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ANTHER CULTURE OF *ORYZA SATIVA* L. × *ORYZA SPONTANEOUS TAIWANIA* HYBRIDS[1]

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Abstract

The anther culture of interspecific rice hybrid aimed to develop genetic recombinants between *Oryza sativa* L. and wild rice *O. spontaneous taiwania* (Kiang and Wu, 1979). A total of four rice varieties Tainan 5, Kaohsiung selection 1, Hung-tsao-sun and Fung-gin was used as female parents and crossed respectively with the wild rice *taiwania*. Anthers of the hybrids F_1 and F'_2s as well as their parentals were cultured on modified MS medium, supplemented with 2 mg/l α-naphthaleneacetic acid and 0.5 mg/l kinetin. The rates of callus induction were considerably high. Approximately 40% of F_1 plant anthers and 60-100% F_2 plant anthers developed calli. The rate of plantlet differentiation ranged 10-70% of the cultured calli. The parental calli differentiated into more green plantlets while the hybrid calli developed more albinos. Most of the anther plants/or lines were vigorous with traits of the cultivated and wild species in field plots. This result gave further evidence that the technique of anther culture is feasible in recovering and fixing genetic recombinants of diverse genetic background.

Introduction

Varietal hybridization with pedigree selection is a general practice for rice breeding. Breeders of this country usually select selfed progenies in the F_2 or later generation of hybrid populations. This method would fix the genetic background of heterozygous structure onto homozygous lines. Selections were made for high yield and well adapted to highly protected habitat. Thus, the current cultivars may have lost their tolerance to environmental stresses. In order to recover the deficiency, new varieties would need to be adapted on less controlled condition and meet the demand of labor shortage. The species of wild rice are preserved in natural habitats; they are usually non-protected by human being. Thus it would retain tolerance to unfavorable conditions. The incorporation of wild germplasm into the cultivated one may

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produce lines capable of growing in less managed field.

Wild rice of Taiwan, *Oryza spontaneous taiwania* previously reported as *formosana* (Kiang and Wu, 1979) has long been adapted in the Taoyuan areas. The habitat of the species is along a creek and close to farmers' paddy fields. It is thus assumed that the wild species has more or less been genetically introgressed by cultivated rices due to spontaneous occasionally hybridization. These wild rices would therefore furnish a good material to cross with cultivated species, and possible genetic recombinants could be fixed and recovered through anther culturing.

Material and Method

Wild rice *Oryza spontaneous taiwania* was self-pollinated for two generations at Taichung District Agricultural Improvement Station. A selection with growth vigor was chosen and crossed respectively with varieties Tainan 5, Hung-tsao-sun, Fung-gin and Kaohsiung selection 1. Anthers of the F_1 plants and some 10–15 F_2 plants selected on the basis of growth vigor and desirable morpho-agronomic characters from individual cross were cultured on defined medium (Murashige and Skoog, 1962). The callus induction medium was supplemented with 2 mg/l α-naphthaleneacetic acid (NAA) and 0.5 mg/l kinetin. For the differentiation medium, the concentration of NAA was reduced to 0.5 mg/l and of kinetin raised to 2 mg/l (Mok and Woo, 1979). Each culture tube contained 5 ml of the stated medium and 20–25 anthers. The inoculation of anthers was carried out in a homemade laminar air flow cabinet with a paper filter of 0.5 μm. The inoculated anthers were incubated at 25 °C under light intensity of 1,500 lux with light/dark period of 16/8 h. Callus usually developed 4–6 weeks after incubation. When calli reached approximately 2 mm in diameter, they were individually transferred to the differentiation medium and grown at 25 °C under 16 h light of 3,500 to 4,000 lux. The rates of callus induction and of albino and green plantlet differentiation were then recorded and analyzed.

Experimental Result

The calli of parental lines and their progenies are given in Table 1. Since the total number of anthers cultured varied with F_1 plants, total number of calli induced also differed from one F_1 plant to the other. The rate of callus induction for variety Tainan 5 was 32%, for Hung-tsao-sun 55%, and for the wild rice *O. spontaneous taiwania* 48%. All of the induction rates were over 30%. The largest difference in induction rate was 13% between varieties Tainan 5 and Hung-tsao-sun. The phenotype of F_1 plants was rather similar. On the other hand, the rate of callus formation among F_1 plants varied 7–14%.

Table 1. *Callus induction of cultivated varieties and wild rice,* ***Oryza spontaneous taiwania*** *crosses.*

	Cultured anthers	Callus	Rate % S. D.	Range* %
Tainan 5	1,273	407	32.00±28.76	
Wild rice	2,831	1,374	48.53±12.98	
F_1	2,983	1,498	50.22±10.22	
F_2	6,212	3,826	61.59±30.36	20-124
Kaohsiung sel. 1	534	283	53.00±22.49	
F_1	1,568	576	36.73±14.27	
F_2	2,985	1,836	61.51±24.71	30-109
Fung-gin	253	110	43.48±39.53	
F_1	3,098	1,233	39.80± 7.28	
F_2	3,399	2,132	62.72±10.86	50-85
Hung-tsao-sun	1,288	720	55.90±26.68	
F_1	2,028	1,079	53.20±10.61	
F_2	3,133	2,089	66.67±27.33	42-98

* F_2 plants with lowest and highest callus formation, including multi-callusing anthers.

F_1 anthers of cross Hung-tsao-sun and *O. spontaneous taiwania* were highly amenable to culture. About 53% of the cultured anthers developed calli. A large number of anthers from selected F_2 plants were also cultured, and over 60% of the anthers proliferated calli. Among the crosses attempted, Hung-tsao-sun ×*O. spontaneous taiwania* gave the highest percentage of calli both in F_1s and F_2 selections. In general, the callus induction varied significantly among F_2 plants of a cross. For instance, a F_2 selection from Tainan 5× *O. spontaneous taiwania* gave a very high calli induction of 124%, including a number of multi-callusing anthers. Its parental varieties and F_1 anthers produced less number of calli, however.

