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# USING AN ISOZYME MARKER TO DETECT POLLEN–DERIVED PLANTS FROM ANTHER CULTURE OF WILD RICE[1]

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**Abstract**

An electrophoretic esterase marker was used in conjunction with cytological examination to screen for pollen derived plants from anther culture of Taiwan wild rice (*Oryza perennis* Var. *formosana*). The method can efficiently screen for homogenic plants derived from cultured anther of hybrids, and shorten the process of pure line selection in plant breeding.

## Introduction

The significance of producing haploid plants for plant improvement has been reviewed and emphasized (Reinert and Bajaja, 1977). With the introduction of different techniques of producing haploids by the culture of excised anther (Guha and Maheshwari, 1964; Nitsch and Nitsch, 1969), by the culture of isolated pollen (Reinert et al. 1975) and from leaf protoplast culture (Ohyama and Nitsch, 1972), it has become increasingly evident that tissue culture could considerably speed up the production of haploids for breeding programs. Among these methods, anther culture has so far shown the most promise and applicable technique.

On the basis of work with *Datura* and *Nicotiana* species, various modes of endogenesis leading to the formation of haploids in microspore culture were described (Sunderland, 1974). It was suggested that haploid nuclei fuse occasionally to give diploids and

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that diploid and tetraploid nuclei could arise by endomitosis or endoreduplication. In this way the doubled haploid embryo can be formed in the anther culture. However, a large proportion of the diploid plants produced from anther culture of certain cereals were found to be not doubled haploids (Niizeki and Oono, 1971, Jones and Pickering, 1973). This result might be due to plants derived from anther somatic tissue or unreduced diploid pollen. Therefore extensive screening is required to detect pollen-derived plants from anther culture.

Examination of chromosomes in root tips is a reliable method to screen for haploids from anther culture, but this method cannot distinguish pollen-derived diploid and diploid plants from those derived from other tissues. Genetic markers of various morphological characteristics can be used to determine pollen derived diploid homogenic plants, but this is a laborious way and often need an additional progeny testing for confirmation.

Enzyme polymorphisms are known to be widespread in plant populations, and have been extensively used as genetic markers in genetic and evolutionary studies (Gottlieb, 1971). Several esterase loci were found to be polymorphic in both Taiwan wild rice (*Oryza perennis* var. *formosana*) and in cultivated varieties (Kiang and Wu, 1979). We report on using an isoenzyme genetic marker (esterase) to screen pollen derived homogenic rice plants from anther culture at an early growth stage.

## Materials and Methods

Taiwan wild rice (*Oryza perennis* var. *formosana*) plants, from Taoyuan County, Taiwan, were used for the present study. Leaves of the plants and self- and cross-pollinated progeny were analyzed for esterase isoenzymes using horizontal starch gel-electrophoresis. A 12% starch gel was made in 0.03 borate buffer at pH 8.5, and poured into plexiglass molds with internal dimensions of 2.0 x 0.6 x 20.0 cm. About 0.5 g of leaf tissue was ground with a procelain mortar and pestle. A filter paper strip 1.6 x 0.5 cm was placed on the paste-like homogenate till saturated, and then inserted in the gel 8 cm from the cathodal end. Electrophoresis was carried out at 250 V for 4 hr at 4-6°C. Esterase activity was detected by incubating the gel at 37°C for 1 hr in 100 ml 0.01 M phosphate buffer (pH 6.8) containing 0.03 g α-naphthylacetate and 50 mg Fast Red TR Salt.

For anther culture, anthers from a wild rice plant P10 were placed on solid agar (0.8%) medium (Murashig and Skoog, 1962), supplemented with 2 ppm IAA, 2 ppm Kinetin, 2 ppm 2, 4-D, 30g/l sucrose and 100 ml/l coconut milk, and incubated at 25°C in the dark. After 4 to 6 weeks callus proliferated from the anthers were transfered onto the differentiation medium which had the same composition as described previously but without 2, 4-D and incubated under flourescent light at 25°C. the shoots were formed from the callus 2 to 3 weeks later. When the plantlets were about 10cm tall part of their leaves were used for electrophoresis and than the plants were suspended in Hoagland

nutrient solution (Hoagland and Arnon, 1950) in 25 ml testubes. The root tips produced in the nutrient solution were used fro chromosome counts.

## Results and Discussion

Starch gel-electrophoresis showed that the wild rice had three esterase zymograms, slow (S), fast (F) and 3-banded, which migrated towards the cathode (Fig. 1.) Genetic study shows that these esterase zymograms are governed by a single locus (Cl) with two alleles ($Cl^S$ and $Cl^F$) and the heterozygote forms a heterodimer between the two homodimers to show a 3-banded zymogram (Kiang and Wu, 1979). The zymograms were stable when the leaves of the adult plant, young tillers and the seedlings were examined.

