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DOUBLED HAPLOID RICE FROM *INDICA* AND *JAPONICA* HYBRIDS THROUGH ANTHER CULTURE[1,2]

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Abstract

The progenies of *indica* and *japonica* rice hybrid usually carry sterility and segregate continuously in the subsequent generations. These two barriers limit genetic recombination and fixation. In order to overcome the barriers, the research was aimed to grow haploid plants from anthers of hybrid plants, and the chromosome number of the haploid plants was artificially doubled to become diploid plants. Crosses of the two semidwarf rice of different types, *indica* IR-8 and *japonica* Chianung 242-d_3 were used. Anthers of the F_1 plants were explanted and cultured on a synthetic medium containing 2,4-D for callus development. The calluses were hence transferred to a differentiation medium which was free of 2,4-D.

One haploid plant and six diploid plants were derived from the anther cultures. The haploid one was treated with 0.05% colchicine. Fourteen seeds from a panicle were obtained from the treated plants. All seeds were grown to plants, and thus 14 lines were obtained in the next generation. The progeny plants of both generations were phenotypically identical, and no significant segregation in agronomic characters was observed. However, sterility remained in the doubled haploid plants for two generations. The cause of the sterility should therefore be subjected to further investigation.

Both *indica* and *japonica* varieties have their commercial significance in the Island of Taiwan. Plant breeders of the country have tried to breed new varieties by hybridization, which may recombine promising characteristics of both types. However, hybrid sterility and continuous segregation frequently occur in the progeny lines. These two weaknesses not only eliminate the genetic fixation of the hybrid progeny but also discourage the attempt of subspecific breeding. In order to overcome the barriers, doubled haploid plants are used to undertake the task. Therefore, this research is aimed to grow haploid plants from the pollen cells of hybrid plants, and then to double the haploid chromosomes to get diploid plant.

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Materials and Methods

Indica rice IR-8 was crossed with Chianung 242-d_3 of *japonica* type. Both of the two varieties are semidwarf. The former carried the semidwarfness gene derived from a native variety, Dee-geo-woo-gin, while the latter was induced by ethyl methanesulfonate (EMS) from a tall-culm variety, Chianung 242. The F_1 plants of the cross were grown in 1972-73. Anthers of the plants were explanted and cultured on a synthetic medium which was enriched with coconut milk and 2,4-D 3 mg/l (Woo *et al.*, 1973). After the callus was grown from the induction medium, it was transferred to the differentiation medium of which the 2,4-D composition was omitted. Plantlets derived from the culture were examined cytologically to identify their chromosome complements. Haploid plants were treated with 0.05% colchicine to double the number of chromosomes. Seeds obtained from the haploid plants after colchicine treatments were grown to plants. The morphology, cytology, and fertility of the plants were studied. Flag leaves of the doubled haploid lines and those of their parental varieties were used to analyze their peroxidase isozymes using the techniques as described by Chu (1972).

Results

One haploid plant obtained from the anther culture (Woo *et al.*, 1973) gave 14 seeds after the colchicine treatment. All of the seeds were harvested and grown to matured plants. Their morphological characters are given in Table 1. Plant heights of the doubled haploid lines varied from 77 to 87 cm, which were similar to those of the parent IR-8 rather than Chianung 242-d_3. On the contrary, the leaf color and shape of the doubled haploid lines were close to the parent CN242-d_3. Plant heights of the F_1 plants were over 110 cm. On the other hand, the haploid plants were below 50 cm. Of the tillers, the doubled haploid lines were less abundant than either one of the two parents, F_1 and haploid plants. Panicle lengths of the doubled haploid lines were shorter than both parents and F_1 plants, but slightly larger than that of the haploids. However, this might not affect the number of seeds per panicle since the seeds were tightly borne on the panicles. The cytology of haploid and doubled haploid plants has been examined. No chromosomal aberration and irregular pairing in diakenesis was found from the pollen mother cells of the doubled haploid plants. All cells examined were diploid with the chromosomes of $2n=24$. The fertility of the doubled haploid lines was recovered after the chromosome numbers have been increased. However, it varied with the lines and differed from 42.5 to 74.8%. Fertilities of the two generations are compared in Table 2. The fertility of the selected panicles

Table 1. *Phenotypic comparisons of doubled haploid lines with their parental varieties*

