*Bot. Bull. Academia Sinica* **17**: 18-24 (1976)

# INDUCTION OF RICE PLANTLETS FROM ANTHER CULTURE[1]

CHI-CHANG CHEN and MING-HWA LIN

*Department of Botany, National Taiwan University and Department of Agronomy, Taiwan Agricultural Research Institute, Taipei, Taiwan, Republic of China*

(Accepted for publication October 20, 1975)

**Abstract**

Anthers of 12 varieties of rice (*Oryza sativa* L.) containing uninucleate pollen grains were cultured on a modified White's agar medium with sucrose concentration increased to 6% and supplemented with 5 mg/l naphthalene acetic acid, 2.5 mg/l kinetin, and 15% coconut milk. The pollen grains were successfully induced to develop into calluses. The percentage of anthers that produced calluses varied considerably with the materials: from 0% for three *indica* varieties to 44.7% for one *japonica* variety. After the calluses were transferred to Murashige and Skoog's medium supplemented with 1-2 mg/l NAA, 4 mg/l kinetin, 40 mg/l adenine sulfate, and 15% coconut milk, plantlets developed in 2-4 weeks. A total of 165 plants were obtained, among which 61 were haploids, 81 diploids, and 23 tetraploids.

## Introduction

Since the original discovery of Guha and Maheshwari (1964, 1966) that haploid plants could be produced through anther culture of *Datura innoxia*, other investigators have applied this technique to a variety of crop plants. The work has been most successful with species of Solanaceae but less successful with species of Gramineae (Sunderland, 1973).

The difficulty in anther culture of rice (*Oryza sativa* L.) has been the low capacity of the pollen grains to produce calluses and the subsequent differentiation of the calluses into plantlets (Niizeki and Oono, 1968; Harn, 1969; Woo and Tung, 1972; Iyer and Raina, 1972). However, Niizeki and Onno (1971) reported that the frequency of callus formation could be increased considerably by culturing only anthers containing uninucleate pollen grains. Iyer and Raina

(1) This work was initiated at the Department of Agronomy, Taiwan Agricultural Research Institute and completed at the Department of Botany, National Taiwan University. We thank Miss L. H. Shih for her excellent technical assistance. This work was supported by National Science Council of the Republic of China.

(1972) and Guha-Mukherjee (1973) observed differences in the ability of the pollen to give rise to plantlets in different varieties they studied. Nishi and Mitsuoka (1969) and Niizeki and Oono (1971) found that anther culture of rice produced plants of various ploidy levels. Limited genetic information indicated that some of the diploids were of pollen origin (Niizeki and Oono, 1971) whereas others were derived from the somatic tissues of the anther (Woo and Tung, 1972).

