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Japonica Rice Breeding Using Anther Culture[1]

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Abstract: Anther culture was used in Japonica rice breeding two seasons a year in the past five years. The modified N6 medium was used for callus fromation and a differentiation medium for plant regeneration. About 67,000-159,000 anthers from F_1's of 25-36 rice crosses were inoculated to produce about 1,500-15,000 green plants per season for selection. Percentages of anther callusing ranged mostly from 21% to 31% and at most 66.6%. The number of crosses was later reduced to 2-3 to produce more than 13,000 green plants per season. The seed harvested from them were progeny-tested, and the strains established from the selected progenies were entered in yield trials. Although desirable recombinants were recovered in the progenies as expected, they generally appeared to be weak and lack of vigorous plant growth. In yield tests involving strains derived from anther culture and pedigree method, only 1-2 among 6 top-yielding strains were from anther culture. So far, 2 strains have reached the final selection and got into regional tests. It took only 3 seasons to complete a breeding cycle by the anther culture, saving 1/4-1/2 and 2/5 in average the time needed.

It has been almost twenty years, since the first success in the production of green plants from pollen by using anther culture (AC) in rice (Niizeki and Oono, 1968). Segregation, independent assortment and recombination of marker genes in anther-derived progenies could be expected as those obtained in the ordinary F_2 populations(Chen, 1984; Chen *et al.*, 1983a, b and Chen *et al.*, 1983b). However, various mutations occurred after this in-vitro procedure as reported in many mutagenic treatments, although two thirds of the AC progenies were genetically identical (Oono 1975) and 84% had a grain yield equal to the control (Shaeffer *et al* . 1984). The AC_1S_1 lines obtained from anther culture of Tainung 67 yielded equal with those from the mother variety, except 5.8% that had a distinctively lower yield due to the segregation and lowering of seedset percentage and panicle weight (Huang *et al.*, 1985). The strain group of rice derived from anther culture showed no significant yield difference from the strain group developed from ordinary pedigree method in the first rice crop but not in the second crop due to lower seedset percentages and panicle weight in the AC group (Huang *et al.* 1986). Taking the advantage of fairly high percentages of callus formation in Japonica rice,

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anther culture has been adopted in rice breeding in Taiwan since 1980 collaborated closely with tissue culturists. The results of our efforts on the application of this line of biotechnology in rice breeding will be evaluated here.

Materials and Methods

Hybrid materials used for anther culture

Tainung 67, that showed a breakthrough in grain yield and many other traits, is now grown on 60%-70% of paddy fields in Taiwan. Since its development, the objectives of rice breeding have been concentrated on the improvement of disease and insect resistances and rice qualities of this variety. Many resistance genes existed in Indica varieties have been or are being transferred to Tainung 67 by backcross method. The developed new lines from it generally possessed the Tainung 67 genetic background having only one resistance to a disease or insect that was independently transferred by a separate backcross breeding program. Anther culture has been used to combine these resistances and other desirable traits as the next step of rice breeding. Since Tainung 67 was used as the material for screening the media necessary for anther culture by our tissue culturists, the callusing and regeneration percentages generally reached a fairly acceptable level.

Anther culture technique

The hybrid materials used for anther culture were prepared and selected by the breeders each season. Only the most promising crosses were selected and used. The tissue culturists took the young panicles for anther culture in the generations of F_1, stubble F_1 and BC_4F_1 or BC_5F_1. Only single cross F_1's were used after 1984 (Table 1). The young panicles having uninucleate microspores, as indicated by a distance of 3-5cm between the auricles of the flag leaf and the next lower leaf, were taken, sterilized with 70% alcohol for 30 seconds then with a 0.5% solution of sodium hypochlorite for 3 mi-

Table 1. Results of anther culture (AC) in the past 5 years.

