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ANTHER CULTURE OF RICE HYBRIDS 1854 AND 1856

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Keywords: Oryza sativa, Anther culture, Callus induction, Plant regeneration.

RINGKASAN

Pokok-pokok padi Hybrid 1854 telah dibiak melalui teknik-teknik didik debunga dengan jayanya. Dari didik debunga Hybrid 1854 atas media yang berlainan, kadar induksi kalus yang diperolehi adalah sebanyak 27.42% dan kadar frekuensi tumbuhan pokok sebanyak 10.67 peratus. Debunga-debunga dari Hybrid 1856 mengeluarkan kurang kalus dan tidak didapati apa-apa perbezaan terhadap pokok jika dibandingkan dengan Hybrid 1854 di dalam keadaan kultura yang sama. Peratus pokok-pokok bulai (albino) yang tinggi juga didapati antara pokok-pokok Hybrid 1854 yang telah tumbuh.

Anak-anak pokok aruhan pada mulanya dipindahkan dan ditanam di rumah kaca. Titisan H2 dan H3 pokok-pokok ini seterusnya ditanam di ladang, di mana ciri-ciri agronomik titisan Hybrid 1854 dilapor dan dibincangkan.

INTRODUCTION

Since NIIZEKI and OONO (1968) regenerated plants from pollens of *Oryza sativa* cultured *in vitro*, there has been a steady accumulation of information and improvement of techniques on rice anther culture. The potential value of anther culture in rice breeding has been widely recognized. It has been used as a tool to generate homozygous plants directly from the pollens of an F_1 plant. Considerable amount of time, manpower and field space could be saved if the technique is properly employed. However, the application of this technique has always been hampered by the inconsistency in callus production rate and plant regeneration frequency from one variety to another (CHEN and LIN, 1976). This paper reports on some of the results obtained from works carried out on MARDI's rice Hybrids 1854 and 1856.

MATERIALS AND METHODS

The parentage of Hybrids 1854 and 1856 is (MR13/MR16)//Khao Saard 108 and IR1561//(MR1[2]/Pongsu Seribu 2)[2] respectively. All parents are of indica type. The F_1 seeds were obtained from MARDI Bumbong Lima Rice Research Station and were sown and planted in the glasshouse in Serdang.

Panicles were collected when the pollens were predominantly in the uninucleate stage. Microscopic observation showed that this occurred one to two days before ear emergence. At this stage, the distance between flag-leaf auricles and the second leaf auricles was approximately 4–8 cm in length. Panicles were surface sterilized in 10% chlorox(R) solution for ten minutes, and rinsed several times in sterilized water. Anthers were then excised and transferred to the medium under aseptic conditions. Cultures were incubated under 1000 lux fluorescent tubes for 16 hours light cycle at 25°C ± 3° Centigrade.

Modified GAMBORG (1968) and N6 (1978) media were used for callus production. Both N6(30) and N6(50) media had their sugar contents at 30 g/litre and 50 g/litre respectively, both contained 2 ppm of 2, 4 D. GAMBORG's E 24 medium contained Benzylamino purine, BAP (0.5 ppm), IAA (0.5 ppm) and 2, 4 D (1 ppm) while GAMBORG's J19 medium contained kinetin (1 ppm) and NAA (1 ppm). The pH of N6 and GAMBORG's media were adjusted to 5.8 and 5.6 respectively prior to autoclaving. All media tested were in liquid and solid form.

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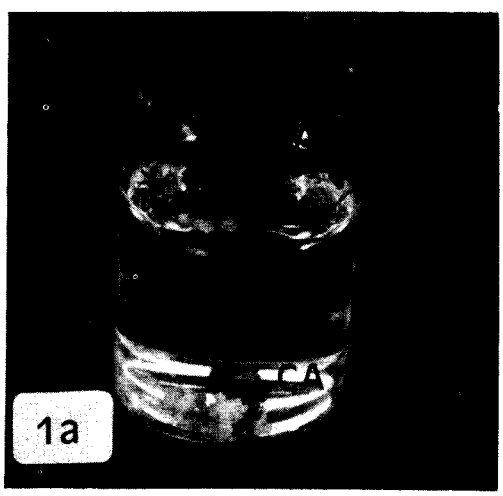
Callus induced from anthers were sub-cultured soon after they have grown to a transferable size (approximately 2–3 mm in diameter), in a modified N6 medium. The auxin 2, 4 D was replaced with either BAP (2 ppm) or Kinetin (2 ppm) for the induction of plantlets.