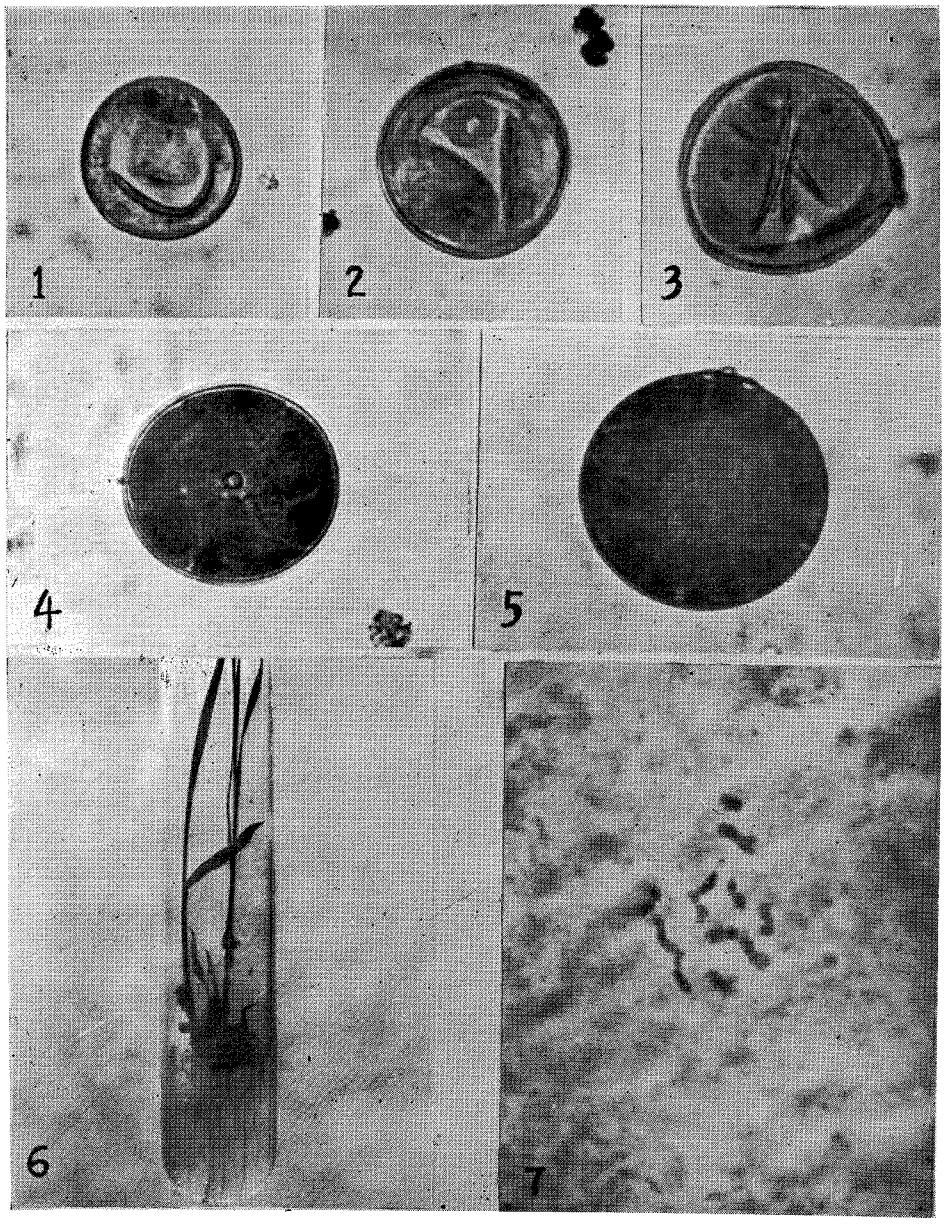
The present paper reports our results from anther culture of 12 varieties of rice.

## Materials and Methods

The varieties of rice used for this study are presented in Table 1. All plants were grown in the Experimental Farm of Taiwan Agricultural Research Institute during March-July 1974. The first few tillers of the plants were collected when the inflorescences were still enclosed within the sheath. Florets were surface-sterilized in 6% calcium hypochlorite for 5 minutes, and then rinsed thoroughly in sterile distilled water. Anthers containing uninucleate pollen grains (Fig. 1) were carefully removed from the florets and transferred to the surface of agar solified medium comprising modified White's basic constituents (Abo El-Nil and Hildebrandt, 1971) with sucrose concentration increased to 6% and supplemented with 5 mg/l naphthalene acetic acid (NAA), 2.5 mg/l kinetin, and 15% coconut milk (CM). The cultures were incubated in

**Table 1.** *Frequency of callus formation in anther culture of 12 varieties of rice*

| Variety | No. of anthers inoculated | % anther forming callus |
|---|---|---|
| *Japonica* type: | | |
| H-124 | 456 | 44.7 |
| Tainan 5 | 386 | 15.3 |
| Tainung 61 | 380 | 7.6 |
| Taichung 65 | 1,123 | 6.1 |
| Taichung Yu 196 | 404 | 5.7 |
| *Indica* type: | | |
| Kabara | 346 | 8.9 |
| Chianung Shien 8 | 331 | 0.6 |
| Chianung Shien Yu 12 | 236 | 0.4 |
| Hsinchu Ai-Chueh Chien | 329 | 0.3 |
| Chianung Shien 11 | 220 | 0.0 |
| Kaoshiung Shien 2 | 348 | 0.0 |
| Taichung Native 1 | 440 | 0.0 |




Figs. 1-5. Stages in the development of pollen calluses. Fig. 1. Uninucleate pollen grain before culture. Fig. 2. Pollen grain in mitosis, 10-12 days in culture. Fig. 3. Pollen grain with two equal nuclei, 14 days in culture. Fig. 4. Multicellular pollen grain, 21 days in culture. Fig. 5. Multicellular pollen grain, 28 days in culture. Fig. 6. Plantlets developed from pollen calluses. Fig. 7. Haploid cell with 12 chromosomes at somatic metaphase.

a growth chamber at a constant temperature of 25±1°C, with a 16-hour photoperiod and 1000 lux light intensity.

