Omonrice 9: 41-45 (2001)

Study on anther culture of F_1 plants from crosses between aromatic and improved rice cultivars

Ong Tai Thuan[1], Vuong Dinh Tuan[2] and Bui Ba Bong[2]

ABSTRACT

Anthers of F_1 plants derived from four crosses of aromatic and improved rice cultivars were cultured in N6 and MS media supplemented with 2,4-D (0.5mg/L)+ NAA (1.0mg/L)+ BAP (0.5mg/L) for callus induction. The anther-derived calli were subsequently subcultured in MS and N6 media supplemented with BAP (1.0mg/L) and NAA (1.0mg/L) for plant regeneration. Frequency of callus formation were better in N6 medium as compared to MS medium (11.9% and 7.95%, respectively). Anther-derived calli from the cross of Khao Hom Suphanburi and DS15 exhibited the highest response to the N6 medium in plant regeneration (7.57% with 37 green plants). Green plants could be regenerated in N6 eight times higher than in MS medium.

Key words: anther culture, callus (calli), haploid, double haploid, aromatic rice

INTRODUCTION

Since the first haploid plants were regenerated from rice anthers in 1968 (Niizeki and Oono 1968), anther culture has been widely integrated in rice breeding programs. This allows rapid production of homozygous double haploid lines from F_1 hybrids and incorporation of new genes into breeding materials. In China, a large number of rice varieties have been developed through anther culture and released for cultivation over several thousand hectares (Chen 1986). However, number of green plants obtained through anther culture did not meet demand of practical exploitation through this technique for genetic improvement (Zhu et al. 1990). Several influencing factors on anther culture have been studied such as genotype of explants (Shen et al. 1982, Li 1991), growth condition of donor plants (Chen 1988), culture methods (Qu and Chen 1983). Though different protocols have been proposed to improve efficiency of anther culture, the green plant regeneration frequencies still remained rather low, especially for indica cultivars and aromatic rice. The aim of this study is to evaluate the culture efficiency of anthers derived from F_1 plants of several crosses between aromatic and improved rice cultivars.

MATERIALS AND METHODS

The indica genotypes were used in this study as parental materials and anthers of F_1 plants of Khao Hom Klong Luang1 / AS996, Khao Hom Klong Luang1 / OM1723; Khao Hom Suphanburi / OM997 and Khao Hom Suphanburi / DS15 crosses were obtained to study. All materials were grown under the greenhouse condition.

Panicles were sampled in the morning when the base of the flag leaf was 5-7cm above the base of the next lower leaf. Cold shock treatment was performed by wrapping the panicles within moist germinating papers and stored in refrigerator at 7-10^{0}C for 10 days. Florests with microspores at the mid to late-unicleate stage were selected and panicles containing selected florests were disinfected by dipping into 70% ethanol for 1 minute and 0.1%$HgCl_2$ for 15 minutes prior to rinsing three times by sterile distilled water. Approximately 120 anthers were then inoculated onto a 100-ml cornical flask containing 30ml nutrition medium and kept in the darkness at 25^{0}C for callus induction. The experiment was conducted in RBC design with three replications, 10 flasks per each treatment.

Culture media

The basic media employed in this study were N6 medium of Chu (1978) and / or Murashige and Skoog (MS 1962) suplemented with 2.4-D (0.5mg/L), NAA (1.0mg/L) and BAP (0.5mg/L).

Calli reached a diameter of 2-3 mm were regenerated in nutrition medium containing N6

[1] Department of Science, Technology and Environment, Soc Trang province

[2] CuuLong Rice Research Institute, Omon, Can tho.

or MS-based medium and BAP (1.0mg/L)+ NAA (1.0 mg/L) at 25^0C and illuminated with a 16h/8h cycle for day and night. Regenerants were transferred to MS medium without phytohormones for root induction. The rooted plants were then cultivated in the greenhouse for further observation and evaluation.

