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ANTHER CULTURE OF POLLEN PLANTS DERIVED FROM CROSS *ORYZA SATIVA* L. × *O. GLABERRIMA* STEUD.*

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Abstract

A total of 17 calli was derived from a cross of *Oryza glaberrima* Steud. and *O. Sativa* L., and 79 anther plantlets were regenerated. Ploidy levels of haploid, diploid and tetraploid were found. Plant heights of these anther plants were somewhat shorter than those derived from *glaberrima* selfed-plants. The anther culture of anther plants derived from the cross did not increase the rates of callus induction and plantlet regeneration. The results did not reveal that the genetics of microspores had played a major role in anther culture. The culture of F_2 selections of the cross also did not give promising result. It may be due to the poor plant growth of the interspecific cross progeny.

Introduction

The improved rice varieties of Taiwan were predominantly developed by the method of varietal hybridization. The breeding stocks used mainly carried germplasm from *indica* and *japonica* rices. Through the effort of over 80 years, most of the released varieties are high yielders. Further improvement will be aimed at growth vigor and tolerance to stresses. This effort would be dependent upon the genetic incorporation of less related species of *Oryza*. *O. glaberrima* Steud. which is grown as cultivated rice in Africa (Oka and Morishima, 1967). This rice species grows vigorously in Taiwan. Probably it may be tolerant to high temperature and to drought, since both of these mentioned conditions existed at the African habitat. Therefore, we would like to assume that the genetic recombination between these two species of *O. glaberrima* and *O. sativa* would bring good growth and promising field tolerance. This study is a continuous survey of our previous program to evaluate the fixed progeny from the interspecific crosses.

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Several lines of the mentioned anther plants were then subjected again to anther culture. The primary goal of the second passage anther culture is to identify whether the proliferation of calli and regeneration of plantlets have been subjected to the genetic selection of microspores during anther culture.

Material and Method

Cultivated variety Taichung 65 (*O. sativa*) was reciprocally crossed with *O. glaberrima*. The fixed progeny lines were derived from F_1 plants through anther culture (Woo and Huang, 1980). The anther plants were selfed and no phenotypic segregation found in the subsequent generations. Thus, the progeny lines were verified to be genetically pure and originated from microspores. A number of the selfed lines were subjected to the second passage of anther culture. The preparation of media and the method of anther inoculation were identical to our previous study (Woo *et al.*, 1978).

Experimental Results

The results of cross *O. glaberrima* (W492) × Taichung 65 is shown in Table 1. A total of 3 F_1 plants was investigated. The F_1-142 plant has developed 2 calli. One of the two calli regenerated 5 haploid plants. The other callus developed 1 diploid plant. The diploid plant was dwarf. It was only 36 cm tall, slightly taller than the haploids. The F_1-144 plant developed 2 calli and regenerated 4 plants. All of the 4 plants were diploids and averaged 82.3 cm tall. For plant F_1-145, a total of 13 anther calli proliferated, and 37 diploid plantlets regenerated from 7 calli. Their plant height averaged 65.8 cm. In addition, 2 calli from the F_1-145 plant gave 27 haploid plant. They were rather short (about 40 cm tall). The other 4 calli of the same plant source developed 5 tetraploid plantlets. They averaged 64 cm, slightly

Table 1. *Plantlets developed from* ***Oryza sativa*** *and* ***O. glaberrima*** *crosses*

	Cross number	Callus cultured	Plt. height cm. S.D.	Ploidy level	No. of plantlets
glab. × Taichung 65	142	1	31.20± 7.4	1n	5
glab. × Taichung 65	142	1	36.00± 0	2n	1
glab. × Taichung 65	144	2	82.25± 3.5	2n	4
glab. × Taichung 65	145	7	65.18± 3.4	2n	37
glab. × Taichung 65	145	4	39.27± 5.5	1n	27
glab. × Taichung 65	145	2	64.40± 1.2	4n	5
glaberrima		7	85.9 ±13.7	2n	16
glaberrima		1	66.0 ± 4.2	1n	2

shorter than those of diploids. For those plantlets derived from *O. glaberrima*, a total of 16 diploid plants was obtained. They averaged 87 cm, much shorter than that parental plants of 120–140 cm tall. Besides, 2 haploid plants with 66 cm were also identified. They were taller than those haploids from hybrid plants.

Hybrid plant F_1-144 has generated four anther plantlets. The anther plants were then subjected to anther culture again during bolting stage. Table 2 indicates that the plants 801–305 and 801–306 were regenerated from a single callus as well as the 801–307 and 801–308 plants. However, the number of calli from plants 801–305 and 801–306 were low, and their induction rates were 2.7 and 4.5% respectively. Calli of these two plants regenerated few albinos only. For the other two plants 801–307 and 801–308, only the former one induced 4 calli, and no plantlets were found.

Since *O. glaberrima* and *O. sativa* (variety Taichung 65) were hybridized, F_2 seeds were harvested in great number. Severe segregation remained in all agronomic traits. A total of 4 F_2 plants carrying fairly good performance of the cross and 5 F_2 plants from the reciprocal cross were selected for anther culture (Table 3). Plants with the *O. glaberrima* as female parent induced more calli than those plants with *O. sativa* as the female parent in reciprocal cross. In general, the rates of callus induction and plantlet regeneration were rather low. The two calli of plant 102–1 regenerated 7 green plantlets. The other 3 plants with a total of 110 calli regenerated no green plantlets but albinos. Overall, the second passage of anther culture and the anther culture of F_2 selection did not raise culturability. These results may elucidate that the growth of microspores to callus and plantlet would seem to be likely dependent upon the physiological condition of plant material. It also seems to indicate that the genetic segregation of microspore does not play a significant role in pollen culture.

