ISSN 1996-0700

Asian Journal of Biotechnology

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

Asian Journal of Biotechnology 3 (5): 470-477, 2011
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2011.470.477

Anther Culture Response in Boro Rice Hybrids

Chaitali Sen and R.P. Singh
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi -221005, India

Corresponding Author: Chaitali Sen, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi -221005, India

ABSTRACT

The objective of the present study was to determine the effect of genotype and culture media in anther culture of boro rice hybrids. Anthers from three f_1 hybrids of boro×high yielding *indica* rice varieties Gautam×BPT 5204, Krishna Hansa×NDR 359 and IR64×NDR 359 were cultured on modified SK media and modified N6 media. Modified SK media was found to be more suitable for callus formation in all the f_1 hybrids. The cross Gautam×BPT 5204 showed maximum callus induction frequency followed by Krishna Hansa×NDR 359. Only the cross of Krishna Hansa×NDR 359 was responsive to green plant regeneration while IR64×NDR359 and Gautam×BPT 5204 resulted in only albino plants. Anther culture response is influenced by the genotype and media composition in *indica* rice hybrids.

Key words: Anther culture, boro rice, genotype, callus, plant regeneration

INTRODUCTION

Traditional boro rice is a unique type of *Oryza sativa* grown during the winter season (minimum temperature around 4-10°C) in the depressions around rivers and lakes (Singh *et al*., 2003). It makes a significant contribution in enhancing the overall rice production in eastern India. Genetic improvement in boro rice can be made by the application of biotechnological tools like anther culture in superior hybrids of boro rice.

Androgenesis in flowering plants provides an understanding of the biological basis of single cell microspore embryogenesis to the production of a doubled haploid plant. This system provides an excellent opportunity to shorten the breeding cycle for rapid production of doubled haploids and fix agronomic traits (Suriyan *et al*., 2009). There are many factors that can affect the success of anther culture, such as the maturity of the donor plant (Afza *et al*., 2000; Jacquard *et al*., 2006), panicle pretreatment (Trejo-Tapia *et al*., 2002), microspore developmental stages (Afza *et al*., 2000; Cha-Um *et al*., 2009). In almost all species genotype no doubt is a deciding factor in achieving success in anther culture response (Ramakrishnan *et al*., 2005; He *et al*., 2006). The genetic makeup of *indica* subspecies makes them recalcitrant to *in vitro* anther culture.

However, components of tissue culture media are also important (Faruque *et al*., 1998; Asaduzzaman *et al*., 2003) and have been demonstrated to have a crucial role in coaxing an *in vitro* response from cultured anthers of otherwise recalcitrant genotype. Monirul *et al*. (2004) observed that addition of 1 mg L^{-1} 2, 4-D and 1 mg L^{-1} kinetin to callus induction medium improved the callus induction and regeneration potential of the responsive hybrid rice line IR-69690. Hassan *et al*. (2001) observed direct regeneration of maize hybrid M95×S 95 and inbred S2-9 on N6 medium supplemented with 2, 4-D (2 mg L^{-1}) and kinetin (1.5 mg L^{-1}).

Genotype may differ widely in their basal media requirement for dedifferentiation and redifferentiation. Therefore, in the present investigation, efforts were made to analyze the effect of genotype and different media formulations on callus induction and plant regeneration in three boro rice hybrids of Gautam×BPT 5204, Krishna Hansa×NDR 359 and IR 64×NDR359.