Callus induction percentage, plant regeneration frequency, green plant percentage were calculated and statistically analysed.

RESULTS AND DISCUSSION

a. Effect of genotypes on callus induction from anthers of F_1 plants:

Anther culture of rice is influenced by the genotypes of the explants (Niizeki and Oono 1968, Li, 1991) and general trend has been reported as follows: japonica / waxy > japonica / japonica > japonica > indica / japonica > indica / indica (Shen et al. 1982). The number of anther formed calli was varied from 7.27 to 15.00, it depended on a given cross at 30-days after inoculation (Table 1).

Table 1: Effects of genotypes on callus induction in N6 medium

Designation	No. of anthers formed callus	Percentage of anther formed callus (%).
Khao Hom Klong Luang1 / AS996.	13.67a	11.39a
Khao Hom Klong Luang1 / OM1723.	11.72b	9.77b
Khao Hom Suphanburi / OM997	7.27c	6.06c
Khao Hom Suphanburi / DS15	15.00a	12.50a
(%)CV	13.11	13.12
LSD 0.05	1.389	1.159
Sx-	0.4509	0.3761

Number of anthers formed callus was the highest in the cross of Khao Hom Suphanburi and DS15 (15.00). However, it was not significantly different from the cross of Khao Hom Klong Luang1 and AS996 at the level of 0.05. The lowest callus induction was recorded in Khao Hom Suphanburi / OM997. Percentage of anthers forming callus was therefore the highest in Khao Hom Suphanburi / DS15. Earlier report by Quimio and Zapata (1990) suggested that genotype affected callus induction, green plant regeneration and culture efficiency. A recent study by Chen et al. (1991) also reported that frequency of anthers producing callus, capacity of callus to differentiate plants and chromosome number of regenerated plants are all related to the genotype of the plant providing the anthers.

b. Effect of nutrion media on callus induction:

Anthers from different crosses were cultured in the N6 and MS media supplemented with same combinations of phytohormones. It was observed that an average frequency of callus induction from all crosses in MS medium was lower than that in the N6 medium as 7.95 and 11.9%, respectively (table 2). This once again confirmed the advantage of N6 medium in rice anther culture. The reason may be attributed to the presence of low concentration of ammonium and high concentration of nitrate ions in the medium. It was also reported by Chen et al (1991) that they have obtained callus induction from rice anthers at four times higher than those cultured in MS medium with the same plant growth regulators.

Table 2: Effects of nutrition media on callus induction from anther culture.

Media	Designation	Anthers forming callus (%)
MS	Khao Hom Klong Luang1 / AS996.	5.47 fg
	Khao Hom Klong Luang1 / OM1723	6.20 f
	Khao Hom Suphanburi / OM997	4.86 g
	Khao Hom Suphanburi / DS15	12.28 b
	Mean	**7.95**
N6	Khao Hom Klong Luang1 / AS996.	17.31 a
	Khao Hom Klong Luang1 / OM1723	13.33 c
	Khao Hom Suphanburi / OM997	7.25e
	Khao Hom Suphanburi / DS15	9.72 d
	Mean	**11.9**
	(%)CV	13.12
	LSD 0.05	0.19
	Sx-	0.265

Anthers from Khao Hom Suphanburi/ DS15 produced highest frequency of callus induction (15.28%) in MS medium (table 2), whereas a very low frequency was obtained (9.72%) in the N6 medium. Otherwise, the N6 medium was considered to be very suitable to callus induction in case of Khao Hom Klong Luang1 / AS996 and Khao Hom Klong Luang1/OM1723. The frequencies of callus formation of Khao Hom Klong Luang1/ AS996 and Khao Hom Klong Luang 1/ OM1723 were 17.23% and 13.33%, respectively. Variation among crosses within a medium and with different media was statiscally different at the level of 0.05. Anthers of F_1 plants from Khao Hom Suphanburi/OM997 expressed neither good response to MS medium nor to N6 medium in forming callus. The lowest frequency of callus induction of the cross was offered in both media MS and N6 as 4.86% and 7.25 %, respectively (table 2). The difference on anther response to different media in term of callus induction was explained by the control of different genotypes as well as main additive genetic effects. Similar results have been reported by Yan et al (1996), Miah et al (1985).

