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ANTHER CULTURE OF *ORYZA SATIVA* L. AND *ORYZA PERENNIS* MOENCH HYBRIDS

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Abstract

The objective of this study was to enrich the germplasm of cultivated rice with genetic introgression of wild species through anther culture. Hybrid plants derived from single- and backcrosses between *Oryza sativa* L. cultivar Taichung 65 (S) and *O. perennis* Moench (W) were used. Anthers having microspores at the single nuclear stage were inoculated on various defined media enriched with coconut milk. The anthers of reciprocal single-cross hybrids (SW and WS) produced only calluses and albinos while those of backcrosses to Taichung 65 (SWS and WSS) developed green and albino plantlets. Backcrossed hybrids SWS with *sativa* rice as original female parent showed enhanced callus induction and plantlet regeneration. The frequency of normal green plantlet differentiation was 13.6 percent from SWS and 5.5 percent from WSS. Media with coconut milk induced more calluses than those without it, though the difference was statistically insignificant. Glucose appeared less essential than sucrose in plantlet regeneration. The different response of donor plants in callus induction to a single medium indicated that the genetics of experimental materials played a predominant role. A total of 96 normal green plantlets were obtained from 1,297 anthers excised from backcrossed hybrids, and an averaged 2.1 percent of anther was effective for normal plantlet formation. Plantlets of haploid, diploid, and triploid have yet been found.

Introduction

The use of pollen plants has been found to be an efficient approach to the enhancement of genetic recombination and rapid fixation in hybrid rice (Woo and Su, 1975). Further application of this practice to culturing the anthers of hybrid rice may help incorporate the genetic traits from distantly related species into the cultivated ones. Thus, the germplasm of cultivated rice will then be enriched with the new genetic introgression and a breakthrough in varietal impovement can be expected. This paper reports an introductory

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result in the culturing of anthers derived from crosses between cultivated and wild rice species.

Materials and Methods

Hybrid plants derived from single- and back-crosses between *Oryza sativa* L. cultivar Taichung 65 and *Oryza perennis* Moench (W120 Oka) were developed at the Institute of Botany, Academia Sinica, Taipei, Taiwan. They included eight reciprocal F_1 plants from Taichung 65×W120 (SW) and W120×Taichung 65 (WS), four BC_1 plants from (Taichung 65×W120)×Taichung 65 (SWS), and five plants from (W120×Taichung 65)×Taichung 65 (WSS). When the plants reached the booting stage at which panicles were still covered with leaf sheaths, the anthers were taken and examined microscopically. Anthers having microspores at the single nuclear stage were inoculated aseptically on an inducing medium and incubated at 25 ± 1°C. Anther calluses were transferred to a differentiating medium before their diameter reached 0.4 cm. Culture tubes with the calluses were then placed under a white fluorescent light 5,000 luxes with 16/8 hr daylength and 28/22°C daynight temperature. Chlorophyll development on calluses or albinos could be seen in two weeks after the transfer.

The compositions of three inducing media and one differentiating medium (RD-7) are given as follows (mg/liter).

RI-13 medium

Mineral salts: KNO_3, 2275; NH_4NO_3, 600; $CaCl_2 \cdot 2H_2O$, 294; $NaH_2PO_4 \cdot 2H_2O$, 172; $MgSO_4 \cdot 7H_2O$, 247; KI, 0.75, H_3BO_3, 3.0; $MnSO_4 \cdot H_2O$, 10; $ZnSO_4 \cdot 7H_2O$, 2.0; $Na_2MoO_4 \cdot 2H_2O$, 0.25; $CuSO_4 \cdot 5H_2O$, 0.025; $CoCl_2 \cdot 6H_2O$, 0.025; Na_2EDTA, 37.3; $FeSO_4 \cdot 7H_2O$, 27.8

Organic substances: inostol, 100; nicotinic acid, 1.0; pyridoxine-HCl, 1.0; thiamine-HCl, 10.0; 2,4-Dichlorophenoxyacetic acid (2,4-D), 2; kinetin, 0.5; sucrose, 60,000

The medium was adjusted to pH 5.8 with 0.5 N KOH or HCl and solidified by 0.9% Bacto-agar before autoclaving.