MATERIALS AND METHODS

Hybridization and anther culture experiments were carried out in three f_1 hybrids Gautam× BPT 5204, Krishna Hansa×NDR 359 and IR 64×NDR 359, in 2008-2009. Crosses were made between boro rice varieties Gautam, Krishna Hansa and IR64 (females) possessing cold tolerance and high yielding rice varieties BPT 5204 and NDR 359 (males) in Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Boots (Panicles) were collected from the primary tillers of the selected hybrids in morning hours (7.00, 8.00 a.m.) when the middle florets of the panicles contain uninucleate microspores in their anthers. Boots were wrapped in moist tissue paper covered with aluminum foil and were kept in refrigerator for 8-10 days for cold pretreatment. Sterilization of panicles was carried out by dipping intact boots in 70% alcohol for 2 min. Panicles were surface sterilized with 0.1% aqueous $HgCl_2$ solution for 8 min and rinsed several times with distilled water. The outer covering of the panicle was removed with sharp scalpel and spikelet was cut at the base to excise the anthers. Anthers from f_1 hybrids were inoculated for callus induction on modified N6 and modified SK media (Table 1). Thirty anthers

Table 1: Media composition for callus induction

Constituents	Modified N6 in mg L^{-1}	Modified SK mg L^{-1}
KNO_3	2,830	3150
$(NH_4)_2 SO_4$	463	230
$MgSO_4 7H_2o$	185	185
$KH_2 PO_4$	400	540
$CaCl_2 2H_2O$	166	440
H_3BO_3	1.6	6.2
$MnSO_4. 4H_2O$	22.3	22.3
$ZnSO_4.\ 7H_2O$	1.5	1.5
$Na_2MoO_4.2H_2O$	0.25	0.25
$CuSo_4 .5H_2O$	0.025	0.025
$CoCl_2.\ 6H_2O$	0.025	0.025
KI	0.83	1.0
$FeSO_4. 7 H_2O$	27.8	27.8
$Na_2EDTA. 2H_2O$	37.3	37.3
myo-inositol	100	100
Thiamine HCl	2.5	2.5
Nicotinic acid	2.5	2.5
Pyridoxine HCl	2.5	2.5
Glycine	2.0	2.0
AgNO3	-	8
Kinetin	0.5	0.5
2,4-D	1.0	1.0
NAA	2.0	2.0
Casein hydrolysate	-	500
Sucrose	30000	-
Maltose	-	30000
Agar	8000	8000

The-sign in the blank boxes *indica*te the absence of that particular compound in the media composition. N6 Media by Chu *et al*. (1975) SK media by Raina and Zapata (1997)

were inoculated in each culture tube containing 10.0 mL medium. There were ten test tubes per replication and three replications were used for a particular media concentration for a particular hybrid. The cultures were incubated in complete darkness at 25±1°C for 4-5 weeks for callus induction. The culture tubes were examined periodically at weekly intervals to observe the progress in respect of callus formation. Data on percentage of callus regenerating was recorded. Calli of at least 2 mm diameter were transferred to culture tubes containing 20 mL regeneration medium consisting of MS media supplemented with 2 mg L^{-1} BAP, 1 mg L^{-1} Kinetin, 1 mg L^{-1} NAA. The pH of the medium was adjusted to 5.8 with 1 N HCl or 1 N NaOH before adding agar and autoclaving. The culture tubes were plugged with non-absorbent cotton wrapped in cheese cloth. Cultures were inoculated for four weeks under 16/8 light/dark at 25±2°C. Green and albino plantlet regeneration percentage were observed and recorded. The unrooted green shoots of transferable sizes were transferred into rooting medium (half strength MS medium supplemented with 2 of NAA and 0.1 mg L^{-1} kinetin). The cultures were kept in growth chamber at 4000-lux[1] cool fluorescent light at 25±1°C for plantlet (shoot with root) regeneration.

RESULTS

The responsive anthers showed slight swelling around it and subsequently started callusing asynchronously after 3-4 weeks (Fig. 1a, b). Table 2 exhibits the callus induction frequencies. Callus induction was observed in all the three f_1 hybrids however, induction frequency varied with the genotype and medium. The anther culture response was better on modified SK medium as compared to modified N6 media for all the rice hybrids studied. Out of three crosses evaluated, maximum callus induction frequency from anthers was observed in the cross Gautam×BPT 5204 (5.77) on modified SK media and minimum was found in IR64×NDR 359 (0.22) on modified N6 medium.

