ISSN: 1511-3701
Pertanika J. Trop. Agric. Sci. **32 (2)**: 185 - 194 (2009)

Somatic Embryogenesis from Scutellar Embryo of *Oryza sativa* L. var. MR219

Syaiful Bahri Panjaitan[1], Siti Nor Akmar Abdullah[1,2*], Maheran Abdul Aziz[2], Sariah Meon[1] and Othman Omar[3]

[1]Laboratory of Plantation Crop, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[3]Rice and Industrial Research Centre, Malaysia Agriculture Research and Development Institute (MARDI), Seberang Prai, Penang, Malaysia
[*]E-mail: sakmar@agri.upm.edu.my

ABSTRACT

Somatic embryogenesis is an efficient plant regeneration system and it is a potentially useful tool for genetic transformation. An experiment was carried out on somatic embryogenesis from scutellar embryo of rice var. MR219. High intensity of callus formation (100%) was initiated through culturing the scutellar embryo on modified MS medium, with the macro nutrients reduced to half-strength and supplemented with different 2,4-D concentrations (1, 2, 4 and 6 mgL^{-1}). Meanwhile, the highest percentage of embryogenic callus formation (80%) was obtained on the modified MS medium containing 4 mgL^{-1} 2, 4-D. The calli produced were yellowish and friable with nodular structures on the surface. Rounded cells with highly dense cytoplasm were also observed under an inverted microscope and their viability was confirmed based on the apple green fluorescence staining in the fluorescein diacetate (FDA) solution. High mean number of somatic embryos was also produced in this treatment, at 85 somatic embryos per explant. Upon transferring the somatic embryos onto the modified MS medium with 2 mgL^{-1} BAP and 0.05 mgL^{-1} NAA for germination, 82.5% of the somatic embryos were germinated into the seedlings.

Keywords: **Rice (*Oryza sativa* L.) var. MR219, 2,4-Dichlorophenoxyacetic acid, α-Naphthalenacetic acid, 6-Benzylaminopurine**

INTRODUCTION

Rice is the most important food crop in developing countries. In fact, it is the basic food for nearly half of the world's population, which is mostly concentrated in Asia. The production of rice has surged over the past 30 years, driven in the beginning by the doubling of yields and the expansion of the cultivated areas. Irrigated rice, which accounts for more than 75% of the global rice production, has been responsible for most of this production increase (IRRI, 1993).

Although rice production has so far kept up with the population growth, new studies suggest that an additional increase of 50 – 70% of the current supply is needed to meet the demand until 2025 (Pinggali *et al*., 1997). As the land resources are shrinking, the present trends

Received: 9 September 2008
Accepted: 24 March 2009
[*]Corresponding Author

suggest that tomorrow's rice land will be under even greater pressure (Greenland, 1997).

Tissue culture of rice, which has been developed in the last six decades, was started by Fujiwara and Ojima (1955), and Amemiya *et al.* (1956), by culturing the excised root and immature embryo on nutrient medium. High concentration of strong synthetic auxin, such as 2-4-Dichlorophenoxy acetic acid (2,4-D), has overcome the limitation of rice tissue culture by culturing the undifferentiated and meristematic tissue or the organ tissue explants at defined stage of development in nutrient medium using organogenesis as a pathway for plant regeneration (Ammirato, 1983). Furthermore, rice plant regeneration has been achieved from embryogenic calli derived from immature embryos (Heyser *et al*., 1983), mature seeds (Harke and Lorz, 1989), scutellum (Gupta *et al*., 1989), coleoptiles (Oinam *et al.*, 1995), and microspore (Datta *et al*., 1990). However, according to Bajaj and Rajan (1995), the establishment of the regeneration system of *indica* rice varieties requires a longer period as compared to *japonica* rice.

The MR219 variety is an indica rice hybrid which was produced by the Malaysian Agricultural Research and Development Institute (MARDI). The rice is very good in terms of its quality (shape and taste) and also high in yield. However, this variety is sensitive to environmental changes. In Malaysia, one of the major problems which limit high production of rice is water deficiency. Moreover, several other abiotic stress factors have also been found to affect the cultivation of rice; these include excess soil salinity (Zhu *et al.,* 2000) and temperature stress which affect all the stages of growth and development (Perales *et al.,* 2008).

