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Efficient Induction of Diploidized Plants in Anther Culture of Rice by Colchicine Pretreatment of Cold-Preserved Spikes

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Effects of colchicine pretreatment of cold-preserved spikes on the increase in ratio of diploidized fertile regenerants in anther culture of rice were studied. After 10 days of storage at 10°C, cut ends of the spikes including uninucleate pollen grains were dipped into colchicine solution at the same temperature. When the spikes were treated with 0. 8% colchicine solution for 3 days, more than 50% of the regenerants, obtained in subsequent anther culture, were fertile.

Introduction

Plant breeding programs for rice as well as tobacco using anther culture techniques have progressed remarkably. Many cultivars have been distributed for actual cultivation in China[1]. Also several cultivars were registered in Japan[2]. The most important point in the case of the so called "haploid method of breeding" using anther culture is how many diploidized plants with different genotypes can be obtained from a certain cross combination.

Several problems are still left in this respect; that is: the difference in response to culture media between cultivars[3-6], the lack of a common method with which callus and plants can be formed at a high frequency[7], the regeneration of albino plants[3,8-10], and the emergence of genetically aberrant plants[3,9]. In addition to these problems, insufficiency in the number of diploidized regenerants obtained may be another important problem in the application of this method to plant breeding. Generally, the frequency of natural diploidization during anther culture is aronud 20 to 30% in rice[11]. Therefore, many of the haploid plants obtained with anther culture are either discarded immediately or preserved for a few months in expectation of natural diploidization during their culture. Though diploidization of callus cells and haploid regenerants have been examined, it is difficult because of the low frequency in diploidization and the high rate of chromosomal chimera[3,11,12].

In order to avoid genetic chimera and to obtain a high regeneration rate of diploidized plants in anther culture, it is considered to be important to induce chromosome doubling at the first pollen division. In the present study, we could obtain a high proportion of fertile regenerants by employing the simple and efficient chromosome doubling method in which rice spikes were treated with colchicine solution before anther culture.

Materials and Methods

Spikes of cv. Nipponbare, which contain glumous flowers corresponding to the pollen stage of uninucleate, were harvested without detaching the flag leaf sheaths at the beginning of June. They

were preserved for 10 days at 10°C by wrapping them in plastic film and aluminum foil. The cold-preserved spikes were then treated with colchicine solution by dipping the cut ends into the solution at the same temperature (Fig. 1). After the colchicine pretreatment, selected glumous flowers were sterilized with 70% alcohol for 20 sec and 2% sodium hypochloride for 8 min, then anthers were picked out aseptically and inoculated on the callus induction media. As a basal medium, N_6 formula[13] supplemented with 5% sucrose and 0.8% agar was used. For callus induction, 10^{-5}M 2, 4-D and 0.05% casein hydrolysate (CH; Casamino Acid, Vitamin free) were added to the basal medium. For the plant regeneration, 5×10^{-7}M naphtalene acetic acid (NAA), 5×10^{-6}M kinetin and 0.1% CH were added. Regenerated plantlets were transplanted in pots after acclimation, then plant length at mid growth stage (about 1 month after transplanting) and seed setting at maturing stage were investigated. We defined completely sterile plants as sterile plants, and others as fertile plants.

Results and Discussion

The Results of anther culture are shown in Table 1. The frequency of callus formation was around 65% in treatments 5 to 8 where cold treatments were continued for 3 days after 10 days-cold preservation. This was higher than for treatments 1 to 4. Since it is well known that callus formation is affected by duration of cold preservation[12], this result seems to have been caused by different imbibition terms. On the other hand, no significant difference was observed among frequencies of callus formation within the treatments with same additional cold storage despite of different concentration of colchicine. This fact indicates that cell division in a pollen grain can take place during anther culture without suffering physiological damage in spite of high concentration colchicine treatment.