RI-14 medium

Coconut milk (15%, v/v) was added. Other components were identical with medium RI-13.

RI-16 medium

A total of 4 macronutrients (mineral salts) varied from RI-14 medium, *i.e.*, $CaCl_2 \cdot 2H_2O$, 220; $NaH_2PO_4 \cdot 2H_2O$, 0; KH_2PO_4, 136; and $MgSO_4 \cdot 7H_2O$, 185

RD-7 medium

The components differed from RI-13 medium are as follows: KNO_3, 2730; NH_4NO_3, 720; $CaCl_2 \cdot 2H_2O$, 350; $NaH_2PO_4 \cdot 2H_2O$, 0; KH_2PO_4, 180; $MgSO_4 \cdot 7H_2O$, 296; N-Z-Amine type A (Sheffield Chemical, Norwith, N. Y.), 250; sucrose (glucose), 30,000; 2,4-D, 0; naphthaleneacetic acid (NAA), 0.2; kinetin, 2.0.

Results

The number of anthers which has developed onto calluses from hybrid plant SW is given in Table 1. The rate of callus induction ranged from 6.67 to 28.29 percent averaging 13.90±6.9 percent in medium RI-13 and from 6.25 to 34.62 percent averaging 16.21±10.0 percent in medium RI-14. In general, medium RI-14 containing coconut milk induced more calluses from anthers than medium RI-13, though the difference was statistically insignificant. The result of reciprocal hybrid WS plants is shown in Table 2. The callus induction rates on medium RI-16 were ranged from 7.83 to 36.36 percent averaging 14.02±9.6 percent. Though the composition of medium RI-16 differed with medium RI-14 in several mineral salts, a similar result was obtained. Results of table 3 indicated the high variation of callus induction among individual plants of a cross.

Table 1. *Anther calluses developed from Taichung 65×W120 (SW) on media RI-13 and RI-14*(1)

Plant No.	Medium			
	RI-13		RI-14	
	No. of anthers cultured	% Anther forming callus	No. of anthers cultured	% Anther forming callus
1	275	10.55	198	17.68
2	54	11.11	52	34.62
3	119	15.13	80	17.50
4	121	7.44	123	21.14
5	96	17.71	60	6.67
6	135	28.29	—	—
7	49	14.29	146	9.59
8	30	6.67	32	6.25
Total	879	13.90±6.931	692	16.21±10.009

(1) The F-values obtained by using analysis of variance calculated between media, and between plants, were 0.14 and 0.44, respectively. The results were statistically insignificant.

For finding out the results of backcrossed hybrids—SWS and WSS, 1,297 anthers were cultured on media RI-13, RI-14, and RI-16. The data with *sativa*

Table 2. *Anther calluses developed from W120×Taichung 65 (WS) on the RI-16 medium*

Plant No.	No. of anthers cultured	% Anther forming callus
1	99	36.36
2	90	16.67
3	121	8.26
4	115	7.83
5	119	15.13
6	37	10.81
7	175	9.14
8	63	7.94
Total	819	14.02±9.64

Table 3. *The Chi-square test of significance of callus induction among the hybrid plants within each cross on single medium*

Hybrid	No. of plants	Medium	X^2
SW	8	RI-13	24.19**(1)
SW	7	RI-14	37.08**
WS	8	RI-16	40.36**
SWS	2	RI-13	50.97**
SWS	2	RI-14	6.19*
SWS	3	RI-16	5.41
WSS	5	RI-16	61.53**

(1) *,**: Significant at 5% and 1% level, respectively.