Conventionally, rice plant can be genetically improved through crossing. However, it takes a long period of at least 2 years for a new variety to be released through such approach. Tissue culture technique, coupled with genetic transformation, can therefore be an alternative approach for improving the rice crop.

This study describes the establishment of somatic embryogenesis, from scutellar embryo of *Oryza sativa* L. var. MR219, which is a potential tool in genetic transformation of rice for high yield and quality improvement.

MATERIALS AND METHODS

Explant Materials and Surface Sterilization Procedure

Explant materials for this research were rice seeds variety MR219, obtained from the Rice and Industrial Research Centre, Malaysia Agriculture Research Development Institute (MARDI) Seberang Perai, Malaysia. Rice caryopses, containing scutellar region of embryo, were isolated by removing lemma and palea from the seeds. The caryopses were sterilized using 70% alcohol for 1 minute, followed by shaking in 40% Clorox containing a drop of Tween-20 on an orbital shaker, at 120 rpm for 40 minutes. Finally, the explants were rinsed with sterile distilled water for 5 times and cultured onto the medium with the different treatments tested in the study.

Basic Media and Treatments

Two basic media used in this study were modified N6 medium supplemented with 500 mgL^{-1} (w/v) L-glutamine and modified MS (Murashige and Skoog, 1962) medium with the macro nutrients reduced to half-strength and supplemented with 500 mgL^{-1} (w/v) of glutamine, 100 mgL^{-1} (w/v) of proline. Both the media were solidified with 0.2% (w/v) phytagel agar. The pH of the media was adjusted to 5.8. Different concentrations of 2,4-D [0, 1, 2, 4, and 6 mgL^{-1} (w/v)] were used as the treatments for embryogenic callus induction. These cultures were then kept at 25 $\pm 2^{\circ}C$ in the growth room (incubation room) in a dark condition for one week and followed by transferring the cultures under 16 hours lighting, provided by fluorescent bulbs with 15.75 $\mu molm^{-2}s^{-1}$ light intensity until the eighth week of culture. Meanwhile, the MS medium containing different concentrations of BAP (0, 0.5, 1, 2 and 4 mgL^{-1}), in combination with different concentrations of NAA (0, 0.01, 0.05 and 0.1 mgL^{-1}) were used as treatments for the

germination of somatic embryos. The cultures were kept at 25 ± 2˚C in the growth culture, with 16 hours of light, provided by fluorescent bulbs and a light intensity of 15.75 μmolm^{-2}s^{-1} for eight weeks.

Experimental Design and Statistical Analysis

The experiments were arranged in a Completely Randomized Design (CRD) as a single factor experiment, with four replications and each replication per treatment contained ten explants. The gathered data were analyzed using the analysis of variance (ANOVA), while the Duncan New Multiple Range Test (DNMRT), at $\alpha = 5\%$, was employed to carry out a comparison between the treatment means.

Data Recorded

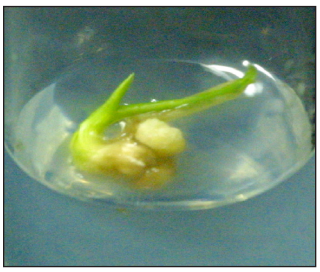
Data recorded in the somatic embryogenesis include the percentage of explant which responded to form callus (%), determination of callus viability based on fluorescein diacetate (FDA) method, the percentage of explant which responded to form embryogenic callus (%), and the mean number of somatic embryos produced per explant. Data were collected every two weeks until the eighth week of culture, while the growth characteristics were observed every week. The parameters on the somatic embryo germination recorded were the percentage of somatic embryo germination (%), the percentage of the normal plant (%) and the percentage of abnormal plant (%), produced after the eighth week of culture.

Assessment of Viability of Callus

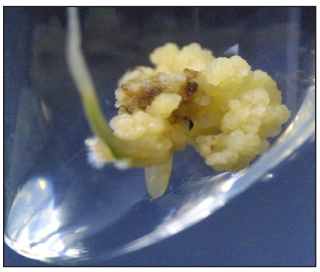
The fluorescein diacetate (FDA) method was used to assess the viability of the callus cells. Stock solution of FDA, at a concentration of 0.5%, was prepared in acetone and stored at 0˚C. Firstly, the scutellum derived callus produced was transferred to liquid MS medium containing 1 mgL^{-1} 2,4-D and the FDA solution was added to the cell suspension at a final concentration of 0.01 %. The mixture was incubated for 5 minutes, and the cells were finally illuminated with UV light and visualized under an inverted microscope. The viable cells gave green fluorescence.