In the regeneration culture, vigorous regeneration of green plantlets occurred in all the treatments (Fig. 2). In the present study, the regeneration rate of callus could not be estimated exactly because of too many inoculants in one test tube (about 7 calli per tube). However, the rate appeared to reach about 80% in each treatment. Though the numbers of green and albino plantlets obtained in each treatment differed slightly, they had no relation to either concentration or days of colchicine

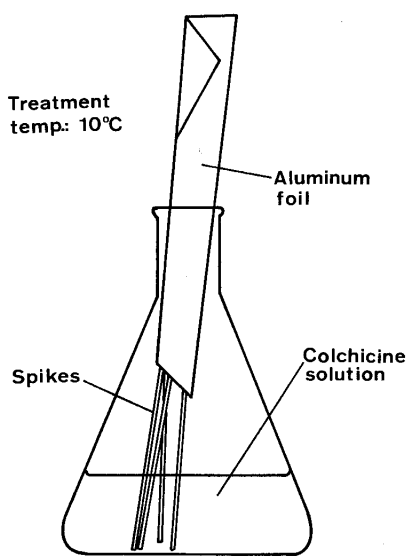

Fig. 1 The method of colchicine pretreatment of rice spikes employed in this study.

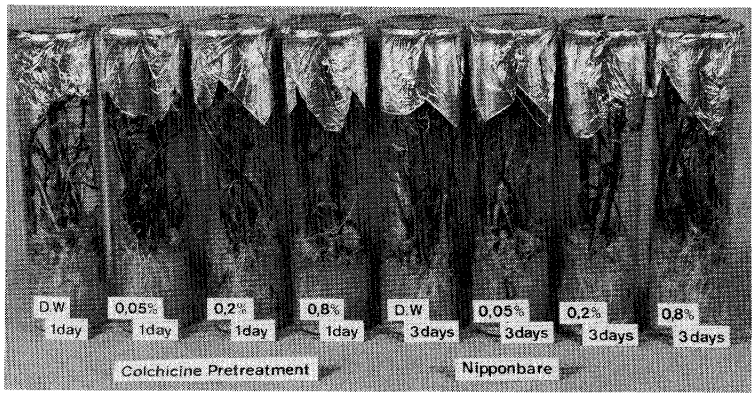

Fig. 2 Plant regeneration in anther culture of rice after colchicine pretreatment (cv. Nipponbare). Vigorous plant regeneration were shown in any treatments.

Table 1. Effects of colchicine pretreatment of spikes on the callus formation and plantlet regeneration in anther culture of rice (cv. Nipponbare)

Treatment*			No. of anthers cultured (A)	Callus-formed anthers (B)	B/A (%)	No. of calli cultured	No. of plantlets	
No.	Colch. (%)	Days					Green	Albino
1	0	1	262	126	48.1	67	193	54
2	0.05	1	318	155	48.7	74	224	20
3	0.2	1	362	184	50.8	71	159	29
4	0.8	1	326	169	51.8	71	120	27
5	0	3	332	216	65.1	72	150	31
6	0.05	3	268	187	69.8	75	142	69
7	0.2	3	334	210	62.9	65	147	25
8	0.8	3	297	186	62.9	62	154	52
	Total		2,499	1,433	57.3	557	1,289	307

* After 10 days of storage at 10°C, cut ends of spikes were dipped into respective solution for 1-3 days at 10°C.

treatment (Table 1). For example, 154 green plantlets were regenerated from 62 calli inoculated in the most intensive treatment, 8. The proportion of albino plantlets to all regenerants was about 19% in the total average, and varied considerably among treatments, but did not correlate with intensity of treatment. From the results obtained in this study, colchicine treatment by the present method is considered to have no adverse influence on the regeneration from callus.

Induced plantlets were potted in order after acclimation. Although more than 50% of regenerants died during the early stages of cultivation due to insufficient acclimation, 210 plants developed to flowering stage. Distributions of plant length and seed set ability of regenerants from each treatment are shown in Fig. 3. It seems possible to identify almost all of the diploid plants by the assessment of plant length, because completely sterile plants were distributed in the range of 20-60 cm. The several fertile exceptions which fell in the short plant length group are considered to be naturally diploidized lines during cultivation in pots, since these plants reached lengths of more than 60 cm at maturation. This result indicates that the frequency of natural diploidization during pot cultivation is considerably low.