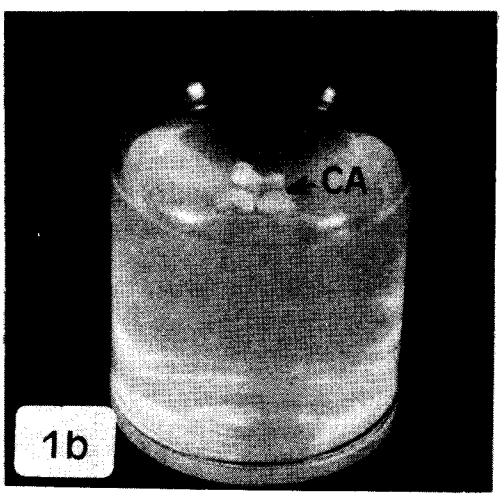
Plants derived from different anthers were classified as different anther culture lines. They were first transplanted in pots in glasshouse and seeds collected (H2 seeds) were planted in the field to produce the H3 seeds. The H2 and H3 lines were planted side by side in Bumbong Lima MARDI Station. Each line had 20 plants, five of which were randomly picked at maturation to collect data on culm height, panicle number per hill and panicle length.

RESULTS AND DISCUSSION

Anthers inoculated in liquid medium were first floating on the surface of the liquid. The callus separated spontaneously from the anther wall after its initiation and sank to the bottom of the culture-vessel *(Figure 1a)*.

N6 and Gamborg's formulae were chosen as basal media for their low level of nitrogen contents. Lower concentrations of nitrogen in the media had been reported to be beneficial to the production of callus from pollen (CHU, WANG, SUN, HSU, YIN and CHU, 1975; CHU, 1978). In *Table 1,* N6(30) and N6(50) media both of liquid and solid form had equally high callus production rate ranging from 25.00% to 27.42% from Hybrid 1854 anthers. These results agree with CHALEFF (1978) who found that the amount of sucrose in the media was not critical for callus formation. Medium E24 achieved a moderate callus production of 14.29% in liquid and 12.50% in solid medium. On the contrary, J19 liquid medium had the lowest callus production rate of only 3.03% for Hybrid 1854 anther. There was no obvious difference in the callus production between liquid and solid media *(Figure 1b). Table 1* also illustrated that callus production rate of Hybrid 1856 in all treatments. The highest callus production rate of Hybrid 1856 anthers was only 9.36% and 8.33% in solid and liquid N6(30) media respectively.





ABBREVIATION

AW : Anther wall
CA : Callus

Figure 1a. Callus produced in liquid medium.
1b. Callus produced on solid medium.

Table 1. Callus production of rice Hybrids 1854 and 1856 anthers on various media

Type of medium		Number of anthers plated		Numbers of anthers producing callus		Callus production rate (%)	
		1854	1856	1854	1856	1854	1856
Solid	N6(30)	174	171	45	16	25.87	9.36
	N6(50)	192	162	48	2	25.00	1.23
	E24	216	216	27	8	12.50	3.70
	J19	216	234	9	2	4.17	0.85
Liquid	N6(30)	180	144	45	12	25.00	8.33
	N6(50)	186	153	51	8	27.42	5.23
	E24	210	207	30	2	14.29	0.97
	J19	198	225	6	4	3.03	1.78

N6(30) and N6(50) solid media supplemented with either Kinetin (2 ppm) or BAP (2 ppm) were used in plant regeneration test. Callus from Hybrid 1854 anthers cultured on these media differentiated into shoots as early as 19 days after being subcultured. However, no plant regeneration occurred from Hybrid 1856 callus throughout the studies. Results on the frequency of plant regeneration of Hybrid 1854 callus within 40-day observation period are summarized in *Table 2*.

Among four medial treatments tested, percentage of green plants recovered from Hybrid 1854 callus was between 8.54% in N6(30) + BAP medium and 10.67% in N6(30) + Kinetin medium. A report by the International Rice Research Institute (ANON, 1981) indicated that an average rate of plant differentiation from callus of rice anther ranged between 3% and 10 per cent. Results from these trials demonstrated a comparatively high plant regeneration frequency from Hybrid 1854 callus.