AC year & season	AC generation	No. of crosses	Anthers inoculated	% of calli	Green plants	
					Num.	100 anthers
1982-II	F1, BC4F1	25	67,291	31.1	1,567	2.3
1983-I	F1(stub.)	38	159,104	23.7	2,591	1.6
1983-II	F1, BC3F1	33	107,743	21.5	4,517	4.2
1984-I	F1, BC4F1	36	143,820	16.7	1,988	1.4
1984-II	F1, BC5F1	25	148,531	26.6	11,692	7.9
1985-I	F1	18	151,123	21.0	15,147	10.0
1985-II	F1	10	110,897	11.9	16,986	15.3
1986-I	F1	9	119,700	24.7	13,183	11.0
1986-II	F1	2	110,666	66.6	13,527	12.2

Stub.: including stubbles.

nutes, and finally washed with sterilized water for 3-4 times. The young anthers were plated on the modified N-6 medium (Chen *et al.*, 1982) for about four weeks in the dark for callus formation. Young calluses that reached about 2mm in size were transferred to the differentiation medium for plant regeneration for about two weeks (Chen *et al.*, 1982). The differentiated plantlets were transferred to the MS medium for subsequent growth for about 14-20 days. Then, the plantlets, removed from test tubes, were thoroughly washed and sterilized with a 1/1000 solution of Benomyl powder about three minutes, finally transplanted to a sterilized peat and soil mixture in a 3 : 1 ratio. The seedlings continued growth in a growth chamber at a 25°C day temperature for 16 hours and at a 20°C night temperature for 8 hours with continuous 3,000 lux illumination for 4-7 days before moving into greenhouse.

Selection and yield testing

The AC_1S_0 plants, that were later transplanted to a paddy field, received investigation by tissue culturists and also by breeders. The breeders selected only desirable plants or the doubled haploid that looked promising and discarded all other undesirable plants. No effort was made to double the chromosome number of those promising haploid plants. The chimeric panicles or branches that set seed were also selected for progeny testing. The seed harvested from each of AC_1S_0 plants were divided into 5-7 portions for progeny testing, including the test for disease and insect resistances cooperated with the scientists of related disciplines at different stations. Those uniform and acceptable AC_1S_1 lines were individually harvested to establish strains for yield testing in the next generation or AC_1S_2. The acceptable lines but still not uniform would receive once more the progeny test in the AC_1S_2. The individual District Agricultural Improvement Station (DAIS) conducted observational and preliminary yield trials for one or two seasons and the advanced yield trial at least for one year. The strains that reached the final selection were recommended to regional tests at seven sites scattered all over the island and conducted by 6 DAIS's for two years. Only the superior entry attested in these tests can be recommended for registration.

Results and Discussion

The number of rice crosses used for anther culture in 1982 was 25, then increased to 36 and dropped to 2 in 1986 (Table 1). The number of anthers inoculated was always more than 100,000 per season except in 1982. The percentage of callus formation ranged mostly between 15%-30% at most 66.6% in 1986, while the number of green plants obtained each season varied greatly from several thousands to more than 15,000 (Table 1). Since the green plants obtained each season during the first four years reached only several thousands with the number of crosses ranging between 25 and 38, the number of green plants per cross obtained was less than 200. The number of doubled haploid plants selected each season was much smaller, far away from the ideal number 2,000-5,000 per cross. Therefore, the number of crosses used each season was gradually reduced to 2-3 after 1985, so that doubled haploid plants per cross selected each season could reach the ideal number. Since very limited number of crosses were allowed to be

anther-cultured, only the most promising crosses were chosen as the materials.

The number of doubled haploid plants in the AC_1S_0 selected each season varied from 230 to 3,460 mostly around 1,500. The percentages of selected green plants reached about 30% in the first three seasons and dropped to about 10% in the latest three seasons (Table 2). This change coincided with the change of anther percentage that produced green plants(Table 1). The higher percentage of anthers that produced green plants, the lower the percentage of doubled haploid or AC_1S_0 plants obtained. The reason for this coincidence is not known. It might be caused by the cold treatment or storage in a refrigerator of the young panicles before inoculation of anthers. Most of the AC_1S_1 looked uniform in agronomic traits, although a low frequency of them appeared to be segregating. In the AC_1S_2, almost all lines were uniform in agronomic characters except a low frequency of lines that continued to segregate the plants having poor seedset. One thing worth to note is that the recombinant types of blast disease and brown planthopper resistance appeared in the AC_1S_2 lines as expected. There was no difficulty in selecting the desirable recombinants except plant types. Most of AC lines appeared to be weak and lack of vigorous plant growth probably due to their very strict homozygosity.