Lines	Plant heights*	Number of tillers	Panicle length*	Fertility %
IR-8	81	25.2	19.0	73.2
CN242-d_3	72	16.0	20.0	86.6
F_1	113	28.0	25.7	65.0
Haploidy	46	24.8	12.5	0
2N 1	83	13	16.4	57.8
2	87	10	16.5	63.2
3	81	10	15.7	53.2
4	79	15	17.4	42.5
5	83	13	17.3	56.5
6	86	10	15.6	57.4
7	80	13	15.2	58.1
8	77	11	18.2	49.2
9	87	16	16.6	74.8
10	84	11	16.0	72.8
11	82	13	18.1	67.3
12	83	17	17.3	64.7
13	87	12	18.3	64.5
14	80	14	16.7	43.8
Average * cm	82.8±3.2	12.7±2.2	16.8±1.0	58.9±9.7

from the first generation ranged from 36.4 to 89.9%, and the average of the second generation was 74.9±4.8%, which was approximately 5% higher than that of the first generation.

The zymogram analysis of peroxidase isozymes is given in Fig. 1. The *indica* parent IR-8 and the F_1 plants of the cross showed 12 bands on the starch gels, while the *japonica* parent CN242-d_3 and the 14 doubled haploid plants revealed 11 bands. The very first band towarded the cathode did not appear. The difference in a single band indicated that the synthesis of that particular isozyme was genetically dominant. The dominance was inherited from the *indica* parent IR-8. However, the lacking of one band in all doubled haploid plants signifies a genetic homozygosity of recessiveness.

Discussion

The culture of generative cells such as pollen grains permits an alternative to the regular form of meiosis. The generation of diploid plants through the haploids as well as those through endomitosis and cell fusions in callus tissues

Table 2. *Comparisons of panicle length and fertility in the first and second doubled generations*

Lines	1st generation		2nd generation	
	Panicle length*	Fertility %	Panicle length*	Fertility %
IR-8	18.9	91.4	19.4	77.3
CN242-d_8	25.2	89.4	23.8	83.0
1	18.2	67.3	18.7	73.8
2	19.1	75.3	19.9	78.1
3	14.5	67.9	18.4	74.4
4	19.1	36.4	18.2	80.8
5	16.7	79.0	17.7	77.8
6	17.2	65.5	17.7	74.3
7	17.3	64.2	17.8	81.7
8	18.5	70.9	17.9	69.6
9	18.2	81.5	17.7	68.0
10	16.0	85.4	17.2	73.6
11	23.0	60.4	16.9	78.6
12	15.0	89.9	18.0	70.7
13	18.5	87.4	17.8	80.8
14	17.1	57.0	17.1	67.3
Average * cm	17.4±2.0	70.6±14.2	17.9±0.8	74.9±4.8

allow the rapid fixation of genetic recombination and thus lead to a homozygosity. These processes not only become useful in varietal development but also open up a new path to the seccess of new genetic recombination in subspecific hybridization. Successful examples of the studies in dicotyledonous plants were reported by Tanaka and Nakata (1969) and Burk *et al.* (1973). They achieved the growth of haploid and diploid tobacco plants in which the chemical and physical characteristics were analyzed and compared with the regular diploid lines. Similar results of the doubled haploid cotton was reported by Feaster and Turcotte (1973). Its yield and yield stabilities were found similar to those of the parental cultivars. All of these findings indicate that the technique of tissue culture can develop the new genetic constitutions and might bring the work of plant breeding to a new era.

Of the monocotyledonous plants successful evidences of producing doubled haploids are rather limited. However, haploid rice developed through anther culture were early reported by Niizeki and Oono (1968), and polyploid as well as doubled haploid plants were later produced by them (Niizeki, 1971). The work of our doubled haploid rice was developed from a hybrid of *indica* and *japonica* cross. The analyses of meiotic behavior, morphological characters