Table 4. *Anther calluses developed from hybrid plants (Taichung 65×W120)×Taichung 65 (SWS) on media RI-13, RI-14 and RI-16*

Plant No.	Medium					
	RI-13		RI-14		RI-16	
	No. of anthers cultured	% Anther forming callus	No. of anthers cultured	% Anther forming callus	No. of anthers cultured	% Anther forming callus
1	41	65.85**(1)	37	35.14**	—	—
2	163	5.52**	175	17.14**	32	34.38**
3	—	—	—	—	58	56.62
4	—	—	—	—	99	46.46
Total	204	35.69±42.66	212	26.14±12.73	189	45.82±11.13

(1) **: Significant at 1% level, by using the Chi-square test.

rice as the original female parent (SWS) are shown in table 4. A total of 605 anthers from hybrid plants were inoculated on three media. Generally, the medium RI-16 induced more calluses than RI-13 and RI-14. The result of the second plant was the most significant one; its induction rates were 5.52, 17.14 and 34.38 percents respectively from RI-13, RI-14, and RI-16 media.

Table 5. *Anther calluses developed from hybrid plant (W120× Taichung 65)×Taichung 65 (WSS) on the RI-16 medium*

Plant No.	No. of anthers cultured	% Anther forming callus
1	88	21.59
2	70	44.29
3	307	1.30
4	37	16.22
5	190	6.84
Total	692	18.05±16.66

Results from hybrid material with *O. perennis* as the original female parent (WSS) are given in table 5. Overall, the average induction rate of WSS plants was 18.05±16.7 percent lower than that of SWS plants. This seems

Table 6. *The formation of normal green and albino plantlets differentiated from anther calluses in relation to the effect of sucrose (S) and glucose (G)*

Cross	Sugar	No. of calluses incubated	No. and % of callus-forming plantlets				Total frequency of plantlet differentiation, (%)[3]
			Green	(%)	Albino	(%)	
SW	S[1]	208	0	(0)	122	(58.7)	
	G[2]	45	0	(0)	12	(26.7)	
	T	253	0	(0)	134	(53.0)	53.0
WS	S	80	0	(0)	41	(51.3)	
	G	33	0	(0)	6	(18.2)	
	T	113	0	(0)	47	(41.6)	41.6
SWS	S	145	21	(14.5)	78	(53.8)	
	G	24	2	(8.3)	6	(25.0)	
	T	169	23	(13.6)	84	(49.7)	63.3*[4]
WSS	S	40	4	(10.0)	20	(50.0)	
	G	33	0	(0)	7	(21.2)	
	T	73	4	(5.5)	27	(37.0)	42.5*

(1) S=Sucrose; (2) G=Glucose;

(3) $\% = \frac{\text{No. of Callus-forming Green and Albino Plantlets}}{\text{No. of Incubated Calluses}}$;

(4) *: $\chi^2 = 4.08$ (5% level of significance).

to indicate that the *sativa* rice as the female parent may enhance callus induction. On the other hand, callus frequencies varied widely among individual plants within SWS and WSS crosses (Table 3).

The regeneration of plantlet is shown in Table 6. Approximately, the half number of calluses from SW and WS hybrids developed albino plantlets. No normal plant was recovered from either of these crosses, however. Calluses produced from SWS and WSS anthers generated both albinos and green plants; the total frequency of plantlet differentiation varied considerably, 63.3 and 42.5 percents in the respective crosses. The SWS hybrids generated more normal plantlets than the WSS. Their frequencies were 13.6 percent of the former and 5.5 percent of the latter. Glucose appeared less essential than sucrose in plantlet differentiation. Generally, a cluster of callus regenerated more than one plantlet. A total of 96 normal green plantlets were obtained form anthers of SWS and WSS plants, and an average 2.1 percent of anthers was effective for normal plantet formation. A total of 13 haploid (n=12), 20 diploid ($2n$=24), and 1 triploid ($3n$=36) was found from 34 plantlets examined cytologically.