Table 2. Numbers of the plants and lines derived from anther culture of rice in different generations in the past years.

AC year & season	AC_1S_0 plants			AC_1S_1 line		AC_1S_2		Yield trial	Regional test
	Num.	Select.	%	Num.	Sel.	Pl.	Sel.		
1983-I	2,591	230	8.9	230	61				
1983-II	4,517	1,361	30.1	1,361	107			107	1
1984-I	1,988	590	29.7	590	135	214	13	93	1
1984-11	11,692	3,460	29.6	3,460	486	41	5	504*	(not yet)
1985-I	15,147	1,531	10.1	1,531	160	273	33	106	
1985-II	16,986	1,474	8.7	1,474	366	225	77	467*	
1986-I	13,183	1,692	12.8	1,692 (not yet)					
1986-II	13,527 (not yet)								

*=Tested at 4 locations in the central and northern Taiwan.

Pl.=planted; Sel.=selected.

In 1984, 62 rice strains selected from anther culture were yield-tested with 61 strains selected from ordinary pedigree method. The two strain groups, although derived from different cross origins were under the same nature of breeding project, showed a similar yield performance in the first (spring) crop but not in the second (autumn) crop. Some AC strains tended to segregate the plants low in seedset percentage and panicle weight in the second crop. Thus the number of high-yielding strains in the AC group was much lower than the group selected by pedigree method (Huang *et al.*, 1984). Table 3 shows the mean yield comparison of 6 top-yielding strains of these

Table 3. Mean yield comparison of six top-yielding rice strains and their breeding methods (t/ha, 1984).

Strain No.	First crop		p=5%	Strain No.	Second crop		p=5%
	Method	Yield			Method	Yield	
3840	P	7.697	a	4776	P	7.384	a
3583	P	7.540	a	4067	P	7.377	ab
4215	P	7.522	a	4939	P	7.064	abc
3839	P	7.421	a	3556	P	6.979	abc
4435	AC	7.340	a	3571	P	6.949	abc
4393	AC	7.317	a	4510	AC	6.882	abc
TNG67	CK	6.822	b	TNG67	CK	6.164	c

P=Selected by pedigree method, among 62 (1st crop) and 38 (2nd crop) strains.
AC=Selected by AC, among 62 (1st) and 18 (2nd) strains.

yield tests. Only two and one among 6 top-yielding strains were from anther culture, respectively, in the first and second crops (Table 3). The yield differences between those top-yielding AC and pedigree strains were not significant, only the frequency of AC strains was lower. In the ordinary pedigree method, the desirable genotypes can be accumulated during the course of artificial selections practiced in the F_2-F_5, while no such selection or accumulation is possible by using the AC method, since the hybrid is fixed and the selection is practiced immediately after the AC. Automatically the frequency of desirable genotypes will be much lower.

Starting from 1986, a rice breeding team consisting of all rice breeding stations was established to work together for cooperated breeding efforts in Taiwan. Taiwan Agricultural Research Institute (TARI) and Chiayi Agricultural Experimental Station (AES/TARI) take in charge of breeding work on Japonica rice including anther culture (TARI only), while Taichung DAIS in Indica rice breeding. Each of all DAIS's is allotted several rice testing programs such as yield trials and disease or insect resistance testp depending on the nature of the tests and district specialities. The rice strains deve-

Table 4. Number of AC rice strains tested in different levels of yield trials after 1986.

Location	1986 crops			1987 1st crop OYT
	AYT	PYT	OYT	
Taoyuan	5	28	145	120
Taichung	—	—	—	117
Hwalien	3	19	129	—
Lanyang	7	56	158	115
Total	15	103	432	352

AYT=Advanced yield trial. PYT=Preliminary yield trial. OYT=Observational yield trial.

loped by TARI are sent to four northern DAIS's and those developed by Chiayi AES/TARI are to the southern three DAIS's for yield tests. The number of AC strains sent by TARI to DAIS are shown in Table 4. They were simultaneously sent to different stations for testing disease and insect resistances, rice quality and other necessary traits.