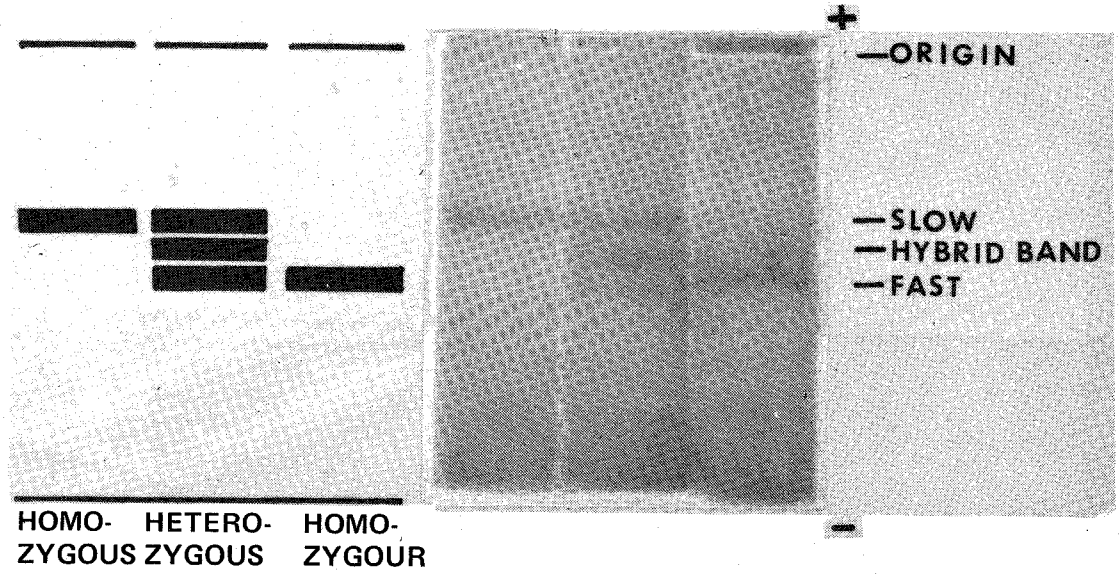


Figure 1. Zymogram showing homozygous and heterozygous esterase Cl patterns in rice plant.

Altogether 32 plantlets were produced from 12 calli derived from different single anthers (Table 1). There were 10 plants showing the S zymogram, 7 the F zymogram and 15 heterozygotes (Table 1). Cytological studies showed that the heterozygous plants were all diploid with 24 chromosomes and were most likely derived from somatic cells of anthers. The plants with the F zymogram were haploid with 12 chromosomes. Among 10 S plants, 3 were diploid (No. 16, 24) and 7 were haploid (No. 1, 3, 9, 31 Table 1). The plants with the F and S zymograms were derived from gametic cells. It is evident that the homogenic diploid plants produced in anther culture can be detected by using the esterase genetic marker. In the present study we did not find any polyploid plant from the anther culture. Albinos, which constitute about 25% of the plantlets, were found to be either haploid or heterogous diploid (Table 1).

**Table 1.** *Esterase zymogram and chromosome numbers of plantlets obtained from wild rice P10 anther culture.*

| Callus no. | Number of plantlets | Esterase zymograms | Chromosome[(1)] number | Green or albino |
|---|---|---|---|---|
| 1 | 1 | S | 12 | G |
| 3 | 1 | S | 12 | A |
| 3 | 3 | He | – | A |
| 7 | 4 | F | 12 | G |
| 9 | 4 | S | 12 | G |
| 10 | 1 | F | 12 | G |
| 16 | 2 | S | 24 | G |
| 18 | 4 | He | 24 | G |
| 24 | 1 | S | 24 | A |
| 27 | 6 | He | 24 | G |
| 31 | 1 | S | 12 | G |
| 42 | 1 | He | 24 | A |
| 42 | 1 | He | – | A |
| 43 | 2 | F | 12 | A |

(1) Chromosome number 2n = 24
S = slow migrating zymogram. F = fast migrating zymogram. He = 3-banded zymogram.

Although a relatively small number of plants was obtained from the anther culture, the present study demonstrates that the use of electrophoretic genetic markers in conjunction with cytological examination, is a reliable and easy method for screening pollen derived plants produced in anther culture at early growth stage. Although the present study used a naturally occurring heterozygote, it is clear that if appropriate isoenzyme markers are available, the method can efficiently screen for homogenic plants derived from anther culture of hybrid plants. Therefore a combination of anther culture and electrophoresis can considerably shorten the process of pure line selection in plant breeding.

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# 利用同位酵素爲遺傳標記篩選野生稻花藥培養所得之花粉植株

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利用水稻酯化同位酵素(Esterase isoenzyme)爲遺傳標記。配合染色體數目的鑑定。有效的篩選台灣野生稻 (*Oryza perennis* var. *formosana*) 花藥培養所得之花粉植株。應用此法可在花藥培養分化植株生長的早期，鑑定所得之純系。因此可有效地縮短育種所須的時間，及簡化繁複的過程。