Plantlets were visible in two weeks after calli were transferred to the differentiation medium. Both albino and green plantlets were found from the calli. In addition, a small number of calli produced both albinos and greens in the same culture tube. However, the albinos did not survive and eventually die. A number of calli have formed roots but no shoots. The four parental varieties produced more green plants than albinos. On the contrary, the wild rice, the F_1s, and the F_2 plants of crosses gave more albinos than green plantlets. The exception to the finding was found in cross Tainan 5×*O. spontaneous taiwania*. Field observation showed that most of the anther lines/plants were vigorous with traits of the cultivated and wild species.

Table 2. *Plantlet and root regeneration for anther callus derived from hybrid between cultivated varieties and wild rice,* ***Oryza spontaneous taiwania***

	Cultured callus	Plantlet callus	% S. D.	Regeneration		Root % S. D.
				GP* %	ALP* %	
Tainan 5	268	30	11.19	10.82	0.37	13.43
Wild rice	273	31	11.35	4.40	6.95	16.48
F_1	148	23	14.80	8.60	6.70	0.60
F_2	1,762	244	13.84±9.4	3.18	10.67	12.09±5.13
Kaohsiung sel. 1	145	19	13.10	10.34	2.07	24.83
F_1	97	26	26.80	7.21	19.58	16.30
F_2	792	204	25.75±10.0	11.74	14.01	13.30±11.57
Fung-gin	81	6	7.41	7.41	0	23.46
F_1	301	205	68.40	11.96	56.50	4.00
F_2	762	183	24.01±15.2	8.21	15.80	20.80±11.80
Hung-tsao-sun	355	116	32.68	16.62	14.08	31.27
F_1	246	106	42.90	10.20	27.70	4.00
F_2	933	204	21.80±13.8	6.90	14.90	12.60±8.41

* Green plant (GP), Albino (AlP).

Discussion

Woo and Su (1975) early used hybrids of *indica*×*japonica* for anther culture. A number of fixed lines had been developed in that time. Most of the lines were fast growing and early maturing, and seemed tolerant to drought. Their preliminary study was encouraging, though a few drawbacks, such as seed shattering and poor seed setting, were encountered. To incorporate the genetic recombinants from different species of *Oryza*, wild species *perennis* W120 was crossed with *sativa* Taichung 65 (Woo *et al.*, 1978) to develop anther lines. Those F_1 plants backcrossed to *sativa* developed anther lines. The fixed anther lines having genetic background from wild species *perennis* were highly rationable. And, surprisingly, the seed setting was quite normal over 80%. However, the shattering habit remained as a major deficiency.

Pure lines developed from hybrids between *O. sativa* and *O. glaberrima* (W492) were reported by Woo and Huang (1980). The anthers of *glaberrima* were highly amenable to culture, though the phenotypes of the developed lines vary drastically. The variation may be due to the genetic impurity of the *glaberrima* lines used. Though the *glaberrima* anthers are highly culturable, its hybrid anthers, on the contrary, are difficult to be cultured. A number of anther plants were developed from a large anther population. However, most of them have poor seeds and low fertility which may have been caused due to

the so-called hybrid weakness between *glaberrima* and *sativa* (Morishima *et al.*, 1963). In general, anthers of vigorous F_1 hybrids are highly culturable. On the contrary, those plants with growth weakness are not desirable for anther culture. In addition, growth conditions also plays a role in the success of the culturing. Field materials are superior to those from greenhouse for anther culturing. Thus, an identical material, for instance F_1 derived from two highly homozygous lines, may reveal variable culture results because of the growing conditions of the anther donor plans.

Oryza spontaneous taiwania is usually a self-pollinated but somewhat cross-pollinated species, and has been growing by the neighborhood of the cultivated rice for decades. Therefore, genetic introgression may have proceeded. Evidences could be found in such traits as grain shape, fertility, seed maturation pattern, and tillering habit. However, the colored epidermis, long purple awns, and grain shattering remain as its progenitors'. The successful crossing between the wild and cultivated rice has favored the exchange of genetic material and a great number of new recombinants would enrich the breeding stocks. Field observation showed that the shattering habit of the lines is most likely to be controlled by the formation of abscission layers. Though the existence of abscission layer and seed shattering can not be overcome with tissue culture technique. It does remain as a problem to be studied. This study concludes that anther culture may be used as an efficient way of incorporating germplasm of diverse origins. The culture process does not contribute genetic variability however. Therefore, the successful application of anther culture technique would depend upon the genetics of parental stocks, the proper time of excision and the culturing of anthers.

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稻屬種間花藥之培養

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本研究之目的，在應用花藥培養促進栽培稻 *Oryza sativa* 和臺灣野生稻 *O. spontaneous taiwania* 之遺傳結合，所用之栽培品種是：臺南五號，高雄選一號，蠶早生及豐錦。雜種第一代及第二代植株之花藥用以培養於合成培養基，所產生之癒合組織，綠株，白株皆分析之。癒合組織之誘導率甚高，雜種第一代約有百分之四十，第二代可達百分之六十以上，少數選株之癒合組織率可達百分之百。

癒合組織之小株分化率，因組織顆粒而異，差別頗大。親本花藥之綠株數多於白株，而雜種花藥白株數目多於綠株，部份癒合組織祇能分化稻根，不能產生完整之小株。多數花藥植株生長快速而健旺，並且具有栽培稻和野生稻之特徵。研究結果可以證明種間遺傳質之轉移及重組可藉花藥培養之技術完成之。