Discussion

Since the rice improvement of this country depends upon the use of

Table 2. *Anther callus and albino induction of four anther plants derived from cross,* ***Oryza glaberrima*** *x Taichung 65*

Parental material	Callus series	Cultured anther	Callus induced	Induction rate %	Subcultured callus	Differentiation of albino	%
801-305	1,300-1	539	13	2.65±5.54	13	1	7.69
801-306	1,300-2	724	31	4.49±8.91	31	6	19.35
801-307	1,301-1	340	4	1.23±4.44	4	0	0
801-308	1,301-2	502	0	0	0	0	0

Table 3. *Anther culture of F_2 selections from cross* ***O. glaberrima*** *and* ***O. sativa***

O. glaberrima × Taichung 65	Anther Cultured	Callus induced		No. of callus regen. plt.				No. of green plantlets
				albino		green		
		No.	%	No.	%	No.	%	
102-1	1,834	86	4.47± 9.92	4	4.65	2	2.32	7
-2	1,238	36	2.91± 9.48	1	2.78	0		0
-3	959	45	4.69±13.11	2	4.44	0		0
-6	1,090	29	2.66± 9.67	3	10.34	0		0
Taichung 65 × *O. glaberrima*								
107-1	730	5	0.68± 1.6	0		0		0
-2	333	0	0	0		0		0
-3	585	0	0	0		0		0
-4	202	5	2.25± 4.13	2	40.0	0		0
-5	122	37	30.32±14.08	2	5.4	0		0

varietal hybridization, most of the genetic recombinants derived from cultivated rice have been evaluated. Further improvement would then be dependent on the utilization of less related source of germplasm. Interspecific hybridization of *Oryza* seems to be the most likely way of constructing new rice genotypes. The practice of anther culture seems to provide a direct approach to make the construction of new genetic structures possible. The work of rice anther culture was firstly reported by Niizeki and Oono (1968) on induced haploid rice. Woo and Tung (1972), Woo *et al.* (1973), and Woo and Su (1975) applied anther culture technique to *indica* and *japonica* rice hybrids. A series of fixed lines was developed from the pollen of F_1 plants of the subspecific crosses. Later, Woo *et al.* (1978) and Woo and Huang (1980) applied the same method to less related species of *O. sativa* × *O. perennis* and *O. sativa* × *O. glaberrima*. Fixed lines obtained from the same approach were genetically homozygous, and no visual segregation appeared in the subsequent generations. The fixed lines carried genetic traits from both parents gave vigorous growth. However, their seeds shattered rather easily. Some lines gave rather low fertility and immature seeds. These weaknesses were not overcome by genetic purity through anther culture. These two undesirable traits remained in the anther progeny lines as those of segregating hybrid progenies derived from less related crosses. So, it would be reasonable to postulate that the poor seed filling would be caused by the genetic interaction between the genetic material of nucleus and cytoplasm.

The primary aim of this study was verifying whether the anther culture was conditioned by the segregating genotype of microspores. Results of the anther culture of anther plants seems to indicate that no improvement of culturability was found. On the other hand, the low rate of callus induction and plantlet regeneration would be considered as due to the growth condition of the material plants. This finding would also indicate that no direct evidence of genetic selection would be involved in the anther culture.

Hybrid progenies of *O. glaberrima* and *O. sativa* hybrids usually carried poorly filled seeds. Same phenomenon appeared in their anther progenies. However, self-pollinated *O. glaberrima* and its anther lines developed normal seeds (Woo and Huang, 1980). The poor seeds could be caused by the genetic interaction between these two species of genera *Oryza*. Thus, the genetic backgrounds of the two species have been incorporated into a fixed line; the interaction still remains in their progeny lines.

For the change in chromosomal number, Chen and Lin (1976) and Chen (1977) studied the chromosome of anther calli. They discovered that the doubling of chromosomes was caused by the duplication of a single nucleus and the fusion of generative and vegetative nuclei. Their findings agree with our result that no visual segregation was found, since the duplication and fusion of nuclei would raise the genetic structure to homozygosity. Hsu and Chen (1977) emphasized that triploidy and polyploidy were less frequent, however.

Results of this study seem to reveal that the hybrids derived from widecross would raise the growth of progeny plants. Hybrid lines derived from parents with severe trait difference would be vigorous. However, the vigorous progeny does not always give abundant seeds. In addition, poor fertility frequently exists in progeny lines. Thus, it limits the exchange of genetic material and the selection of genetic recombinants. A successful anther culture would reveal the genotype of microspores; thus new genetic recombination could be discovered. Nevertheless, the rate of developing microspore plant is still low; the limited number of anther plants obtained has not yet played a major role in genotypic selections. To recover the limitation, more emphasis should be paid on the selection of F_2 and F_3 plants with promising performance. And those with ideal traits are then subjected to culture for genetic fixation. This process would make anther culture to be more likely in rice breeding.

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亞非洲稻作之花藥培養

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非洲栽培稻 *Oryza glaberrima* Steud. 和亞洲栽培稻 *Oryza sativa* L. 雜交，培養雜種第一代植株之花藥，共獲得癒合組織十七顆。經分化培養後得到花藥植株七十九株。染色體羣數目有：單倍 $n=12$，雙倍 $2n=24$ 及四倍體 $4n=48$。雜種花藥植株之桿高均矮於 *Oryza glaberrima* 親系。花藥植株之花藥再用人工培養，其癒合組織之產生及再分化率均偏低，不能證明花藥培養受到花粉選擇之作用。雜種第二代之選株做花藥培養時，其效果亦未臻理想，這可能是該批材料在當時生育欠佳，影響花藥中花粉之發育不够健全之緣故。