RESULTS AND DISCUSSION

Based on the observation, the callus started to grow from scutellar embryo of rice, after three days of culture (Plate 1A). The scutellar embryo derived callus subsequently started to enlarge and some yellowish to greenish nodules grew around the explants after ten days of culture (Plate 1B). After six weeks of culture, calli almost covered the explants surface (Plate 3A). Nodular structure and globular somatic embryo-



(A)

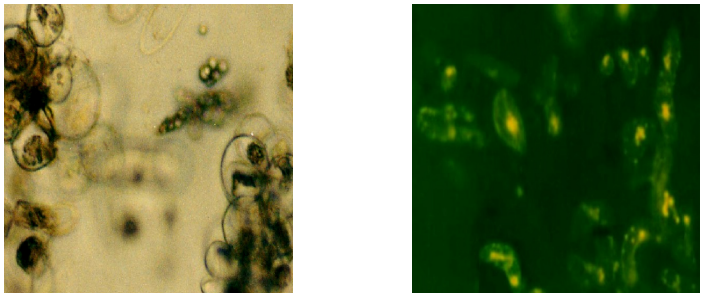


(B)

Plate 1: The developmental stages of embryogenic callus formation from scutellar embryo of rice (Oryza sativa L.) *var. MR219. Callus formation from the scutellar embryo of rice after three days of culture (A), and the scutellar embryo derived callus started to enlarge and some yellowish to greenish nodules grew at explants, after around ten days of culture (Bar=1cm)*

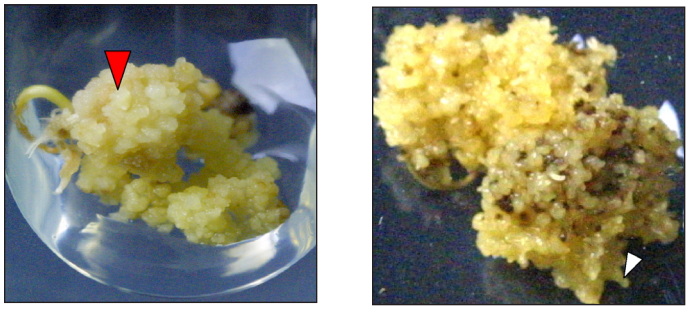
like structures were formed from the calli (Plate 3B). The somatic embryos were then transferred onto the MS medium containing different concentrations of BAP, in combination with different concentrations of NAA for germination (Plate 4A). After three weeks, the somatic embryos were germinated into seedlings (Plate 4B) and grew into a complete plant after sixth week of culture (Plate 4C).

Fig. 1 shows the effect of different media (MS and N6) containing different 2,4-D concentrations on the percentage of explant forming callus after the eighth week of culture. The presence of different 2,4-D concentrations (2, 4, 6, 8 mgL^{-1}) in the media tested gave a significant response for 100% callus formation; meanwhile the absence of 2,4-D (control) inside the media did not produce any callus (*Fig. 1*). The result indicated that 2,4-D was the most suitable to stimulate the formation of callus. However, no significant difference was observed between the 2,4-D treatments. Bonga and Aderkas (1992) stated that in large amount, phenoxy auxin (2,4-D) is a strong promoter of callus formation, while Matsuta and Hirabayashi (1989) stated that suitable concentration of 2,4-D would promote somatic embryogenesis. Although all the 2,4-D concentrations tested produced



(A) (B)

Plate 2: The assessment of callus viability. Rounded cells with highly dense cytoplasm confirmed the embryogenicity of the callus (A) and viable cells with dense cytoplasm fluoresced apple green when assessed using the FDA solution (B)



(A) (B)

Plate 3: The developmental stages of somatic embryo formation, whereby calli with nodule structures (red arrow) almost covered the explants surface after six weeks of culture (A) and nodular structure and globular somatic embryos (white arrow) formed from the callus (Bar= 1cm)

(A) (B) (C)

Plate 4: Stages of somatic embryos germination. Somatic embryos on MS medium containing different concentrations of 2 mgL^{-1} BAP in combination with the different concentrations of 0.05 mgL^{-1} NAA for germination (A) Somatic embryos germinating into seedlings after the third week on germination medium (B), and complete plants regenerated, after the eighth week of culture (C), (Bar=1cm)

