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IDENTIFICATION OF POLLEN PLANTS REGENERATED FROM ANTHERS OF AN INTERSUBSPECIFIC RICE HYBRID

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Abstract

Anthers of a subspecific hybrid between *indica* and *japonica* rice (*Oryza sativa* L.) were cultured for callus development. Plantlets of various ploidy levels were successfully regenerated. The plants differentiated from a single cluster of callus exhibited identical, similar or different phenotypes. The semidwarf stature of the parents was used as a genetic marker for identifying the origin of regenerated plants. The spikelet fertility of those plants differed considerably. The time of heading ranged from 50 days to nine months, however some plants grew vigorously for more than one year without heading.

Introduction

The induction of germplasm from distantly related sources is always considered to be an important approach in rice breeding. Attempts have been made to transfer genes between *indica* and *japonica* crosses in order to recombine promising traits into a cultivar. However, the germplasm initiated from the two types of rice may interact to cause a continuous segregation in subsequent generations. Thus, true breeding lines are difficult to obtain in early hybrid generations. This phenomenon has discouraged breeders from adopting the induction approach even though highly vigorous progenies may result from the interaction of different rice genes.

The utilization of haploids for the development of true breeding lines has become an important tool in specific hybridization since Niizeki and Oono (1968) succeeded in plantlet induction from rice anthers. Woo and Su (1975) used F_1 anthers as experimental material. They obtained a pure line with semidwarf stature from an *indica-japonica* cross. Their preliminary success has furthered the researches in identifying the origin of regenerated plants with the aid of

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marker genes as well as in studying the genetics of developing true breeding lines from intersubspecific hybrids.

Materials and Methods

A reciprocal hybridization was made between *indica* rice IR-8 and *japonica* rice Chianung 242-d_3 in the summer of 1973. Both parents carry semidwarfness genes which are non-allelic to each other (IRRI, 1971). F_1 plants were grown in the spring of 1974 and found to grow vigorously with heights over 125 cm tall. Anthers of the F_1 plants were removed during the uninucleate stage of microspores. They were incubated in dark at 28°C on a solid medium modified from Murashige and Skoog (1962) but supplemented with 0.5–2.0 mg/l IAA, 2.0 mg/l 2, 4-D, 0.5–2.0 mg/l kinetin, 15% coconut milk and 0.8% agar. The pH of the medium was adjusted to 6.0. Calluses of pale-yellow color which proliferated from anthers after a six-week incubation were transferred to the differentiation medium which had the same composition as the aforementioned one but without 2, 4-D, and with a kinetin content raised to 4 mg/l. The cultures were placed under fluorescent light of 3,000 luxes, 14 hours/day at 28±2°C. Chlorophyll was found two weeks after transfer, and plantlets were formed ten days afterwards. After one-month in culture, the plantlets were transplanted into pots 10 cm in diameter and grow under 8,000 lux-light in a culture room for two weeks, and then to 30 cm pots in a greenhouse for maturity.

For chromosome determination of the plantlets, root tips or panicles with pollen mother cell were fixed in acetic-ethanol (1:3, v/v) overnight, and stained by the Aceto-Carmine squash method.

Results

A total of 25,200 anthers were excised, only 122 or 0.48% of them formed calluses. Among the 35 calluses from which plantlets were differentiated, 15 grew to green plantlets, 4 produced green and albinoes, and 16 gave only albinoes, altogether 229 green plantlets were obtained as shown in Table 1. Their chromosome numbers and morphology that was further analysed in this study are given in Table 2, except the 17 plantlets which died in early stage. The semidwarf stature was used as the genetic marker for identifying the origin of such plants. Plants having semidwarf or dwarf stature, either haploid, diploid or polyploid, were of pollen origin while those having tall culms (over 100 cm) might have originated either from the pollens or somatic cells of anthers. Thus, Table 2 indicated that almost all the plants were of pollen origin. The plants that regenerated from calluses No. 34, 38 and 112 problably originated either from the pollens or somatic cells, whereas those

Table 1. *The number of anthers planted, calluses formed and plantlets derived*

Crosses	No. of anthers		No. of calluses forming plantlet				No. of green plantlets
	planted	forming callus	Albino	Green	Albino & Green	Total	
IR-8 × CN242-d_3	21,600	88	9	13	3	25	182
CN242-d_3 × IR-8	3,600	34	7	2	1	10	47

Table 2. *The plant stature and chromosome number of the plantlets*

Crosses	Callus No.	No. of Plantlets	Chromosome Number (n=12)	Plant Stature(1)	Spikelet Fertility(2)
IR-8 × CN242-d_3	1	18	24	Semi-D	b
〃	2	2	12	Smei-D	a
〃	13	31	24	Semi-D	b
〃	14	3	24(3)	Semi-D	a
〃	21	3	24	Semi-D	b
〃	22	1	24	Semi-D	b
〃	34	2	48	Tall	b
〃	37	22	12	Dwarf	a
〃	〃	3	36	Semi-D	a
〃	38	14	24	Tall	b
〃	〃	3	48	Tall	b
〃	42	3	24	Semi-D	b
〃	47	1	24	Semi-D	a
〃	〃	2	36	Semi-D	a
〃	57	6	24	Semi-D	a
〃	58	8	28-32	Dwarf	a
〃	73	6	24	Semi-D	*
〃	〃	10	24	Dwarf	*
〃	112	34	24	Tall	c
CN242-d_3 × IR-8	78	22	24	Tall	17* 5a
〃	〃	14	36	Semi-D	1a 13*
〃	91	2	24	Semi-D	*
〃	105	2	24	Semi-D	a
Total	18	212			

(1) Tall: over 100 cm; Semidwarf (Semi-D): 60-90 cm; Dwarf: less than 50 cm.
(2) a: 0%; b: 0-10%; c: 50-80%; *: non-heading.
(3) with chromosomal translocation.

differentiated from No. 78 originated either from the pollens or both pollens and somatic cells.