There have been, so far, two AC strains reached the regional yield tests, namely TNGY 6696 and 50727. The performance of TNGY 6696 in regional tests was rather disappointing (Table 5), although it is resistant to the blast and brown planthoppers. It is hoped that the next strain, TNGY 50727, that entered in the regional tests in 1987 will perform better. The results of this strain in advanced yield trial are shown in Table 6. The chronological history in developing the two rice strains is shown below.

Table 5. Performance of the first AC strains in regional tests (average of 7 sites in 1986).

Crop	Entry	DAT	Pl. hgt. (cm)	Grain yield		Remarks
				(kg/ha)	(%)	
I	TNGY6696 (AC)	125	110	5,717	93.6	BL, BPH resist.
	TNG67 (CK)	127	103	6,110	100	BL, BPH suscept.
II	TNGY6696 (AC)	109	109	4,101	91.0	
	TNG67 (CK)	111	99	4,509	100	

DAT=Days after transplanting to harvest.

Table 6. Performance in the advanced yield trial of the second AC strain, TNGY 50727, to be entered in the regioanl tests (1985, TARI).

Crop	Entry	DAT	Pl. hgt. (cm)	Grain yield		Remarks
				(kg/ha)	(%)	
I	TNGY50727 (AC)	121	95	6,975	99.4	BL, BPH resist.
	TNG67 (CK)	121	101	7,020	100	BL, BPH suscept.
II	TNGY50727 (AC)	104	109	5,281	100.4	
	TNG67 (CK)	104	109	5,260	100	

DAT=Days after transplanting to harvesting.

Strains : TNGY6696	TNGY50727
Parents : HCW4//F_4(TNG67^2/CNY243)/// TNGY8814/CNY252////TNG67^5/ Tetep//TNG67^5/Dawn	TNGY8005//F_2(TNG67$_4$/Tetep)
Anther culture : F_1, 1983 2nd(II) crop	F_1, 1983 1st(I) crop
AC_1S_0 1984 I	1983 II
AC_1S_1 1984 II Line	1984 I Line
AC_1S_2 1985 I Preliminary YT	1984 II Preliminary YT
AC_1S_3 1985 II Advanced YT	1985 I Advanced YT

AC_1S_4 1986 I Regional tests	1985 II Advanced YT
AC_1S_5 1986 II Regional tests	1986 I Advanced YT
AC_1S_6 1987 I Regional tests	1986 II Advanced YT
AC_1S_7 1987 II Regional tests	1987 I Regional tests

The F_1 of TNGY 6696 was anther-cultured later than that of TNGY 50727 for one season but was entered to regional tests earlier than that by one year. This was primarily due to the difference in the number of generations spent for yield trials. Starting from the F_1, both strains took three seasons to get into the preliminary yield trial regardless of when the anther culture was made. In the ordinary cross breeding, this period or a breeding cycle generally takes 5 or 6 and at least 4 seasons in Taiwan. Therefore, we can save 1/4 to 1/2 in average 2/5 of the time necessary to obtain pure lines from the F_1 hybrids. In the cross breeding using rapid generation acceleration (RGA) method, 3 generations can be advanced in one year. Since 5 or 6 generations are needed to complete a breeding cycle, it still takes longer time than the anther culture method to complete a breeding cycle.

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應用花藥培養的粳稻育種[1]

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摘 要

以改良N 6 培養基誘導癒合組織之形成而分化培養基引發植株之分化，將花藥培養應用於粳稻育種，每年二期作，繼續五年。每期作從25～36雜交組合第一代雜種取約 67,000至 159,000個花藥，培養得約1,500～15,000綠色植株。癒合組織之形成變異於 21%至31%之間，最高有 66.6%。每期作所用之組合數後來減少至 2 ～ 3 ，並培養 13,000 以上之綠色植株。由此等植株所得種子用於後裔檢驗及篩選。當選的後裔成立爲品系，列入於產量比較試驗。如所預料，重組的基因型出現於後裔，但這些品系多數顯示軟弱缺乏旺盛的生長力。花藥培養品系與譜系法育成品系一起參加產量試驗，6 個最高產品系中只有 1 ～ 2 品系來自花藥培養。至目前爲止有 2 品系通過產量比較試驗，進入區域試驗。應用花藥培養只需三期作即可完成育種一個循環，節省時間約¼至½，平均約⅖。

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