Calli derived from the anthers of three rice hybrids were transferred to regeneration (MS) media for shoot regeneration. Anther calli on differentiation gave rise to shoots in all the genotypes under study but shoot differentiation varied with the genotype (Fig. 1c, d). It is evident from Table 3 that green shoot regeneration was observed only in Krishna Hansa×NDR 359 (8.95%) (Fig. 1e), Maximum shoot regeneration was observed in Gautam×BPT 5204 but all the shoots were albino. Minimum shoot regeneration was observed in the cross IR 64×NDR 359 however, shoots devoid of chlorophyll (albino) regenerated in all the three crosses (Fig. 1f). Initiation of roots in rooting media is shown in Fig. 1g and hardening and acclimitazation of green plants is shown in Fig. 1h.

Table 2: Callus induction frequency

		Modified N6 media		Modified SK media	
F_1 hybrid	No. of anthers plated	No of anthers responded	Callusinduction frequency	No. of anthers responded	Callus induction frequency
GAUTAM×BPT 5204	900	38	4.22	52	5.77
KRISHNA Hansa×NDR 359	900	13	1.44	28	3.11
IR64×NDR 359	900	2	0.22	7	0.78

[1]The lux (symbol: lx) is the SI unit of illuminance and luminous emittance measuring luminous power per area. It is used in photometry as a measure of the intensity, as perceived by the human eye, of light that hits or passes through a surface. One lux is equal to one lumen per square metre: $1\ lx = 1\ l\ m/m^2 = 1\ cd \cdot sr \cdot m^{-2}$

Table 3: Green and albino shoot regeneration frequency

		Calli regenerating green shoots		Calli regenerating albino shoots	
F_1 hybrid	No. of calli plated	No. of calli regenerating green shoots	Green plant regeneration frequency	No. of calli regenerating albino shoots	Albino plant regeneration frequency
GAUTAM×BPT 5204	75	-	-	17	22.67
KRISHNA Hansa×NDR 359	67	6	8.95	11	16.41
IR64×NDR 359	45	-	-	5	11.11

The blank (-) represents no regeneration of plants from calli in that particular hybrid