Discussion

In the anther culture of rice plants earlier conducted by Niizeki and Oono (1968, 1971), haploid plants were induced and then diploidized with colchicine treatment. The use of hybrid rice in anther culture was reported by Woo and Su (1975). In their study pure lines were developed from *indica*×*japonica* rice. The origin of regenerates was identified with semidwarf genetic markers (Mok and Woo, 1976). The further application of anther culture to cultivated×wild rice hybrids may incorporate genetic materials between the less related germplasms. Though the anther calluses of F_1 plants derived from single cross did fail to generate green plantlets, they differentiated albinos.

For the albino plantlets of cereals, artificial amphidiploid of *Aegilops caudata*×*Ae. umbellulata* was reported by Kimata and Sakamoto (1972) and *Triticale* by Ono and Larter (1976). The course of albinism was investigated by Edelman and Hanson (1971). They demonstrated that sucrose suppression of chlorophyll synthesis occurred in carrot callus tissue culture, and glucose did not cause a similar effect. Pamplin and Chapman (1975) confirmed these observation in carrot root tissue cultures. Oono (1975) examined the effect of sugars to the regeneration of green and albino plantlet from pollen callus of rice, and no significant difference was found. In the present study, the calluses of SW and WS anthers developed only albinos from differentiating media containing either sucrose or glucose. The use of glucose as the carbon

source instead of sucrose for both callus initiation and plantlet differentiation requires a further investigation.

The anther culture of hybrid rye was reported by Wenzel *et al.* (1977). Approximately 1/4 green plants and 3/4 albinos were developed from *Secale cereale*×*S. vavilovii* crosses. The result was similar to our study that a number of normal green plantlets were recovered and grown into adult plants. Calluses derived from SWS plants with *sativa* rice as the original female parent were found easier to generate more plantlets than those with *perennis* rice as the females (WSS). The difference in plantlet differentiation might be related to the cytoplasmic effect of *sativa* and *perennis* rices.

Guha-Mukherjee (1973) in assessing the embryoid ability and haploid plantlets of 20 rice varieties in anther culture, discovered that a wide difference existed in the response of anther culturing and that the primitive Assam rice was the most responsive. They denoted that genotypic differences of rice plants have a definite role in shifting the normal course of development of the pollen. Thus the inconsistent responses of anther donor plants to a single medium are likely to be controlled by the genetics of the plants. Since the species *O. perennis* is heterozygous, the hybrids developed from the crosses used would be differed from one plant to another. This result can be referred to the work of Wenzel *et al.* (1977) in *Secale*. They observed that the genome of the donor parents used for producing F_1 anthers appeared to be very important in determining the success of culture. They suggested that a second cross with a parent carrying good regeneration capacity would be necessary. The anther culture is then applied and an extended screening for desired genotypes followed. The different findings may suggest that a full-scale use of anther culture technique to develop a large number of plantlets for studying specific problems in genetics and plant breeding would have to depend upon the nature of parental materials.

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栽培稻與野生稻雜種之花藥培養

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本研究之目的在於引導野生稻之遺傳質以增加栽培稻之種源，栽培粳稻臺中65號（簡稱S）與野生稻 *Oryza perennis* Moench（簡稱W）之雜種爲試驗材料。當單交及回交第一代植株之花粉發育至單核期時，將花葯接種於含有或不含椰子水之合成培養劑上。單交雜種之花葯可以產生癒傷組織及白苗，而回交栽培稻之花葯則尙能產生綠苗。(SW)S 雜種之花葯培養較易產生小株，其中正常綠株之分化率爲癒傷組織之 13.6%，而 (WS)S 雜種之綠株分化率只爲癒傷組織之 5.5%。培養劑中含有椰子水時癒傷組織較易形成，但效果並不明顯；葡萄糖對小株分化之效用亦不如蔗糖。由於各培養植株對同一培養劑都有顯著之不等反應，因而材料之遺傳結構實爲培養成效之關鍵。培養回交雜種花葯1,297個，其中只有 2.1% 經癒傷組織而形成正常綠株，經檢查染色體後，發現該等綠株有單元體、二元體及三元體者。