Albinism is reported to be a common phenomenon in anther culture of plants belonging to Gramineae family (SUN, WUE, WANG and CHU, 1979). A fairly high percentage of albino plants was also observed among generated progenies. As high as 18.67% of albino plants were induced from callus cultured on N6(30) + Kinetin medium, while those cultured on N6(30) + BAP produced less albino plants (1.22%). The actual cause for albinism is still unknown. SUN and his co-workers (1979) suggested that the inability of the development of plastid into chloroplast could be the direct cause.

Table 2. Plant regeneration from callus of Hybrid 1854 on N6(30) and N6(50) solid media supplemented with 2 ppm of kinetin or 2 ppm of benzylamino purine (BAP)

Regeneration medium	Number of callus plated	Plants regenerated			
		Number of green plants	(%)	Number of albino plants	(%)
N6(30)+Kinetin	75	8	(10.67)	14	(18.67)
N6(30)+BAP	82	7	(8.54)	1	(1.22)
N6(50)+Kinetin	81	7	(8.64)	4	(4.94)
N6(50)+BAP	145	15	(10.34)	12	(8.28)

Roots were naturally induced from differentiated green shoots *(Figures 2* and *3)* without further subculture. A total of 13 plants regenerated from Hybrid 1854 anthers were successfully planted out in the field. Some of the agronomic data collected from the 13 lines (derived from the 13 plants) of the H2 and H3 generation are presented in *Table 3.*

Taking the H2 and H3 population as a whole, all lines appeared to be quite uniform in their appearance. A statistical test of their population means, however, shows that there are significant differences at 5% level in culm height and panicle number between the two generations. Similar results were presented by YIN, HSU, CHU, WANG, LIN, CHU, WANG and SUN (1976), FUJITA and UCHIYAMADA (1981). Closer scrutiny of the data shows that six out of 13 lines had no significant difference in their culm height and five in their panicle number, indicating that the characters in these lines were already fixed.

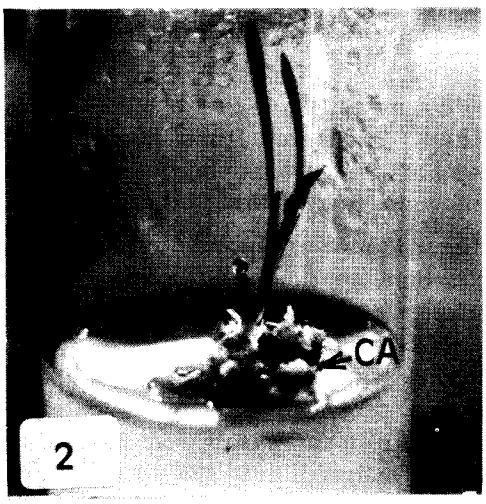


Figure 2. Shoot differentiated from callus tissue of Hybrid 1854.

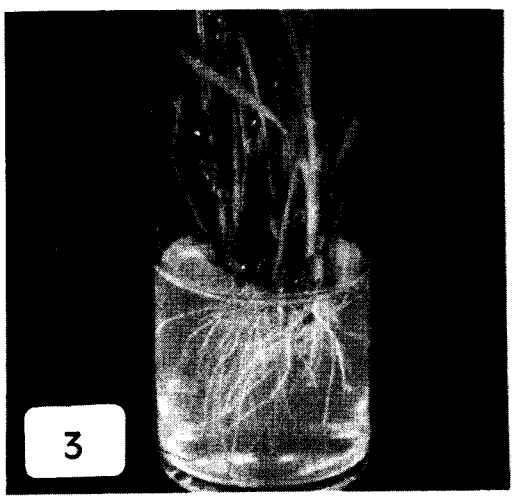


Figure 3. Well-rooted plantlets ready to be transplanted to soil.

Figure 4. H2 and H3 lines of anther culture plants were planted simultanetously in the field for observations.

