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ANTHER CULTURE OF *ORYZA GLABERRIMA* STEUD. AND ITS HYBRIDS WITH *ORYZA SATIVA* L.[1,2]

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The African rice *Oryza glaberrima* Steud. is commercially cultivated in Africa. Its plants have broad leaves, long stems and panicles, which usually are associated with vigorous growth. This species is genetically less variant than *O. sativa* L. (Chang, 1975). Hybridization between *O. glaberrima* and *O. sativa* (cultivar Taichung 65) has been made at the Institute of Botany, Academia Sinica, Taipei, for studying their genetic recombinants.

Since the F_1 plants of reciprocal crosses are completely sterile, no F_2 seeds have been obtained. Thus, the anthers of F_1 hybrids were used as experimental materials for developing anther lines. Anthers were cultured on defined medium to produce callus tissue and to regenerate plantlets. The study method is the same as described in our previous research reports (Mok and Woo, 1976; Woo and Su, 1975; Woo *et al.*, 1978). Results are briefed as follows:

Anthers of *O. glaberrima* easily grew to callus tissue (Table 1). A high 43% rate of cultured anthers containing mainly uninucleate pollen proliferated into callus tissue. On the other hand, the F_1 hybrid anthers from reciprocal crosses showed poor callus formation. Anthers of *O. glaberrima* x Taichung 65 gave a low 2.99% rate of callus formation, and those of Taichung 65 × *O. glaberrima* 5.35%. Although the callus formation from hybrid anthers was low, 16-72% of calli regenerated plantlets. The proliferation of anther calli differed from one hybrid plant to anothers.

Albinos appeared over two-fold more than the normal green plantlets (Table 2). This phenomenon was also found in the anthers of callus of reciprocal hybrids. Crosses with *O. glaberrima* as the female parent seemed to have more albinos than those with *O. sativa* as the female. Nevertheless, the

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Table 1. *Number of calli derived from rice anthers*

Source	Anther cultured	No. of calli	Induction (%)	$\bar{x}$
781-142 *O. glaberrima*/Taichung 65	589	37	6.28	
781-143 *O. glaberrima*/Taichung 65	538	6	1.11	
781-144 *O. glaberrima*/Taichnng 65	1,126	18	1.60	2.99 ± 2.85
781-145 Taichung 65/*O. glaberrima*	2,739	191	6.97	
181-747 Taichung 65/*O. glaberrima*	1,532	57	3.72	5.35 ± 2.30
781-149 *O. glaberrima*	4,692	2,030	43.26	

Table 2. *Plantlets regenerated from anther calli*

Source	Callus generated plantlets				Number of green plantlets	Total rate of differentiation
	Albino		Green			
	Number	%	Number	%		
781-142 *O. glaberrima*/Taichung 65	2	5.41	4	10.81	14	16.22
781-143 *O. glaberrima*/Taichung 65	3	50.00	1	16.67	5	66.67
781-144 *O. glaberrima*/Taichung 65	9	50.00	4	22.22	19	72.22
781-145 Taichung 65/*O. glaberrima*	25	13.09	17	8.90	103	21.99
781-147 Taichung 65/*O. glaberrima*	8	14.04	3	5.26	73	19.30

total number of plantlets obtained was considerably small. Some calli, about 10%, initially regenerated roots. None of the calli that regenerated roots prior to shoots would differentiate into plantlets. On the contrary, those calli that initiated shoots would eventually develop into complete plants; in this case, roots appeared after the shooting. Calli that produced only roots died afterwards.

A number of plantlets could develop from a single callus. Their phenotypic appearance was studied according to the magnitude of florets. The plants that carried florets samller than those of diploids with fully blanking panicles were found to be haploids (Woo *et al.*, 1978), and those carrying florets larger than those of diploids with low fertility were tetraploids. A total of 3 chromosomal levels of tetraploidy, diploidy and haploidy were identified. The diploid plants were most frequently found. Each haploid or diploid callus regenerated more than four plantlets, and tetraploid callus produced less than two plantlets (Table 3). The average number of diploid and haploid plantlets derived from a single callus of the control anther of *O. glaberrima* were 1.51 and 1.14 respectively. The genomic level seemed to be associated with plant phenotype. Usually, the haploid plant was short-statured, about 50 cm (Table 4). The plant height of tetraploids (cross 781-145)

Table 3. *Ploidy level of anther plantlets*

Source	Calli	Plantlet		
		Tetraploid	Diploid	Haploid
781-142 *O. glaberrima*/Taichung 65	2	1	0	5
781-144 *O. glaberrima*/Taichung 65	2	0	4	0
781-145 Taichung 65/*O. glaberrima*	15	4	48	19
781-147 Taichung 65/*O. glaberrima*	1	0	0	3
Mean/callus*		1.67/3*	4.72/11*	4.5/6*
O. glaberrima	4	0	53	8
Mean/callus*			1.51/35*	1.14/7*

* Callus-regenerated plantlets.

