

The growth regulators used were auxin 2,4-D (2,4-Dichlorophenoxyacetic acid) and cytokinin (kinetin).The media combinations were tried MS + 2,4-D (1, 1.5, 2.0, 2.5, 3.0 mg/l) + kinetin (0.5mg/l). Seeds were immersed in Teepol (5 per cent) solution for ten minutes, followed by rinsing with sterile distilled water to remove traces of soap solution. Seeds were surface sterilized to laminar air flow chamber. Seeds were surface sterilized with 70 per cent alcohol for 2 minutes, followed by rinsing with distilled sterilized water 2-3 times. Seeds were treated in 0.1 per cent mercuric chloride solution for two minutes and again rinsed 3 to 4 times thoroughly by sterile distilled water. Surface sterilized seeds was carefully inoculated into the callus induction media at the rate of one seed per tube in each treatment with five replications (20 tubes per replication). The culture were incubated in a closed room in which temperature was maintained at 26 ± 2^{o}C, humidity between 60 to 80 per cent and light intensity of 3000lux for 16 hrs daily. They were examined every alternate day. Contaminants were removed. The media combination best suited for seed callus was ascertained from number of days taken for callus induction and the percentage of callus induction. Treatment combination with the highest callus induction percentage was used for further studies.

$$\text{Callus induction percentage} = \frac{\text{Number of calli produced per treatment}}{\text{No. of explants inoculated per treatment}} \times 100$$

Effect of growth regulators combinations on callus induction was statistically analyzed in CRD (Completely Randomised Design). If calculated 'T' value was greater than or equal to table value at 5 per cent level of significance, then the treatments were considered to be

Correspondence to:
R. AMBIKA RAJENDRAN, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA
Authors' affiliations:
DIJEE BASTIAN, ACHAMMA OOMMAN, V.V. RADHAKRISHNAN AND P. SRIDEVI, College of Horticulture, Kerala Agricultural University, THRISSUR (KERALA) INDIA