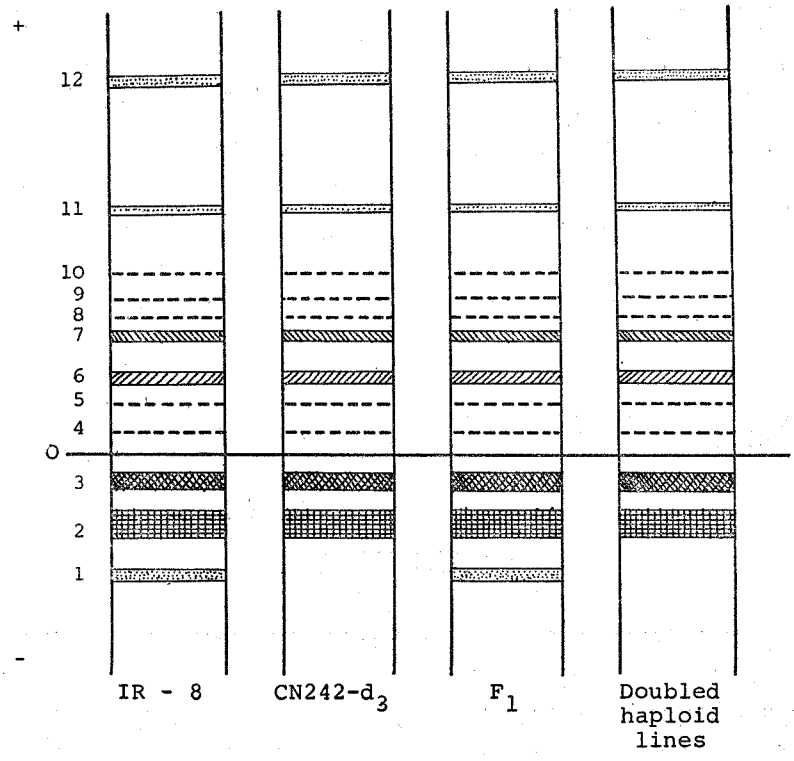

Fig. 1. Peroxidase zymograms of the doubled haploid lines and their parents.

and peroxidase isozymes reveal the genetic purity of the doubled haploid lines. However, whether the traits of the doubled haploid lines are agronomically promising will be subjected to further progeny tests.

Since the progeny of such hybrid does not usually allow genetic fixation in early generations, the development of doubled haploid plants furnishes a successful breakthrough to the limitation of the present genetic recombination between closely related species. Nevertheless, promising methods for the induction of callus cells as well as for the differentiation of haploid plantlets are urgently needed. So far, the very limited number of a single doubled haploid strain would provide a good experimental material to study the genetics of the two subspecific plants. Its contribution to rice breeding could be expected when a large number of haploid plants could be successfully developed.

Literature Cited

BURK, L. G., G. R. GWYNN, and J. F. CHAPLIN 1973. Diploidized haploids from aseptically cultured anthers of *Nicotiana tabacum*. J. Heredity **63**: 355-360.

CHU, Y. E., T. S. CHOU, Y. S. LI, C. Y. SHIH, and S. C. WOO 1972. Identification of Bamboo clones *Dendrocalams latieforus* in Taiwan. Bot. Bull. Academia Sinica **13**: 11-18.

FEASTER, C. V., and E. L. TURCOTTE 1973. Yield stability in doubled haploids of American Pima cotton. Crop Science **13**: 232-233.

NIIZEKI, H., and K. OONO 1971. Rice plants obtained by anther culture. Les Cultures De Tissue De Plantes. Colloques Internationaux Du Centre National De La Recherche Scientifique **193**: 251-257.

TANAKA, M., and K. NAKATA 1969. Tobacco plants obtained by anther culture and the experiment to get diploid seeds from haploids. Japan J. Genetics **44**: 47-54.

WOO, S. C., H. Y. SU, C. M. NG, and I. J. TUNG 1973. Seed formation on induced haploid plant and cytology of anther callus from hybrid rice. Bot. Bull. Academia Sinica **14**: 61-64.

秈粳稻雜種花藥之培養和雙單倍體純系之育成

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秈粳稻之雜種後代時常繼續分離及部份不稔，這兩特性限制了遺傳質的互換和後代的迅速固定。茲爲克服這困難，本研究之目的在應用雜種花藥培養，以期產生單倍體的稻株，進而使用秋水仙精使染色體之數目加倍，而成爲雙單倍體之純系。研究所應用之材料得自IR-8×嘉農 242-d_3 (EMS, 矮生誘變體)第一代雜種之花藥，將花藥首先種植於含有 2, 4-D 之培養劑上，約50天便能產生癒傷組織，嗣後將該組織轉植於分化培養劑，置於日光燈下約三星期部份組織便開始分化成帶有葉綠素之細胞。本研究曾先後獲得單倍體壹株，雙倍體陸株，該單倍體曾使用 0.05%秋水仙精處理生長點後獲得種子十四粒，並發育成十四純系，經二代之試驗，所有之後代形態相同，且無分離現象，諒爲秈粳之純系後代；可是稔實率祇能達到75%，其原因正分析中。