Two to three weeks after calluses initiated, they were transferred to Murashige and Skoog's (1962) basic medium supplemented with 1–2 mg/l NAA, 4 mg/l kinetin, 40 mg/l adenine sulfate, and 15% CM for induced organoid differentiation. The cultures were maintained in the same condition as for callus induction except that light intensity was increased to 2000 lux. After the root system had developed, the plantlets were transplanted into pots.

For observation on the developmental changes of the pollen grains, individual anthers were dissected at various intervals during the *in vitro* culture and the pollen grains were stained in acetocarmine. For chromosome determination of the plantlets, root tips were pretreated in 0.002 M 8-hydroxyquinoline at 18°C for 3 hr, fixed in ethanol-acetic acid solution (3:1, v/v) overnight, and stained by the Feulgen squash technique.

## Results

The uninucleate pollen grains stained only lightly with acetocarmine before culture (Fig. 1). After 10 to 12 days in culture, some of the grains started enlarging and their cytoplasm became densely stained. Many other grains either became hollow by losing their cytoplasmic contents or were abnormally enlarged and packed with starch grains. The pollen grains with dense cytoplasm soon underwent mitosis (Fig. 2) giving rise to two nuclei of equal size (Fig. 3). These two nuclei then procceded to divide independently and asynchronously, and by the time of 21–28 days of culture multicellular pollen grains were formed (Fig. 4, 5). After a few more divisions, the multicellular masses broke open the pollen exine as well as the anther wall, and developed into calluses. Formation of embryoids from pollen was not observed.

There were wide variations in the 12 varieties studied with regard to the capacity of their pollen to produce calluses (Table 1). In the *japonica* varieties,

**Table 2.** *Frequency of organ formation and distribution of chromosome numbers in anther-derived plants of rice*

| Variety | No. of calluses | No. of calluses forming plantlets | Distribution of chromosome numbers | | |
|---|---|---|---|---|---|
| | | | Haploid | Diploid | Tetraploid |
| H-124 | 196 | 44 | 0 | 16 | 9 |
| Tainan 5 | 55 | 29 | 15 | 9 | 9 |
| Tainung 61 | 33 | 12 | 7 | 10 | 1 |
| Taichung 65 | 68 | 35 | 32 | 41 | 2 |
| Taichung Yu 196 | 17 | 6 | 7 | 5 | 2 |

the frequency of anthers that produced calluses ranged from 5.7% for Taichung Yu 196 to 44.7% for H-124. After the calluses were transferred to the differentiation medium, plantlets (Fig. 6) developed in 2-4 weeks. A total of 165 plants were obtained, among which 61 were haploids (Fig. 7), 81 diploids, and 23 tetraploids (Table 2). On the othen hand, anthers of the *indica* varieties either did not produce calluses or produced only with very low frequencies. None of the calluses survived on the differentiation medium. Consequently, no plantlets were obtained from anther culture of the *indica* varieties of rice.

## Discussion

Our success in obtaining haploid plants through anther culture of rice may be attributed to several causes. It has been suggested that in tobacco the the capacity of the pollen grains to produce embryoids decreases with an increase of plant age (Sunderland, 1973). In rice, our preliminary results obtained in 1973 indicated that anthers collected and cultured during the first few days of the flowering period were generally more productive than anthers harvested at the end of this period. For this reason, anthers of only the first few tillers of a plant were used in the present study.

The developmental stage of the pollen is an important factor determining the success or failure of the culture. Niizeki and Oono (1971) and Guha-Mukherjee (1973) found that in rice only uninucleate pollen grains were capable of developing into calluses or embryoids; anthers containing microsporocytes or binucleate pollen failed to grow. Our unpublished results indicated that if the uninucleate stage was further divided into three substages, the early- mid-, and late-uninucleate stages, the peak response occurred at the mid-uninucleate stage (just prior to, in the midst of, or immediately after vacuole formation). Before or after this stage the response declined sharply.