Although the number of the regenerants were not enough to compare each treatment with the others, the rate of fertile plants (B/A) did not increase in the 1 day-treatment group (Table 2). On the contrary, this rate increased up to 53.7% in the 3 day-treatment group. These facts indicate that

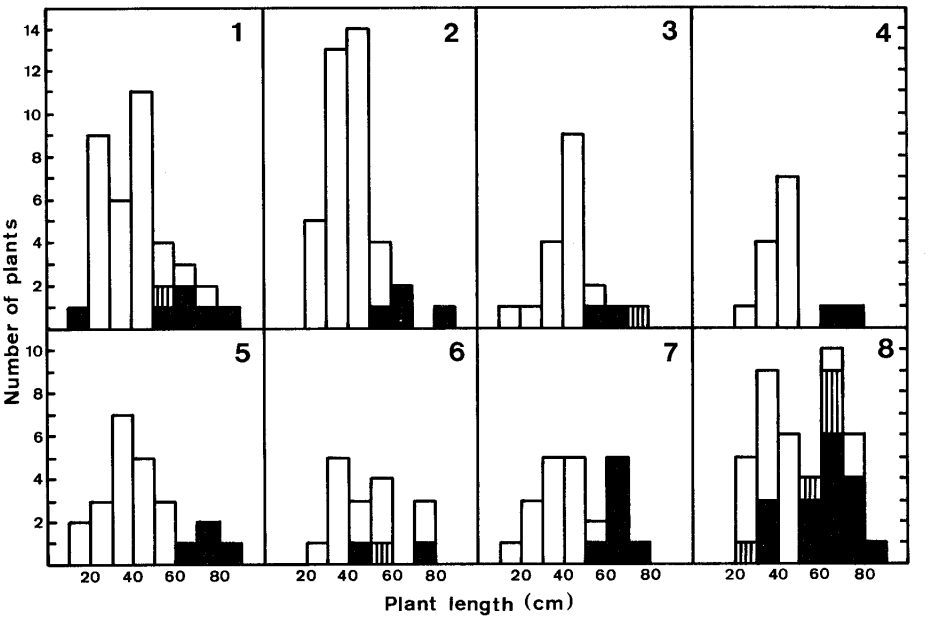

Fig. 3 Plant length and fertility of the regenerated plants obtained in each treatment (cv. Nipponbare). 1-8: Treatment numbers. For the explanation of each treatment, see Fig. 1 and Table 1. □; Sterile plant. ▨: Fertile (less than 50%) plant. ■: Fertile (more than 50%) plant.

Table 2. Effects of colchicine pretreatment of spikes on the formation of fertile plants in anther culture of rice (cv. Nipponbare)

Treat*. no.	No. of plants			B/A (%)
	potted	flowered (A)	setting seeds (B)	
1	60	37	7	18.9
2	80	39	4	10.3
3	50	19	3	15.8
4	42	14	2	14.3
5	60	24	4	16.7
6	50	16	3	18.8
7	53	20	7	35.0
8	65	41	22	53.7
Total	460	210	53	29.5

* For the explanation of treatment, see Table 1.

it takes at least 3 days to obtain the desired results by the present method of colchicine treatment. The number of fartile plants per cultured anther is considered to be the most important index for actual plant breeding efficiency. This value was 2.7 per 100 anthers in treatment 1, while it was 7.4 in treatment 8, being remarkably higher than previous results[2]. We did not transplant all of the calli obtained, and meny of the regenerants in this study failed to aclimate. If we could carry out these processes completely, this value would increase further. In the present study, we did not detect fertility chimera in any plant at all, though some of the fertile plants showed seed set frequency of less than 50%.

Although the diploidization method described in this study might need some improvement in the treatment method and determination of optimum conditions, this method is highly applicable to

actual rice breeding because of its simplicity, no effect on the frequency of callus formation and plant regeneration, the high rate of fertile plants, and no chimera concerning fertility.

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《和文要約》

イネ葯培養における低温保存穂のコルヒチン前処理による倍加個体の効率的誘起

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イネ葯培養における再分化個体の倍加率を高める一方法として，培養前の穂に対するコルヒチン処理について検討した．その結果，10℃で10日間低温前処理した穂を，同温度で0.8%のコルヒチン溶液に3日間浸漬することにより，葯培養で再分化した個体中の稔実個体の割合を50%以上に高めることができた．