Table 4. *Plant height (cm) and ploidy level of anther plantlets*

Source	Tetraploid	Diploid	Haploid
781-142 *O. glaberrima*/Taichung 65	36.0(1)*	—	32.00± 6.28(5)
781-144 *O. glaberrima*/Taichung 65	—	82.50±2.87(4)	—
781-145 Taichung 65/*O. glaberrima*	64.4±1.27(5)	64.84±2.57(38)	41.23± 7.35(28)
781-147 Taichung 65/*O. glaberrima*	—	—	33.70± 4.04(3)
781-149 *O. glaberrima*	—	94.00±14.7(48)	56.83±11.59(12)

* Number of plantlets.

was similar to that of diploids. The tetraploids also have broad leaves, large florets, and highly blanking panicles—seed set less than 2%. Though the diploids may differ in plant height, but were consistent in floret magnitude. Most of the diploid plant were completely sterile, and the highest seed setting did not exceed 25% however. In general, plants derived from a single callus had the same phenotypes. Only one callus from the cross Taichung 65 × *O. glaberrima* differentiated into one haploid and one diploid plantlet. This results agree with our previous conclusion that most plantlets regenerated from a single callus are of single-cell origin.

The fertility of anther plants varied from plant to plant. In general, haploid plants were completely sterile. It might be due to the abnormality of their micro-and megaspores. Nevertheless, two seeds were secured from one haploid plant, though their magnitude was considerably small. The minuteness of seeds was caused by the haploid genome of hostal plants; thus all florets borne on the panicles were rather small. The normal plant stature and normal florets could be recovered in the second generation. Plants having florets of normal magnitude produced fertile seeds. However, the percentage of seed set was very low, about 0-3.12 percent. The poor seed setting was likely caused by the genetic interaction between less related species. Whether the poor fertility can be recovered through another cycle of anther culture needs further investigations.

Interspecific hybridization has been considered as a necessary approach to incorporating the germplasm of wide variability from less related origins. The exchanging of germplasm between wild species and cultivated rice would discover the potential of promising genes preserved in natural habitat. This would open up the possibility of increasing genetic variability and growth vigor as well. Research in potatoes along this line was earlier reported by Hawkes (1977); he investigated the resistances first to cyst nematode and then to frost from wild species into the cultivated varieties of *Solanum tuberosum*. Gentry (1969) transferred the resistance to *Fusarium* from *Phaseolus vulgaris* subspecies *aborigineus* to cultivated forms. Similiar results were reported by Budin (1973) on the development of barley resistant to *Helminthosporium* from hybrid between *Hordeum spontaneum* and *H. vulgare*. The interspecific hybridization of rice has not yet been identified in disease resistance, since the resistance needed can still be found in our present cultivated rice. On the other hand, the Taiwan wild rice, *O. perennis formosana* is highly introgressed with cultivated rice. It contains a large amount of genetic variability which may be valuable for the improvement of present rice cultivar (Kiang *et al.*, 1979). Pure line developed from the cross (*O. perennis* × *O. sativa*) × *O. sativa* (Woo *et al.*, 1978) through anther culture have been identified as having outstanding vigor and free from disease infection under field condition. In addition, the stock lines have good ratooning ability. Several pollen lines grew horizontally in the first three weeks after transplanting and then turned upright. This kind of characteristic, which has never been observed in our present materials, may help compete with weeds.

Overall, the utilization of the less related species *O. glaberrima* for varietal improvement of *O. sativa* may become a long tedious work. For one thing, this African rice also carries a number of undersirable characteristics, such as shattering, awning, low fertility, and panicle blanking, although it shows a promising growth vigor. For another, the use of interspecific rice in the breeding program needs series of backcrossings to recover the parental genotype. However, the genetic recombinants developed from a single hybridization and the fixation through the diploidization of haploid plantlets may incorporate a sequence of genetic materials which would raise the growth vigor to a higher level. The processes of genetic incorporation, recombination and possibly introgression would then bring the rice crop to a healthy and tough stand tolerant to environmental stresses, less susceptible to diseases, and high yielding.

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亞非洲水稻花葯之培養

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非洲稻 *Oryza glaberrima* Steud. 之花葯用人工培養，約有43%可以產生癒合組織。非洲稻/臺中65號之雜種花葯，其癒合組織形成率爲 2.99%，臺中65號/非洲稻則爲 5.35%。雜種癒合組織形成率雖然偏低，但是小株分化率可達72%。白苗數目約爲綠苗之兩倍，非洲稻作雜交母本時，白苗數目比以臺中65號作母本時爲多。一顆癒合組織分化成之許多小株，具有相同之遺傳結構，但是亦發現一顆癒合組織分化單倍體和雙倍體小株各一株。小株之總數以具有雙倍體24條染色體最多，單倍體12條染色體次之，四倍體48條染色體最少。一般而言，單倍體小株皆不孕，不能結實，但是亦發現一株單倍體，結種子兩粒，所生長成之兩株後代均屬雙倍體，並皆結實。該雜種花葯雙倍體小株，多數亦不孕，稔實率最高祇有百分之3.12。