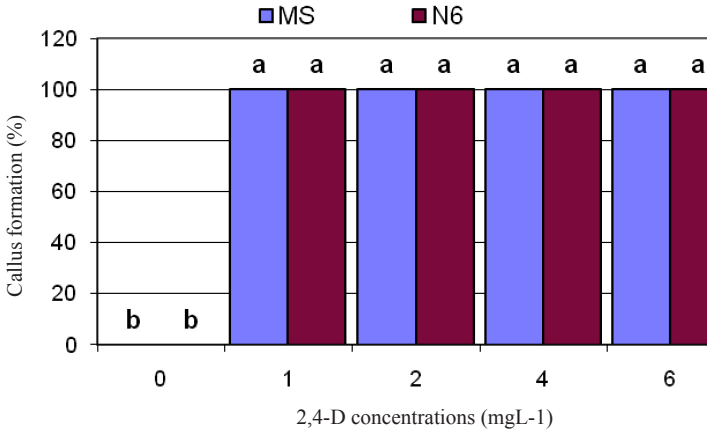


Fig. 1: The effect of the different media (MS and N6) containing different 2,4-D concentrations on the percentage of callus formation after eight weeks of culture

100% formation of callus, the cell viability test performed on the callus showed that not all callus produced were embryogenic. *Fig. 2* illustrates the effect of the different media (MS and N6) containing different concentrations of 2,4-D on the percentage of scutellar embryo of rice explants, producing embryogenic callus after the eighth week of culture. The highest percentage of embryogenic callus formation (80%) from scutellar embryo was observed on the modified MS medium containing 4 mgL^{-1}. Based on the data presented in *Fig. 2*, the growth trend on the percentage of explants produced embryogenic callus showed a quadratic pattern, whereby the addition of 2,4-D up to 4 mgL^{-1} in modified MS and N6 medium showed an increment on the percentage of the scutellar embryo of rice explant producing embryogenic callus. Nevertheless, when the 2,4-D concentration was increased to more than 4 mgL^{-1}, it resulted in a decrement on the percentage of explants forming embryogenic callus. The results indicated that the optimum concentration of 2,4-D was 4 mgL^{-1}. It is important to highlight that the embryogenicity

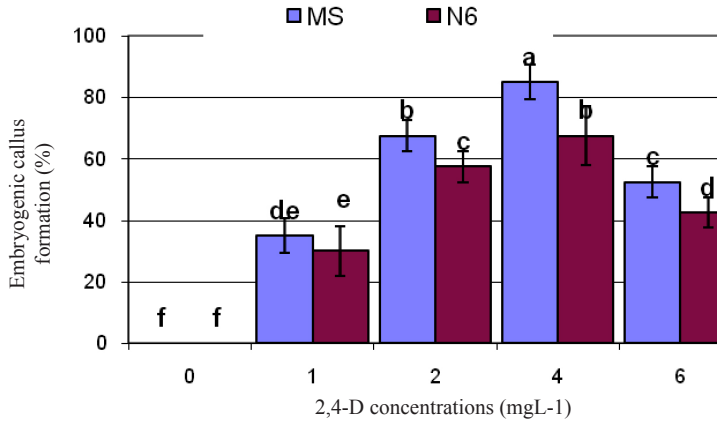


Fig. 2: The effect of the different media (MS and N6) containing different 2,4-D concentrations on the percentage of embryogenic callus formation, after eight weeks of culture

of callus produced is important for plant regeneration. Matsumoto (2003) reported that the selection and differentiation of embyogenic calli is necessary to get efficient regeneration. The cells of the callus were rounded with a highly dense cytoplasm, confirming the embryogenicity of the callus produced (Plate 2A) and furthermore, the cell viability of the callus was confirmed with the fluorescein diacetate (FDA) staining, visualized under an inverted microscope, illuminated with UV luminescence. The viable cells, with a dense cytoplasm and a healthy nucleus fluoresced apple green when assessed using the FDA solution (Plate 2B).