Table 3. Some agronomic characters of Hybrid 1854 lines derived from anther culture

Line	Culm height (cm)		Panicle number per hill		Panicle length (cm)	
	H2	H3	H2	H3	H2	H3
AC 1	65.8	70.0	13.4	13.2	23.6	23.6
AC 2	64.0	70.4	15.2	14.0	22.8	23.2
AC 3	69.4	69.6	13.6	14.2	23.0	23.4
AC 4	66.0	69.6	14.4	12.6	22.0	22.4
AC 5	67.0	69.6	12.8	13.0	22.8	22.6
AC 6	69.8	73.2	13.8	12.4	23.2	22.0
AC 7	68.4	69.4	12.6	13.0	22.6	22.4
AC 8	65.8	66.0	13.4	13.4	22.8	22.6
AC 9	70.0	70.0	13.4	14.6	22.2	23.0
AC 10	68.6	69.0	14.0	11.8	19.2	20.2
AC 11	65.2	69.0	14.0	13.0	19.4	19.2
AC 12	69.0	73.6	13.8	11.8	19.2	18.0
AC 13	69.2	69.6	14.4	13.0	19.6	18.2
Mean	67.6	69.9	13.8	13.1	21.7	21.6
LSD (5%)	1.57		0.62		1.50	

Note: Data presented are the average of five plants randomly picked from the population of 20 plants of each line.

The above results showed that anthers of Hybrid 1854 responded well to various treatments in both callus production and shoot differentiation while anthers of Hybrid 1856 did not. It has been reported that anthers from different rice varieties, when cultured *in vitro*, do not respond equally in producing callus and in regenerating plants. NIIZEKI and OONO (1968) and GUHA-MUKHERJEE (1973) postulated that this could be due to the genetic make up of the different varieties and their differential responses to cultural conditions. This study also reveals that anther culture may be effective in the early fixation of certain agronomic characters. In addition, inferior lines can be detected and discarded early in the breeding programme.

Subsequent works to monitor the ploidy levels of the callus and the regenerated plants are being carried out. Further improvement on callus production rate and green plant regeneration frequency should also be looked into if the technique is to be adopted for use as an efficient and effective tool in rice breeding.

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SUMMARY

Progenies of rice Hybrid 1854 were successfully raised through anther culture technique. A 27.42% callus induction rate and 10.67% plant regeneration frequency were obtained from anthers of Hybrid 1854 cultured on various media. Anthers of Hybrid 1856 produced less callus and no plant regeneration as compared with Hybrid 1854 under same cultural conditions. High incidence of albino plants were also found among generated Hybrid 1854 plants.

The induced plants were first transplanted and grown in the glasshouse. H2 and H3 lines of these plants were subsequently planted in the field. Some of the agronomic characters of Hybrid 1854 lines were reported and discussed.

REFERENCES

ANON, (1981). Tissue Culture work promises faster, lower cost breeding, IRRI Reporter 2/81.

CHALEFF, R.S. (1978). Anther culture as a rice breeding technique. *IRRI* **3**, pp. 2–3.

CHEN, C.C. and LIN, M.H. (1976). Induction of rice plantlets from anther culture. *Bot. Bull. Acad. Sin.* **17**, 18–24.

CHU, C.C., WANG, C.C., SUN, C.S., HSU, C., YIN, K.C. and CHU, C.Y. (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sci. Sinica* **18**, 659–68.

CHU, C.C. (1978). The N6 medium and its application to anther culture of cereal crops. *Proc. of Symposium on Plant Tissue Culture 1978, Peking.*

FIJITA, Y. and UCHIYAMADA, H. (1981). Morphological variation in differentiated rice plants induced by anther culture. *Jap. Breed.* **31** (Suppl. 2), 46–7.

GAMBORG, O.L., MILLER, R.A. and OJIMA, K. (1968). Nutrient requirements of soybean root cells. *Exp. Cel. Res.* **50**, 151–8.

GUHA-MUKHERJEE (1973). Genotypic differences in the *in vitro* formation of embryoids from rice pollen. *J. exp. Bot.* **24**, 139–44.

NIIZEKI, H. and OONO, K. (1968). Induction of haploid rice plants from anther culture. *Proc. Acad.* **44**, 554–7.

SUN, C.S., WUE, S.C., WANG, C.C. and CHU, C.C. (1979). The deficiency of soluble proteins and plastid ribosomal RNA in the albino pollen plantlets of rice. *Theor. Appl. Genet.* **55**, 193–7.

YIN, K.C., HSU, C., CHU, C.Y., WANG, S.T., LIN, T.Y., CHU, C.C., WANG, C.C. and SUN, C.C. (1976). A study of the new cultivar of rice raised by haploid breeding method. *Sci. Sinica* **14**, 227–42.

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