c. Effect of nutrition media on green plant regeneration:

Regeneration of green plants is greatly influenced by age and size of callus. It was reported that rice callus induced in early stage e.g. 30-50 days after anther inoculation, offers high differentiation for green plants (Wang et al. 1977, Chen et al. 1986).

Frequency of green plant regeneration was higher in N6 medium than in MS supplemented with the same concentration of plant growth regulators as 4.64% and 1.94%, respectively (table 3). Of all these crosses, Khao Hom Suphanburi / DS15 expressed its excellent response to N6 medium in plant regeneration. Its frequency and number of green plants was the highest as 7.57% with 37 plants (table 3).

Poor response was expressed in case of anther-derived calli of Khao Hom Suphanburi / OM997 under both conditions. This may be due to its genotype effect. In this study, Khao Hom Suphanburi / DS15 did not show good response to MS medium, while it could produce the highest number of green plants in N6 medium. Similarly, Khao Hom Suphanburi / DS15 could obtained the highest frequency of callus formation in MS medium, whereas the lowest frequency of callus was noticed in both media in case of Khao Hom Suphanburi / OM997. This occurrence could be attributed by the presence of OM997 which may pose its great influence on callus induction as well as plant regeneration. The exact explaination is still needed.

Statistical analysis of culture efficiency on the N6 and MS media indicated that green plants could be regenerated in N6 eight times higher than in MS medium. The difference is highly significant at the level of 0.05.

Table 3: Effect of nutrion media on green plant regeneration.

Media	Designation	Percentage of callus forming green plants.	Number of green plants
MS	Khao Hom Klong Luang1 / AS996.	4.71bc	6.00c
	Khao Hom Klong Luang1 / OM1723.	1.27 cd	1.33c
	Khao Hom Suphanburi / OM997	1.00 cd	1.67c
	Khao Hom Suphanburi / DS 15.	0.78 d	4.67
	Mean	1.94	3.42
N6	Khao Hom Klong Luang1 / AS996.	4.99b	36.33a
	Khao Hom Klong Luang1 / OM1723.	3.69b	28.67b
	Khao Hom Suphanburi / OM997	2.30 c	5.00c
	Khao Hom Suphanburi / DS 15.	7.57 a	37.00a
	Mean	4.64	26.75
	(%)CV	62.96	
	LSD 0.05	5.99	
	Sx-	1.947	

The relationship between culture efficiency and percentage of callus regenerating green plant has been analysed. There was a close correlation between percentage of callus regenerating plant and culture efficiency. A high positive correlation was also obtained between culture efficiency and number of green plants regenerated (table 4).

Table 4: Correlation analysis of culture efficiency and percent callus regenerating green plants

Parameter	r	Regression equation
Percentage of callus regenerating green plants	0.999**	Y = -0.02 + 0.834 X
Number of green plants / callus	0.822**	Y = 1.01 + 0.113 X

** significant at the level of 0.01

This study indicated that though green plant regeneration from anther of indica cultivars was much lower than that of japonica, a considerable frequency of green plants from anthers of F_1 plants in four different crosses of indica cultivars was possibly obtained. In addition, it is interesting that these parents including two aromatic cultivars Khao Hom Klong Luang1 and Khao Hom Suphanburi were able to regenerate to some extents. Success in plant regeneration will pave the way for further investigation in improvement of biotic and abiotic resistance of these cultivars through gene manipulation.

Green plants obtained from anther-derived calli of F_1 plants have been cultured in green house of Cuu Long Rice Research Institute for further evaluations and studies.