Fig. 3 depicts the effect of the different media (MS and N6) containing different concentrations of 2,4-D on the mean number of somatic embryos formed per explant. The highest mean number of somatic embryos formation (85) was found on the modified MS medium containing 4 mgL^{-1} 2,4-D. Based on *Figs. 2* and *3*, in comparison to N6 medium, the modified MS medium

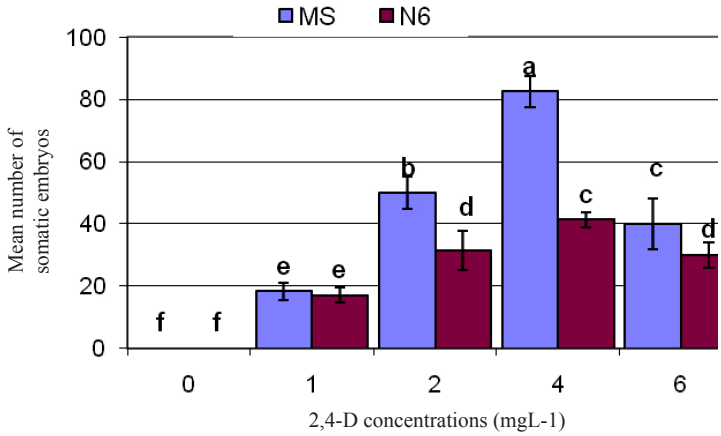


Fig. 3: The effect of the different media (MS and N6) containing different 2,4-D concentrations on the mean number of somatic embryos formed per explant after eight weeks of culture

combined with all the 2,4-D concentrations tested show a higher percentage of embryogenic callus formation from the scutellum embryo of rice explant and the highest mean number of somatic embryos formed per explant. This indicates that the MS medium, which is rich with nutrients is more suitable for the tissue culture of rice, as compared to the N6 medium (Table 2). Minocha (1987) reported that the presence of 2 mgL^{-1} 2,4-D, in the culture medium (MS) of *Pinus radiata* cotyledon explants, caused the formation of callus, while Sane *et al*. (2000) used the MS medium containing 2,4-D at 9.05µM for the induction of somatic embryo on *Acacia tortilis*. Ronald *et al.* (2005) made use of 1mgL^{-1} of 2,4-D for the somatic embryogenesis on slash pine (*Pinus ellotii* Engelm.) and Liu *et al*. (2001) reported that the presence of 4 mgL^{-1} 2,4-D, in the culture medium of rice seeds, caused the formation of embryogenic callus.

Table 1 shows the effect of the different BAP concentrations, in combination with the different concentrations of NAA, on the percentage germination of somatic embryo as well as the percentage of the normal and abnormal plants produced after the eighth week of culture. The normal plant was characterized by the normal growth with bipolar structure, which was indicated by containing shoot and root structures. Meanwhile, the abnormal plant was characterized by the stunted growth. In this study, the highest percentage of somatic embryos

TABLE 1
Effect of different BAP concentrations in combination with different concentrations of NAA on percentage of somatic embryo germination, percentage of normal plant and percentage of abnormal plant produced after the eighth week of culture

Plant Growth Regulators		Somatic embryo Germination (%)	Normal Plant (%)	Abnormal Plant (%)
BAP (mgL^{-1})	NAA (mgL^{-1})			
0	0	20.00 e	18.00 cd	2.00 f
0.5	0	22.50 de	15.50 cd	7.50 ef
1	0	25.00 cde	16.38 cd	8.62 ef
2	0	32.50 c	17.75 cd	14.75 bcd
4	0	23.75 cde	12.63 d	11.12 de
0.5	0.01	25.00 cde	16.75 cd	8.25 ef
1	0.01	27.50 cd	18.75 cd	8.75 ef
2	0.01	60.00 b	39.25 b	20.75 a
4	0.01	32.50 c	20.25 c	12.25 cde
0.5	0.05	21.25 de	12.63 d	8.62 ef
1	0.05	55.00 b	37.00 b	18.00 ab
2	0.05	82.50 a	66.00 a	16.50 abc
4	0.05	30.00 cd	18.50 cd	11.50 cde
0.5	0.1	25.00 cde	13.25 d	11.75 cde
1	0.1	60.00 b	39.00 b	21.00 a
2	0.1	60.00 b	41.75 b	18.25 ab
4	0.1	27.50 cde	13.00 d	14.50 bcd
CV (%)		15.90	9.30	12.30

Means followed by the same letter(s) in the same column are not significantly different using the Duncan New Multiple Range Test (DNMRT) at p=0.05

TABLE 2
The composition of the modified Murashige and Skoog (MS, 1962) and the modified N6 media