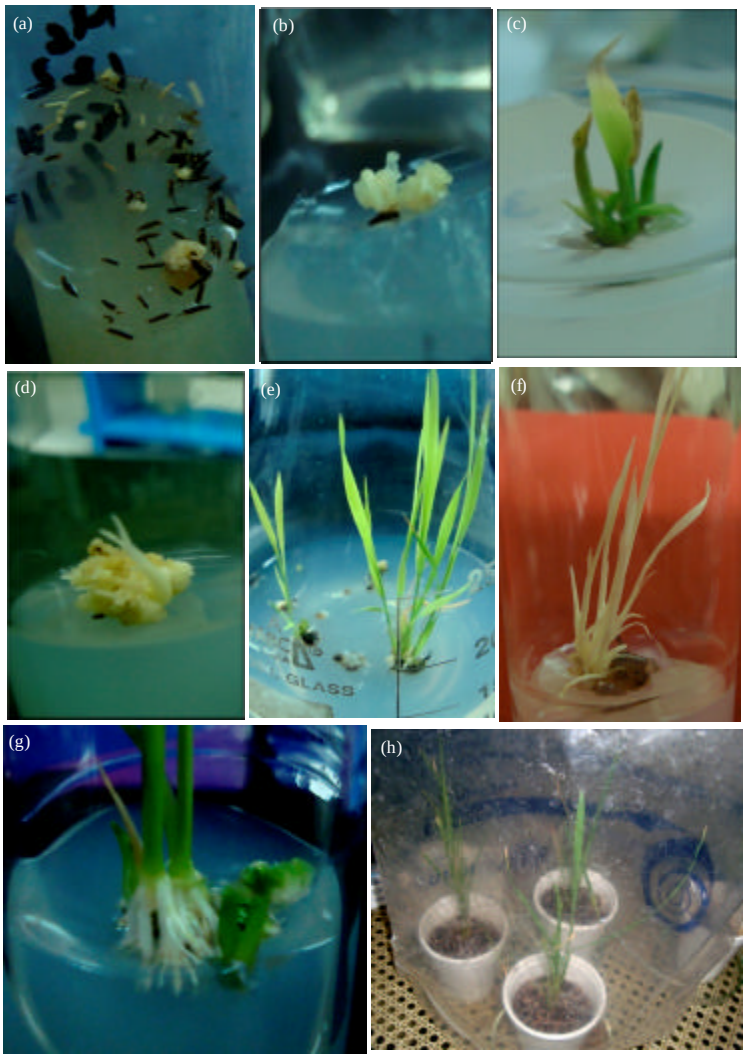

Fig. 1(a-h): Callus induction from anthers ,regeneration, rooting and hardening of boro rice hybrids (a) Callus induction, (b) Callus proliferation, (c) Initiation of green shoots in Krishna Hansa×NDR 359, (d) Initiation of albino shoots, (e) Elongation of green shoots in Krishna Hansa×NDR 359, (f) Elongation of albino shoots, (g) Initiation of roots in rooting media, (h) Hardening and acclimitazation of green plants

DISCUSSION

Genotype and nutrient composition of the culture media are the major sources of variation in *in vitro* culture and regeneration (Bishnoi *et al.*, 2000; Talebi *et al.*, 2007). The presence of significant variation in callus induction due to genotype, media composition and genotype×media interaction was observed by Bagheri and Jelodar (2008). Niroula and Bimb (2009) demonstrated the possibility of enhancing androgenic response by manipulating media composition using responsive genotypes of Nepalese rice cultivars.

N6 medium (Chu *et al.*, 1975) which has been widely used for anther culture was found less suitable for *indica* rice anther culture (Gosal *et al.*, 1997). The *indica* cultivars require lower NH_4^+ ions therefore in the modified SK media amount of $(NH_4)_2\ SO_4$ was reduced.

In the present study the presence of maltose as a carbon source along with $AgNO_3$ and Casein Hydrolysate (CH) in the modified SK media, have enhanced the androgenic potential in boro rice hybrids. The callus induction frequency was enhanced in all the hybrids by replacing sucrose with maltose in the SK medium. This result of increase in the callus induction frequency by the use of maltose have been supported by the study with barley microscope culture (Hunter, 1987) wheat (Mejza *et al.*, 1993) and rice (Xie *et al.*, 1995). One mechanism by which maltose may influence androgenic response is through differences in the breakdown products of the two disaccharides. Sucrose is hydrolyzed to glucose and fructose by invertase, both of these monosaccharides have been found to inhibit anther culture response.

The positive effect of maltose was associated with keeping a high proportion of swollen microspores and increasing their division rate whereas sucrose plasmolyze the microspores. Kasha *et al.* (1990) reported plasmolysis followed by dying of microspores at 3% sucrose containing media. Further maltose stabilizes the culture medium osmotically (Kuhlmann and Foroughi-Wher, 1989) and it releases glucose at a slow rate after degradation (Last and Brettell, 1990).

The 8 mg L^{-1} of silver nitrate which acts as an ethylene antagonist was added in, modified SK media. It has been reported that the presence of ethylene in the culture medium inhibits somatic embryogenesis and shoot regeneration (Biddington, 1992; Vain *et al.*, 1989). Ethylene is produced by plant cells in closed culture vessel (Gamborg and LaRue, 1971) and the gelling agent like agar (Mensuari *et al.*, 1992). Some studies showed that the presence of auxin particularly 2, 4-D stimulates ethylene production (Yang and Hoffman, 1984). $AgNO_3$ has been employed in tissue culture studies as it interferes in the ethylene perception mechanism. Further it is easily soluble in water and is not phytotoxic at effective concentrations (Beyer, 1976). Casein hydrolysate which is a source of calcium, several micronutrients, vitamins and amino acids, was added in modified SK media. Improvement in callus induction and growth by the addition of casein hydrolysate was also reported by Khaleda and Al-Forkan (2006) in deepwater rice.They also stated that callus formation and plant regeneration was influenced by interaction of media components.