Fig. 1: Plant regeneration

REFERENCES

Chen JJ, JY Hsu and HS Tsay. 1986. Effects of iron on rice anther culture. J. Agric. Res China. 35: 244-252.

Chen Y. 1986. Anther and pollen culture of rice. In: Haploids of higher plants *in vitro*. H.Hu and Yang, H (Ed), Spriger-Verlag, Berlin, pp.3-25.

Chen Y. 1988. *In vitro* development of plant frommicrospores of rice. In: Hu.H & Chen Y (Ed): Plant somatic genetics and crop improvemnet (pp. 27-67). Beijing Univ. Press. Beijing.

Chen CC, HS Tsay, CR Huang.1991. Factors affecting androgeneis in rice (*Oryza sativa* L.) in Biotechnology in agriculture and forestry, Vol.14. (Ed. by YPS Bajaj), Springer-Verlag Berlin Heidelberg.195-211.

Chu CC. 1978. The N6 medium and its application to anther culture of cereal crops. *In* Proc. Symp. On Plant Tissue Culture. Science Press. Beijing, China, pp. 43-50

Li MF. 1991. Anther culture breeding of rice. In: Yan CJ (Ed) Tissue culture of field crops (pp 135-152). Shanghai Scientific and Technical Publishers. Shanghai.

Miah MAA, ED Earle and GS Khush. 1985. Inheritance of callus formation ability in anther cultures of rice, *Oryza sativa* L. Theor. Appl. Gent. 70: 113-116;

Murashige T, F Skoog. 1982. A revise medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-496

Niizeki H and K Oono. 1968. Induction of of haploid rice plant from anther culture. Proc. Jpn. Acad. 44: 554-557.

Qu RD and Y Chen.1983. A preliminary research on the function of enhancement of callus induction frequency by cold pretreatment in rice anther culture. Acta Phytophysiol Sin. 9: 375-381.

Shen JH, MF Li, YQ Chen & ZH Zhang. 1982. Breeding by anther culture in rice varieties improvements. Sci. Agricult Sin. 2: 15-19.

Wang CC, CS Sun, CA Chu. 1977. An effect of culture factors *in vitro* on the production of albino-pollen plantlets of rice. Acta. Bot. Sin. 19: 190-198.

Yan Juqiang, Qingzhong Xue & Jun Zhu. 1996. Genetic studies of anther culture ability in rice (*Oryza sativa* L.). Plant Cell, Tissue and Organ Culture. 45: 253-258.

Zhu DY, XH Ding, JH Ying, NQ Jie, and HL Xiong. 1990. Genetic studies on anther culture ability of indica rice. In. Hu H & Wang LH (Ed): Plant cell engineering and crop improvement (pp 38-43). Beijing Industrial Univ Press. Beijing.

SUMMARY IN VIETNAMESE

Nuôi cấy túi phấn con lai f_1 của tổ hợp lai indica x indica giữa lúa thơm và lúa cao sản

Túi phấn của cây F_1 từ bốn tổ hợp lai giữa lúa thơm và lúa cao sản (loại hình indica) được nuôi cấy trong môi trường N6 và MS, có bổ sung thêm 2,4-D (0.5mg/L)+ NAA (1.0mg/L)+ BAP (0.5mg/L) để kích thích tạo mô sẹo. Tần suất hình thành mô sẹo trong môi trường N6 (11,9%) tốt hơn so với môi trường MS (7,95%). Những calli được hình thành từ túi phấn của Khao Hom Suphanburi / DS15 đáp ứng tốt nhất trên môi trường N6, với tần suất tái sinh là 7,57% và có 37 cây xanh được thu hoạch. Ảnh hưởng của từng giống lúa khác nhau đối với sự hình thành callus cũng được ghi nhận. Cây xanh tái sinh trên môi trường N6 cao gấp 8 lần hơn trên môi trường MS.