Plants that originated from a clump of callus could have identical, similar or different phenotypes. In most incidences each clump of callus differentiated into plants that exhibited identical or similar features. But calluses No. 37, 73 and 78 were regenerated into plants of various heights (Table 2).

Overall, the spikelet fertility of plants differed from plant to plant. The haploid plants did not produce any seeds whereas the polyploids (triploids or tetraploid) gave a few. The spikelet fertility of the diploids can be catergorized into three major groups (Table 2): one group did not have any seeds, a second group with less than 10% fertility, the third group includes tall culms with 50 to 85% fertility. Heading dates of the plants varies considerably. The most of them headed normally, but some diploids headed after nine months growth whereas one haploid headed 50 days after transplanted in soil. However, many plants as shown in Table 2 grew vigorously for more than one year without heading. The cause of non-heading has not been identified.

Discussion

Haploid plants have been considered to be useful materials for genetic study. Since only half of their genomes is involved, the function of recessive genes and the interaction of alleles can be studied easily. Plants that were spontaneously or artificially induced can also be used to attain the same goal of the investigation. However, the frequency of haploid occurrence is very low, and it is quite laborious to make such a search. Induced haploids could be obtained though the use of irradiation (Chase, 1969), chemical agents (Simantel and Ross, 1964; Hermsen, 1969) and interspecific hybridization (Kasha, 1974). Though these treatments can greatly increase the occurence of haploids, they may interact with the genetic material and modify the genotype to a certain extent. In order to avoid this possible side-effect, it appears better to use anther culture whereby haploids developed from pollen cells carry an intact genome. This prospect loomed ahead after a successful development of haploids has been made in *Datura innoxia* by Guha and Maheshwari (1964), in *Nicotiana tabacum* by Nitsch and Nitsch (1969) and in *Oryza sativa* by Niizeki and Oono (1968). Their successes open up a new approach in cell physiology as well as in plant breeding.

If haploids were treated with colchicine for induction of diploids, a homozygous line could be obtained. Woo and Su (1975) used anther culture for rice breeding, they obtained a true breeding line from *indica* and *japonica* hybrids with early maturing genes that were recovered from both parents. Though the overall agronomic characters of the progeny thus produced are not promising, their finding does indicate that new genotypes, which do not exist naturally can be built up from hybrid material through anther culture.

The origin of regenerated plants could be identified through their phenotypes. Niizeki and Oono (1971) obtained diploid plants from anthers of an F_1 hybrid between the *japonica* and *indica* rice. Those diploid showed segregation of several characters such as leaf size and leaf color. That could not have occurred if the plants had come from callus derived from the somatic tissues. In this paper, the semidwarf stature of the parents was used as a genetic marker. Since both parents carried semidwarfness genes which were non-allelic to each others, the F_1 plants were tall. Thus, plants that arose from the calluses of somatic cell were similar to the F_1 plants and therefore would not be semidwarf. On the other hand, the plants that regenerated from the calluses of pollen origin might exhibit tall, semidwarf or drawf statures because pollens are meiotic products. The origin of such plants can be verified by growing their progenies out in subsequent generations. Plants from pollen cells are genetically pure thus no segregation would occur among their progenies; on the contrary, those plants from somatic cells would segregate in all traits in the next generation. Further studies will elucidate the premises.

The phenotypes of the plants could be used to reveal whether they came from a clump of cells or a single cell. Plants having identical characters and the same chromosome number are considered to be initiated from a single pollen or somatic cell whereas those carrying distingishable features are regenerated from a clump of cells.

In the present study, evidence of endomitosis and fusion of nuclei in callus development has been observed. They might be the major cause of giving a heterogeneous level of ploidy. However, their actual frequencies have not yet been determined. Therefore, plantlets regenerated from such calluses would carry a different number of genomes or chromosomes.

Aneuploids with 28–32 chromosomes and chromosomal translocations are found in several plants. The major cause of such a chromosomal or genomic abnormality is not yet known. It is very likely to be a result of rapid cell divisions during the callus development. The aneuploids and other euploids thus formed can be used as genetic stocks in studying the product of cell differentiation. Their progenies would eventually segregate to form homozygous lines which are useful in rice breeding.

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秈粳稻雜種花粉植株之識別

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本研究在說明秈粳稻雜種之花葯培養及探討花葯誘出植物之特性。應用雜種第一代之花葯爲材料，經由癒傷組織分化得綠色植株，其染色體數出現多種元性。獲自同一癒傷組織之植株，形態有完全相同、相似或相異三類；兩親之半矮生株形可作爲遺傳標記，以判別花葯誘出植物之來源，發現大多數植株確源於花粉。植株之結實率因株而異，抽穗期爲自盆植後五十天起至九個月不等，也有生長甚至已達一年而未見抽穗者。