Elements	MS (mgL^{-1})	N6 (mgL^{-1})
Macro elements		
Calcium Chloride $CaCl_2$	332.02	166.00
Potassium Dihydrogen Phosphate KH_2PO_4	170.00	400.00
Potassium Nitrate KNO_3	1900.00	2830.00
Magnesium Sulfate $MgSO_4$	180.00	180.00
Ammonium Nitrate NH_4NO_3	1650.00	-
Diammonium sulphate $(NH_4)_2NO_3$		463.00
Micro elements		
Cobalt Chloride $CoCl_2\ 6H_2O$	0.025	-
Cuprum Sulfate $CuSO_4\ 5H_2O$	0.025	-
Boric Acid H_3BO_3	6.20	1.60
Potassium Iodide KI	0.83	0.80
Manganese Sulfate $MnSO_4\ 4H_2O$	16.90	4.40
Sodium Molybdate $Na_2MoO_4\ 2H_2O$	0.25	-
Zinc Sulfate $ZnSO_4\ 7H_2O$	8.60	1.50
Vitamins		
Glycine $C_2H_5NO_2$	2.00	2.00
Nicotinic Acid $C_6H_5NO_2$	0.50	0.50
Pyridoxine $C_8H_{11}NO_3$	0.50	0.50
Thiamine $C_{12}H_{17}CIN_4O_5$	0.10	1.00
Iron		
Disodium ethylenediaminetetraacetic acid Na_2EDTA	37.25	37.25
Ferrous Sulfate $FeSO_4\ 7H_2O$	27.85	27.85
Others		
Myo-inositol	100	100
Sucrose	30,000	30,000
L-glutamine	500	500
L-proline	-	100
Pytagel agar	2000	2000

germinated into the plant (82.5%) was observed, with 2 mgL^{-1} BAP, in combination with 0.05 mgL^{-1} NAA, and this treatment was also found to produce the highest percentage of the normal plant (66%). Yang *et al*. (1996) stated that the combination of auxin (NAA) and cytokinin (BAP) could enhance the percentage of somatic embryo germination in papaya. On the contrary, Craig *et al.* (1997) obtained somatic embryos germination in *Moricandia arvensis* when the somatic embryos from an auxin medium were transferred into an auxin-free medium.

Based on the data presented in Table 1, the MS medium without both BAP and NAA (MS0) could also result in somatic embryos germinating into seedlings (20%) although with the low percentage of somatic embryos germinating into plant, and 18% of the normal plants were obtained from this treatment. These results indicated that the hormone-free MS medium could be used for the recovery of plants from somatic embryos. Nevertheless, the presence of BAP and NAA was most suitable for somatic embryo germination in rice MR219. Based on the findings of this study, the treatment containing 2 mgL^{-1} BAP combined, with 0.05 NAA, could be chosen as the ideal germination medium for this particular variety.

CONCLUSIONS

Callus was successfully induced on the modified MS and N6 media, containing 2,4-D from scutellar region of rice caryopses embryo var. MR. 219. The study found that eighty percent of embryogenic callus and 85 somatic embryos were produced from the scutellar region on the modified MS medium containing 4 mgL^{-1} 2,4-D. Meanwhile, the MS medium containing 2 mgL^{-1} BAP, in combination with 0.05 mgL^{-1} NAA, resulted in 82.5% of the somatic embryo germination into seedlings. The somatic embryogenesis from the scutellum embryo of rice, developed in this study, could therefore serve as a potential tool with an important application in genetic transformation and production of quality planting materials.

REFERENCES

Amemiya, A., Akemine, H. and Toriyama, K. (1956). Culture condition and growth of immature embryo culture in rice plant. *Bulletin of the National Institute of Agricultural Sciences* D6, 1 – 40.

Ammiratio, P.V. (1983). Embrygenesis. In D.A. Evan, W.R. Sharp, P.V. Ammirato, and Y.Y. Yamada (Eds.), *Hand Book of Plant Cell Culture* (Vol. 1, pp. 82 – 113). New York: MacMillan Publishing Co.

Bajaj, S., and Rajan, M.V. (1995). Efficient plant regeneration from long term callus culture of rice by spermidice. *Plant Cell Reports, 14,* 717 – 720.

Bonga, J.M. and Aderkas, P.V. (1992). *In Vitro* Culture of Trees. *Forestry Sciences* (Vol. 38, pp. 75 – 76). The Netherlands: Kluwer Academic Publishers.