Differential response for callus induction was found in the three f_1 hybrids with different media. Callus induction frequency as determined by anthers forming calli varied between 0.22 to 5.77% depending upon the culture medium and the genotype. Shahnewaz *et al.* (2004) also observed a callus induction frequency ranging from 1.42 to 8.06%.

Maximum callus induction was observed in the cross of Gautam×BPT 5204 but it lacked the ability to produce green plants. Krishna Hansa×NDR 359 was the only hybrid that exhibited green plant regeneration potential. This indicates that genotype also acts as an important factor for the production of green plants. This is in accordance with the studies reported by Raina and Zapata

(1997), Zhu *et al.* (1991) and Xie *et al.* (1995) that medium used for callus induction and genotype of the donor significantly affected green plant regeneration potential. Plant regeneration was observed in MS media with different combination and concentration of BAP, Kinetin and NAA by (Jubair *et al.*, 2008). In contradiction to the present study, Roy and Mandal (2011) clearly indicated that higher concentration of BAP had inhibitory effects on microtillering of androgenic plantlets of rice variety IR 72.

The recovery of albino plants from microspore derived calli in rice especially in *indica* rice varieties has been a formidable obstacle to the utilization of rice anther culture for *indica* rice improvement (Chen *et al.*, 1991; Raina and Zapata, 1997; Sripichitt *et al.*, 2000; Chowdhury and Mandal, 2001).

Occurrence of albino plant regeneration seems to be a common phenomenon in rice anther culture, as the albino shoot regeneration frequencies were more in all the crosses as compared to the green shoot regeneration frequencies in the present study. In general, albino plants (e.g., wheat, barley and rice) contain deleted forms of the plastid genome (Day and Ellis, 1985; Harada *et al.*, 1991; Zubko and Day, 2002).

CONCLUSION

The present study indicate that the effect of genotype and media composition significantly affected callus induction frequency in the f_1 hybrids. Nevertheless the modified SK media can be considered more favorable for induction of callus in the f_1 hybrids of boro×high yielding rice varieties. The cross of Krishna Hansa×NDR 359 was observed to be most responsive to anther culture for the production of green shoots and therefore this cross can be further exploited for the production of doubled haploids.

REFERENCES

Afza, R., M. Shen, F.J. Zapata-Arias, J. Xie and H.K. Fundi *et al.*, 2000. Effect of spikelet position on rice anther culture efficiency. Plant Sci., 153: 155-159.

Asaduzzaman, M., M.A. Bari, M.H. Rahman, N. Khatun, M.A. Islam and M. Rahman, 2003. *In vitro* plant regeneration through anther culture of five rice varieties. J. Biol. Sci., 3: 167-171.

Bagheri, N. and N.B. Jelodar, 2008. Combining ability and heritability of callus induction and green-plant regeneration in rice anther culture. Biotechnology, 7: 287-292.

Beyer, E.M., 1976. Silver ion: A potent anti-ethylene agent in cucumber and tomato. HortScience, 11: 175-196.

Biddington, N.L., 1992. The influence of ethylene in plant tissue culture. Plant Growth Regul., 11: 173-178.

Bishnoi, U.S., R.K. Jain, K.R. Gupta, V.K. Chowdhury and J.B. Chowdhury, 2000. High frequency androgenesis in *indica* x basmati rice hybrids using liquid culture media. Plant Cell Tiss. Org. Cult., 61: 153-159.

Cha-Um, S., B. Srianan, A. Pichakum and C. Kirdmanee, 2009. An efficient procedure for embryogenic callus induction and double haploid plant regeneration through anther culture of Thai aromatic rice (*Oryza sativa* L. subsp. *indica*). *In vitro* Cell. Dev. Biol. Plant, 45: 171-179.

