research article | International Journal of Plant Sciences, (July to December, 2009) Vol. 4 Issue 2 : 422-425

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

Accepted : March, 2009

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

significantly different from each other. SEd and CD were calculated to compare the unit means and choose the best treatment. The best MS medium for above observations was used to subculture to effect more calli growth and proliferation. First sub culturing was done in 21 days. Thereafter the calli were allowed to proliferation for 21 days. Once the calli attained the size of 2cm (approximately 500 mg weight) they were taken for regeneration. The basal medium for regeneration was MS. Two regeneration media with two growth combinations were studied MS+ NAA (1- Naphthalene acetic acid) 2mg/l+ Kinetin 4mg/l + BAP (Benzyl amino purine) 0.5mg/l and MS + NAA 2mg/l+ Kinetin 4mg/l + BAP1mg/l, respectively.

Green spots appeared in 35 days. Spots turned into shoots which were allowed to develop for seven days. The shoots obtained were transferred to rooting medium (MS+ NAA2.0 mg/l+ Kinetin 0.5mg/l). The cultures were kept under continuous light (3000lux intensity) at 26±20°C for seven days and were observed for percentage.

$$\text{Percentage of plant regeneration} = \frac{\text{No. of green plants produced}}{\text{Total no. of plantlets hardened}} \times 100$$

Regenerated plants from the above experiment were taken out, washed, dipped in fungicide, again washed and planted in sterilized sand filled in plastic cups. All the plantlets were planted out. Each individual cup with plantlets was covered with transplant polythene cover to develop sufficient moisture inside to prevent decaying of plantlets. After look polythene cover was removed. The plantlets were irrigated with sterile water as and when required. When hardened, the plantlets started growing by forming new leaves and roots. A mixture of dried cow dung and subsoil was added around each plantlet, in the cup. After two weeks time, the plantlets were transplanted to pots and maintained as pot cultures. Observation measured was,

$$\text{Percentage success of hardened plants} = \frac{\text{No. of plantlets surviving}}{\text{Total no. of plantlets hardened}} \times 100$$

RESULTS AND DISCUSSION

Callus indication was achieved in fourteen days after inoculation. Further proliferation occurred when they were subcultured on medium with same level of growth regulators. The value of callus induction percentage ranged from 0.00 per cent to 98.00 per cent. Maximum callus induction percentage was recorded by MS + 2, 4-D 2mg/l+ Kinetin 0.5mg/l (98.00 per cent). The experimental results relating to the effect of growth regulators when statistically analysed in CRD showed MS + 2, 4-D 2mg/l+ Kinetin 0.5mg/l treatment to be highly significant and hence the best (Table 1). All calli induced were off white, creamy and non embryogenic. In the regenerative medium, regeneration percentage was maximum for MS + NAA 2mg/l + kinetin 4mg/l +BAP 0.5mg/l for shooting (90.44 per cent) and hence adjudged best (Table 2). The shoots obtained were transferred to rooting medium MS + NAA 2mg/l + Kinetin 0.5 mg/l. The percentage of success of regenerates to produce rooted hardened was 89.47 per cent.

Totipotency of cell is primarily by the interaction between media constituents and endogenous tissues that determine cell differentiation (Islam *et al.*, 2005). Genotype used in this study was of *indica* type. Several reports confirm high callus induction performance of *indica* culture in MS medium (Abassi *et al.*, 2000; Deepthi *et al.*, 2001). On the contarary, Visarada and Sarma (2002) compared *in vitro* response of several elite *Indica* rice cultivars with that of most responding *japonica* type, *Taipei* 309. Callus induced from mature seeds of *japonica* rice genotypes was more compact and proliferated slowly.

Among the various explants used in plant tissue

Table 1 : Mean performance and the significance test of callusing

Treatments	Mean callusing percentage	Significance test
MS+1mgl^{-1} 2,4-D+0.5mgl^{-1} kinetin	0.00 (0.00)	e
MS+1.5mgl^{-1} 2,4-D+0.5mgl^{-1} kinetin	32.00(34.50)	c
MS+2.0mgl^{-1} 2,4-D+0.5mgl^{-1} kinetin	98.00(81.87)	a
MS+2.5mgl^{-1} 2,4-D+0.5mgl^{-1} kinetin	55.00(47.84)	b
MS+3.0mgl^{-1} 2,4-D+0.5mgl^{-1} kinetin	26.00 (30.66)	cd

Figures in parenthesis indicate arc sine transformed values
SEd = 3.715; CD at error degrees of freedom at 5 per cent of significance = 7.750

Table 2 : Mean regeneration and hardening success percentage

Treatments	Mean regeneration percentage	Hardening success percentage
MS+ NAA 2mgl^{-1}+ Kinetin 4mg l^{-1} + BAP 0.5mgl^{-1}	90.48	89.47
MS + NAA 2mgl^{-1}+ Kinetin 4mgl^{-1} + BAP 1 mg l^{-1}	75.23	81.01

culture such as immature embryo, anther, pollen, ovary and spikelet tried by several rice workers in different genotypes, it was seed that excelled in performance for callus induction and subsequent regeneration in the shortest period. Abassi *et al.* (2000) found callusing from seeds in 10-14 days time interval.

2,4-D is the most commonly used growth regulator for callus induction in cereal tissue culture (Bregitzer *et al.*, 1989). 2,4-D 2mg/l with Kn0.5mg/l was the best for callus and subculturing in this experiment. A critical level of 2, 4-D and kinetin proved to be essential for optimum callus induction (Gonzalez, 2000, Wang *et al*., 2001 and Gomez and Kalamani, 2002). A plateau was reached at 2mg/l level of 2,4 D and therefore the response of 2,4-D in callus growth characteristics declined with every unit increase in its level. However, Singh and Singh (1996) found good callus induction at 2,4-D 8mg/l.