The composition of the basic medium appears to be less critical than pollen age, since various media have been used and all shown to be capable of inducing pollen calluses (Niizeki and Oono, 1968, 1971; Nishi and Mitsuoka, 1969; Harn, 1969; Iyer and Raina, 1972; Guha-Mukherjee, 1973). The modified White's medium used in this study is appropriate for callus induction but poor for callus growth and organ formation. Although 2, 4-dichlorophenoxyacetic acid (2, 4-D) was used by most investigators, we found that it was not essential for anther culture of rice. We avoided using 2, 4-D in our culture medium for two reasons. It may stimulate the somatic tissues to proliferate and render the calluses more difficult to differentiate. Our choice of NAA was primarily based on the finding of Niizeki and Oono (1971) that if this auxin was used in the callus-inducing medium, plantlets developed directly from the calluses without transferring to another medium for organ formation. Although some

workers omitted auxins in the differentiation media (Nishi and Mitsuoka, 1969; Woo and Tung, 1972), we found that NAA at the concentration of 1–2 mg/l was beneficial to callus growth and organoid differentiation.

Cytological information on the anther-derived plants of rice has been rather limited. The present investigation and those conducted by Nishi and Mitsuoka (1969) and Niizeki and Oono (1971) provide evidence of the production of plants with various ploidy levels, but they have not been able to reveal the pattern of the distribution. The plants which have been cytologically studied by Nishi and Mitsuoka (1969) and Niizeki and Oono (1971) are too few. In the present study, many test-tube plantlets died before or after transfer to soil. It is possible that some aneuploids and a higher frequency of haploid plantlets do originate from the pollen grains, but have been eliminated owing to their poor survival.

## Literature Cited

ABO EL-NIL, M. M., and A. C. HILDEBRANDT. 1971. Differentiation of virus-symptomless geranium plants from anther callus. Plant Disease Reporter **55**: 1017-1020.

GUHA, S., and S. C. MAHESHWARI. 1964. *In vitro* production of embryos from anthers of *Datura*. Nature **204**: 497.

GUHA, S., and S. C. MAHESHWARI. 1966. Cell division and differentiation of embryos in the pollen grains of *Datura in vitro*. Nature **212**: 97-98.

GUHA-MUKHERJEE, S. 1973. Genotypic differences in the *in vitro* formation of embryoids from rice pollen. J. Exp. Bot. **24**: 139-144.

HARN, C. 1969. Studies on the anther culture of rice. Korean J. Breed. **1**: 1-11.

IYER, R. D., and S. K. RAINA. 1972. The early ontogeny of embryoids and callus from pollen and subsequent organogenesis in anther cultures of *Datura* and rice. Planta **104**: 146-156.

MURASHIGE, T., and F. SKOOG. 1962. A revised medium for growth and bioassys with tobacco tissue culture. Physiol. Plant. **15**: 473-497.

NISHI, T., and S. MITSUOKA. 1969. Occurrence of various plants from anther and ovary culture of rice plant. Japan. J. Genet. **44**: 341-346.

NIIZEKI, H., and K. OONO. 1968. Induction of haploid rice plant from anther culture. Proc. Japan. Acad. **44**: 554-557.

NIIZEKI, H., and K. OONO. 1971. Rice plants obtained by anther culture. Colloques Internationaux du C. N. R. S. No. 193, Paris. pp. 251-257.

SUNDERLAND, N. 1973. Pollen and anther culture. *In*: Plant Tissue and Cell Culture. (H. E. Street, Ed.) pp. 205-239. Univ. of California Press, Berkeley.

WOO, S. C., and I. J. TUNG. 1972. Induction of rice plants from hybrid anthers of *Indica* and *Japonica* cross. Bot. Bull. Academia Sinica **13**: 67-69.

# 水稻花葯培養

陳其昌 林明華

國立臺灣大學理學院植物學系及
臺灣省農業試驗所農藝系

本研究的目的在利用花葯培養的技術，獲得單元體水稻，以供遺傳研究及育種之用。培養12個水稻品種含單核花粉的花葯，發現含 2.5 mg/l kinetin, 5 mg/l NAA, 6%蔗糖及15%的椰子汁的改良式 White 培養基可誘導水稻花葯產生癒合組織，其誘導率在 0–44.7% 之間。將癒合組織移至含 4 mg/l kinetin, 1–2 mg/l NAA, 40 mg/l adenine sulfate 及15%椰子汁之 MS 培養基，二至四週後癒合組織分化成幼株。由花葯培養共獲水稻165株，其中61株爲單元體，81株爲二元體，23株爲四元體。