Chen, C.C., H.S. Tsay and C.R. Huang, 1991. Factors Affecting Androgenesis in Rice (*O. sativa* L.). In: Biotechnology in Agriculture and Forestry Rice, Bajaj, Y.P.S. (Ed.). Vol. 14, Springer Verlag, Berlin, ISBN: 354051810X, pp: 193-215.

Chowdhury, B. and A.B. Mandal, 2001. Microspore embryogenesis and fertile plantlet regeneration in a salt susceptible × salt tolerant rice hybrid. Plant Cell Tiss. Org. Cult., 65: 141-147.

Chu, C.C., C.C. Wang, C.S. Sun, K.C. Hsu, K.C. Yin, C.Y. Chu and F.Y. Bi, 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sinica, 18: 659-668.

Day, A. and T.H.N. Ellis, 1985. Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr. Genet., 9: 671-678.

Faruque, M.O., T. Farzana, Z.I. Seraj, R.H. Sarker and S.A.A. Khatun, 1998. Variations in green plant regeneration response from anthers of *indica* rice and their hybrids with *japonica* cv. Taipei 309. Plant Cell Tissue Organ Cult., 54: 191-195.

Gamborg, O.L. and T.A.G. LaRue, 1971. Ethylene production by plant cell culture. Physiol. Plant, 48: 394-401.

Gosal, S.S., A.S. Sindhu, J.S. Sandhu, R. Sandhu-Gill, B. Singh, G.S. Khera and H.S. Dhaliwal, 1997. Haploidy in Rice. In: Cereals. *In vitro* Haploid Production in Higher Plants, Jain, S.M., S.K. Sopory and R E. Veilleux (Eds.). Vol. 4, Kluwer Academic Publishers, Dordrecht, pp: 1-35.

Harada, T., T. Sato, D. Asaka and I. Matsukawa, 1991. Large-scale deletions of rice plastid DNA in anther culture. Theor. Applied Genet., 81: 157-161.

Hassan, L., S.D. Ahmad and A. Okumus, 2001. The direct regeneration of maize haploids through anther culture. J. Biological Sci., 1: 900-901.

He, T., Y. Yang, S.B. Tu, M.Q. Yu and X.F. Li, 2006. Selection of interspecific hybrids for anther culture of *indica* rice. Plant Cell. Tissue Organ. Cult., 86: 271-277.

Hunter, C.P., 1987. Plant regeneration method. European Patent Application No. 0245 898 A2. pp: 1-8.

Jacquard, C., R. Asakaviciute, A.M. Hamalian, R.S. Sangwan, P. Devaux and C. Clement, 2006. Barley anther culture: Effects of annual cycle and spike position on microspore embryogenesis and albinism. Plant Cell Rep., 25: 375-381.

Jubair, T.A., U. Salma, N. Haque, F. Aktar, I.J. Mukti, A.K.M.F. Haque and M.R. Ali, 2008. Callus induction and regeneration of local rice (*Oryza sativa* L.) variety topa. Asian J. Plant Sci., 7: 514-517.

Kasha, K.J., A. Ziauddin and E. Simion, 1990. Barley and wheat microspore culture. Abstr. 7th Int. Congress on Plant Cell Tissue Culture, Amsterdam, pp: 187.

Khaleda, L. and M. Al-Forkan, 2006. Stimulatory effects of casein hydrolysate and proline *in vitro* callus induction and plant regeneration from five deepwater rice (*Oryza sativa* L.). Biotechnology, 5: 379-384.

Kuhlmann, U. and B. Foroughi-Wher, 1989. Production of doubled haploid lines in frequencies sufficient for barley breeding programs. Plant Cell Rep., 8: 78-81.

Last, D.I. and R.I.S. Brettell, 1990. Embryoid yield in wheat anther culture is influenced by the choice of sugar in the culture medium. Plant Cell Rep., 9: 14-16.

Mejza, S.J., V. Morgant, D.E. DiBona and J.R. Wong, 1993. Plant regeneration from isolated microspores of *Triticum aestivum*. Plant Cell Rep., 12: 149-153.