Craig, H., Wiegand, A., O'Neill, C.M., Mathias, R.J., Power, J.B. and Davey, M.R. (1997). Somatic embryogenesis and plant regeneration from stem explants of *Moricandia arvensis*. *Plant Cell Reports, 17,* 27 – 31.

Datta, S.K., Peterhans, A., Datta, K. and Potrykus, I. (1990). Genetically engineered fertile *indica* rice recovered from protoplasts. *BioTechnology, 8,* 736 – 740.

Fujiwara, A. and Ojima, K. (1955). Physiological studied on plant root (Part 1). Influent of some environment condition on growth of isolated root and rice and wheat. *Journal of Soil Science, Manuse Japan, 28,* 9 – 12.

Greenland, D.J. (1997). *The Sustainability of Rice Farming*. pp. 273. Wallingford (UK).

Gupta, H.S., Pattanayak, A., Bhuyan, R.N. and Pandey, D.K. (1989). Cytokinin mediated induction of embryogenic calli and plant regeneration in indica rice (*Oriza sativa*). *Indian Journal of Agricultural Sciences, 59*(8), 526 – 528.

Harke, S. and Lorz, H. (1989). Somatic embryogenesis and plant regeneration from various indica rice (*Oryza sativa*) genotypes. *Journal of Genetics and Breeding, 43,* 205 – 214.

Heyser, J.W., Dykes, T.A., Demott, K.J. and Nabors, M.W. (1983). High frequency long-term regeneration of rice from callus culture. *Plant Science Letters, 29,* 175 – 182.

International Rice Research Institute (IRRI). (1993). IRRI rice almanac. PO Box 933. Manila, Philippines, pp. 1 – 10.

Liu, C., Kwanhoon, L., Honda, M.H. and Kobayashi, T. (2001). Enhanced regeneration of rice (*Oryza sativa* L.) embryogenic callus by light irradiation in growth phase. *Journal of Bioscience and Bioengineering*, *91*(3), 319 – 321.

Matsumoto, K. (2003). Micro-propagation of bananas. In S. Mohan Jain and K. Ishii (Eds.), *Micro-propagation of Woody Trees and Fruits* (75, 353 – 380). Kluwer Academic Publishers.

Minocha, S.C. (1987). Plant growth regulators and morphogenesis in cell and tissue culture of forest trees. In J.M. Bonga and D.J. Durzan (Eds.), *Cell and Tissue Culture in Forestry* (1, 125 – 141). General Principles and Biotechnology, Dordrecht: The Netherlands, Martinus Nijhoff Publishers.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum, 15,* 473 – 497.

Oinam, G.S. and Kotharii, S.L. (1995). Totipotency of coleoptile tissue in indica rice (*Oryza sativa* L. cv. Ch1039). *Plant Cell Reports, 14,* 245 – 248.

Perales, L., Penarrubia, L. and Cornezo, M.J. (2008). Induction of polyubiquitin gene promoter by dehydration stresses in transformed rice cells. *Journal of Plant Physiology, 165,* 159 – 171.

Pinggali, P.L., Hossain, M. and Gervacio, R.V. (1997). Asian rice bowls- The returning crisis. Wallingford (UK). CAB International, pp. 341.

Ronald, J.N., Tang, W. and Jain, S.M. (2005). Slash fine (*Pinus elliotii* Engelm.). In M.S. Jain and K. Gupta (Eds.), *Protocol for Somatic Embryogenesis in Woody Plants* (77, 1 – 10). Dordrecht: The Netherlands, Springer.

Sane, D., Borgel, A., Verdeil, J.L. and Gassama-Dia, Y.K. (2000). Plantlets regeneration via somatic embryogenesis in immature zygotic embryo calli from a tree species adapted to arid lands: *Acacia tortilis* Subsp. Raddiana (savi.) Brenan. *Acta Botanical Gallica*, *147,* 257 – 266.

Yang, J.S., Yu, T.S., Cheng, Y.H. and Yeh, S.D. (1996). Transgenic papaya plants from *Agrobacterium*-mediated transformation of petioles of *in vitro* propagated multishoots. *Plant Cell Reports, 15,* 459 – 464.

Zhu, G.Y., Kinet, J.M., Bertin, P., Bouharmont, J. and Lutts, S. (2000). Crosses between cultivars and tissue culture-selected plants for salt resistance improvement in rice, *Oryza sativa*. *Plant Breeding, 119,* 497 – 504.