Subculturing in media with the same hormonal combination produced better callus proliferation and further good regenerants similar to results of Sakila *et al.* (1999) and Abassi *et al*. (2000). Virk *et al*. (1998) reported callus subculturing on media with lower hormonal composition. Callus morphological studies revealed all calli to be cream coloured and compact, hence non embryogenic calli as reported by Virk *et al*. (1998). Successful regeneration occurred in the shooting media MS + NAA 2mg/l + kinetin 4mg/l +BAP 0.5mg/l. The shoots obtained were transferred to rooting medium MS + NAA 2mg/l + Kinetin 0.5 mg/l. Higher percentage of success using above shooting and rooting media was also reported by Lee *et al*. (2002), Revathy *et al*. (2000) and Rodrangboon *et al*. (2002). Acclimatization while hardening is often the critical stage in the overall tissue culture cycle where losses can be high (Jagannathan, 1985).

Acknowledgement:

Junior Research Fellowship of The Indian Council of Agricultural Research, New Delhi, India is humbly acknowledged

REFERENCES

Abassi, F.M., Hamid, R. and Azra, Q. (2000). Regeneration effieciency and embryogenic callus production of three cultivars of rice. *Pakistani J. agric. Res.*, **16** : 97-99.

Bregitzer, P., Somers, D. A. and Rines, H.W. (1989). Development and characterization of friable callus. *Crop Sci.*, **29** : 798-803.

Deepthi, S., Shankhdar, S.C. and Pant, R.C. (2001). Genotypic variation of callus induction and plant regeneration in rice. *Indian J. Pl. Physiol.*, **6** : 261-264.

Gamborg, O.C. and Shyluk, J.P. (1981). Nutrition media and characteristics of plant cell and tissue cultures. Plant tissue culture- Methods and applications in Agriculture (eds. Thrope, T. A.). Academic press, New York, pp. 21-24.

Gomez, S.M. and Kalamani, A. (2002). Variability analysis of traits related to callus growth and plant regeneration in drought resistant local land cultivars of rice (*Oryza sativa* L.). *Asian J. Pl. Sci.*, **1**: 583-584.

Gonzalez, M.C. (2000). Effects of different growth regulators on *in vitro* culture of rice cv. AMISTAD - 82. *Cultivor trop. (Russian)*, **21**: 27-28.

Islam, MM, Ahmed, M. and Mahaldar, D. (2005). *In vitro* callus induction and plant regeneration in seed explants of rice (*Oryza sativa* L.). *Res. J. Agric. & Biol. Sci.*, **1** (1) : 72-75

Jagannathan, V. (1985). Factors influencing the survival of tissue culture plantlets in the field. In : *Biotechnology in Agriculture* (eds. Natesh, S., Chopra, V and Ramachandran, S.). Oxford and IBH Publishers, New Delhi, pp. 185-192.

Katiyar, S.K., Chandel, P.S. and Pratibha, R. (1999). Genetic variation and effects of 2, 4-D on *in vitro* plant regeneration in *indica* rice cultivars. *Oryza*, **36** : 254-256.

Lee, K., Jeon, H. and Kim, M. (2002). Optimization of a mature embryo-based *in vitro* culture system for high frequency somatic embryogenic callus induction and plant regeneration from *japonica* rice cultivars. *Pl. Cell Tissue Organ Cult.*, **71** : 237–244.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco cultures. *Physiol. Pl.*, **15** : 473-497.

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. *Plant Cell Tissue Organ Cul.*, **64** (26) : 185-210.

Revathy, N., Muthusamy, M.K., Sethuraman, Yesuraj, I.E., Rajeswari and Ramanathan, A. (2000). Effect of BAP on regeneration of IR50 and CO43 genotypes. *J. Ecol.*, **3** : 79-81.

Rodrangboon, P. P., Pontongkam, S., Suputtitada, T. and Adachi. (2002). Abnormal embryo development and efficient embryo rescue in inter-specific hybrids, *Oryza sativa* x *Oryza minuta* and *Oryza sativa* x *Oryza officinalis*. *Breeding Sci.*, **52** : 123-129.

Sakila, M., Ibrahim, S.M. and Kalamani, A. (1999). *In vitro* mutagenesis in scented rice cultivars. *Rice Biotech.*, **38** : 30-34

Singh, S. and Singh, H.P. (1996). Somatic cell culture of rice: II Dose effect of 2, 4- D and *in vitro* organ farming potential in different genotypes of *indica* rice (*Oryza sativa* L.). *Biol. Zbl. (German)*, **115** : 300-306.

Virk, G. S., Kaur, R. Grover, I. S. and Thukral, A. K. 1998. *In vitro* regeneration from immature embryo and cotyledon derived cultures of rice variety Pakistani Basmati. *J. Environ. Biol.*, **15** : 275-281.

Visarada, K.B.R.S. and Sarma, N.P. (2002). Qualitative assessment of tissue culture parameters useful in transformation of *indica* rice. *Curr. Sci.*, **82** : 343-347

Wang, Z.B., Pan, X.B., Tang, K.X. and Sun, Z.X. (2001). Studies on tissue culture of *indica* rice cultivars. *J. Yangzlou Univ. Nat. Sci.*, **4** : 37- 41

****
*****