Mensuari, S.A., M. Panizza and E. Tognoni, 1992. Quantification of ethylene losses in different container seal systems and comparison of biotic and abiotic contributions to ethylene accumulation in cultured tissues. Physiol. Plant., 84: 472-476.

Monirul, I.M, K.A. Sanjay, G. Purnendu, Md. R. Mizanur and S. Noor-e-Alam, 2004. Effect of plant growth regulators on callus induction and plant regeneration in anther culture of rice. Pak. J. Biol. Sci., 7: 331-334.

Niroula, R.K. and H.P. Bimb, 2009. Effect of genotype and callus induction medium on green plant regeneration from anther of Nepalese rice cultivars. Asian J. Plant Sci., 8: 368-374.

Raina, S.K. and F.J. Zapata, 1997. Enhanced anther culture efficiency of *indica* rice (*Oryza sativa* L.) through modification of the culture media. Plant Breed., 116: 305-315.

Ramakrishnan, S.H., S. Saravanan, C.R. Anandakumar and J.R. Kannanbapu, 2005. *In vitro* androgenesis in rice (*Oryza sativa* L.). Asian J. Plant Sci., 4: 600-602.

Roy, B. and A.B. Mandal, 2011. Profuse microtillering of androgenic plantlets of elite *indica* rice variety IR 72. Asian J. Biotechnol., 3: 165-176.

Shahnewaz, S., M.A. Bari, N.A. Siddique and M.H. Rahman, 2004. Effects of genotype on induction of callus and plant regeneration potential *in vitro* anther culture of rice (*Oryza sativa* L.). Pak. J. Biol. Sci., 7: 235-237.

Singh, R.K., H. Mahabub and R. Thakur, 2003. Boro Rice. Fine Grains Pvt Ltd., IRRI, New Delhi, pp: 1-3.

Sripichitt, P., T. Ozawa, M. Otani and T. Shimada, 2000. Improved method for anther culture of an *indica* rice cultivar of Thailand. Plant Prod. Sci., 3: 254-256.

Suriyan, C., S. Bootsaya, P. Aussanee and K. Chalermpol, 2009. An efficient procedure for embryogenic callus induction and doubled haploid plant regeneration through anther culture of Thai aromatic rice (*Oryza sativa* L. subsp. *indica*). *In vitro* Cell. Dev. Biol. Plant, 45: 171-179.

Talebi, R., M.R. Rahemi, H. Arefi, M. Nourozi and N. Bagheri, 2007. *In vitro* plant regeneration through anther culture of some Iranian local rice (*Oryza sativa*) cultivars. Pak. J. Biol. Sci., 10: 2056-2060.

Trejo-Tapia, G., U.M. Amaya, G.S. Morales, A.D.J. Sanchez and B.M. Bonfil *et al*., 2002. The effects of cold-pretreatment, auxins and carbon source on anther culture of rice. Plant Cell Tissue Organ Cult., 71: 41-46.

Vain, P., P. Flament and P. Soudain, 1989. Role of ethylene in embryogenic callus initiation and regeneration in *Zea mays* L. J. Plant Physiol., 146: 537-540.

Xie, J., M. Gao, Q. Cai, X. Cheng, Y. Shen and Z. Liang, 1995. Improved isolated microspore culture efficiency in medium with maltose and optimized growth regulator combination in japonica rice (*Oryza sativa*). Plant Cell Tiss. Org. Cult., 42: 245-250.

Yang, S.F. and N.E. Hoffman, 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol., 35: 155-189.

Zhu, D.Y., X.H. Ding and J.H. Yi, 1991. Anther Culture and Breeding in *indica* Rice. In: Tissue Culture of Field Crops, Yan, C.J. (Ed.). Shanhai Press, Shanghai, pp: 153-169.

Zubko, M.K. and A. Day, 2002. Differential regulation of genes transcribed by nucleus-encoded plastid RNA polymerase and DNA amplification, within ribosome-deficient plastids in stable phenocopies of cereal albino mutants. Mol. Gen. Genomics, 267: 27-37.