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Efficient production of haploid wheat (*Triticum aestivum*) through crosses between Japanese wheat and maize (*Zea mays*)

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Summary. Four Japanese wheat varieties, three crossable and one non-crossable with Hordeum bulbosum, were pollinated with maize pollen of 5 genotypes. By the application of 2,4-dichlorophenoxyacetic acid after pollination, embryos kept developing on wheat plants until 14 days after pollination. The frequency of embryo formation was significantly different among the maize genotypes, varying from 18.0% to 31.9%, but not among the wheat varieties. By bagging spikes with flag leaves the frequency of embryo formation was increased by about 7%. Ten- to twelve-day-old embryos gave higher frequencies of plant formation(83.6 %) than 14-day-old embryos(50.0%). All 6 regenerated plants investigated cytologically were found to be haploid. Twelve of the 14 colchicine-treated plants produced florets setting seeds. The overall efficiency of our procedure is considered to be higher than that reported by Laurie and Bennett(1988).

Key words: Wheat- Maize- Intergeneric cross- Embryo culture- Haploid production

Introduction

In wheat, haploid plants can be produced either through anther culture or crossing with Hordeum bulbosum. Inagaki et al.(1987) reported that the efficiency of haploid production was higher using the H.bulbosum technique. However, most wheat varieties, except those of Asian origin, show no crossability with H.bulbosum due to the presence of the Kr1 and/or Kr2 gene(s)(Folk and Kasha 1981), which may restrict the application of this technique in practical wheat breeding.

Recently, the intergeneric cross between wheat and maize has been suggested as an alternative way to haploid wheat production. Laurie and Bennett(1986; 1987) reported that wheat ovules were fertilized by maize pollen and that haploid wheat embryos were produced through the elimination of maize chromosomes. Furthermore, it was demonstrated that haploid wheat plants were regenerated following spikelet culture and subsequent embryo rescue (Laurie and Bennett 1988). In this report, we describe a simplified method for haploid wheat production by means of wheat x maize crosses.

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Materials and Methods

Experiment 1. Four Japanese wheat varieties, Haruhikari, Shirogane-komugi, Asakaze-komugi and Fukuho-komugi, were used. Haruhikari was non-crossable with H.bulbosum, and the other three varieties were crossable(Inagaki 1986). The maize inbred line and F1 hybrid used as pollinators were A619 and A116xA619. Wheat plants were grown under a 15°C and 10-hour daylength regime during the initial 4-week period, then exposed to a 23°C and 16-hour daylength regime. Maize plants were maintained in a green house under natural light conditions(ca.14.5-hour daylength on the average). The emasculation and crossing procedures followed the methods of Inagaki(1986). Wheat plants were emasculated 3 to 5 days before anthesis, and the emasculated spikes were covered with plastic bags. On the flowering day, those spikes were pollinated with freshly collected maize pollen. The spikes were sprayed with a 75ppm gibberellic acid(GA3) solution 1 and 2 day(s) after pollination, and the plastic bags were replaced by parchment bags.

About 0.3ml to 0.5ml of a 100ppm 2,4-dichlorophenoxyacetic acid(2,4-D) solution was injected into the uppermost internodes of the wheat stems twice as follows: Treatment A; immediately after and again 1 day after pollination, Treatment B; 2 and 1 day(s) before pollination.

Two weeks after pollination, the embryos were excised under a microscope and cultured in petri dishes on a half strength Murashige and Skoog basal medium(Murashige and Skoog 1962) supplemented with 20g/l sucrose and 6g/l agarose and maintained at 25°C in the dark for 1 to 2 weeks. The germinated embryos were transferred to the same fresh medium in test tubes and kept under a 16-10°C and 8-hour daylength regime for about one month. The plantlets were then potted into soil and grown at 16-10°C under 8-hour daylength.

At the 3- to 5-tiller stage, the plants were treated with colchicine, according to the method of Inagaki (1985). The treated plants were then kept under a 20-14°C and 8-hour daylength regime for about one month and subsequently grown under a 23°C and 16-hour daylength regime.

The chromosome number of the regenerated plants was determined according to the method of Nishibayashi and Kaeriyama(1986).

Experiment 2. Fukuho-komugi was used as female parent, and 4 maize F1 hybrids, A116xA619, B14xCI64, B73xA188 and Oh545xA34, were used as pollinators. One hundred ppm 2,4-D was applied after pollination(Treatment A). Pollinated spikes were wrapped with flag leaves and bagged together with them. Ten- to twelve-day-old embryos were cultured on the same medium as that used in Experiment 1. The other procedures were the same as those in Experiment 1.

Results and Discussion

Experiment 1.

It has been reported that the application of 2,4-D increased the seed set in wheat x H.bulbosum crosses. The frequency of embryo formation was increased from 24% to 49% by the application of 100ppm 2,4-D before pollination(Inagaki 1986). In this study, when 2,4-D was not applied, the ovaries failed to grow and most of them were shrunken by 2 weeks after pollination(Fig.1.A). No visible embryos were obtained(data not shown). The application of 2,4-D before pollination(Treatment B) did not give much improvement of embryo formation(1.0%, Table 1). On the other hand, the application of 2,4-D after pollination(Treatment A) markedly increased the frequency of embryo formation up to 11.8%(37 embryos / 314 florets, Table 1). Laurie and Bennett (1987; 1988) reported that embryos were found in 23.4% of the wheat florets pollinated with maize pollen 2 days after pollination, but that most embryos failed to survive until 10-14 days after pollination without 2,4-D application. Therefore, it it speculated that the 2,4-D application in Treatment A promoted embryo development rather than fertilization. In contrast, in Treatment B, the ovules had already started to enlarge by the time of pollination. This enlargement was ascribed to cell expansion (Marshall et al. 1983). It is suggested that the morphological or physiological changes induced in unfertilized ovules by the 2,4-D application may be an obstacle to fertilization in the crosses of wheat x maize, unlike in the crosses of wheat x H.bulbosum.

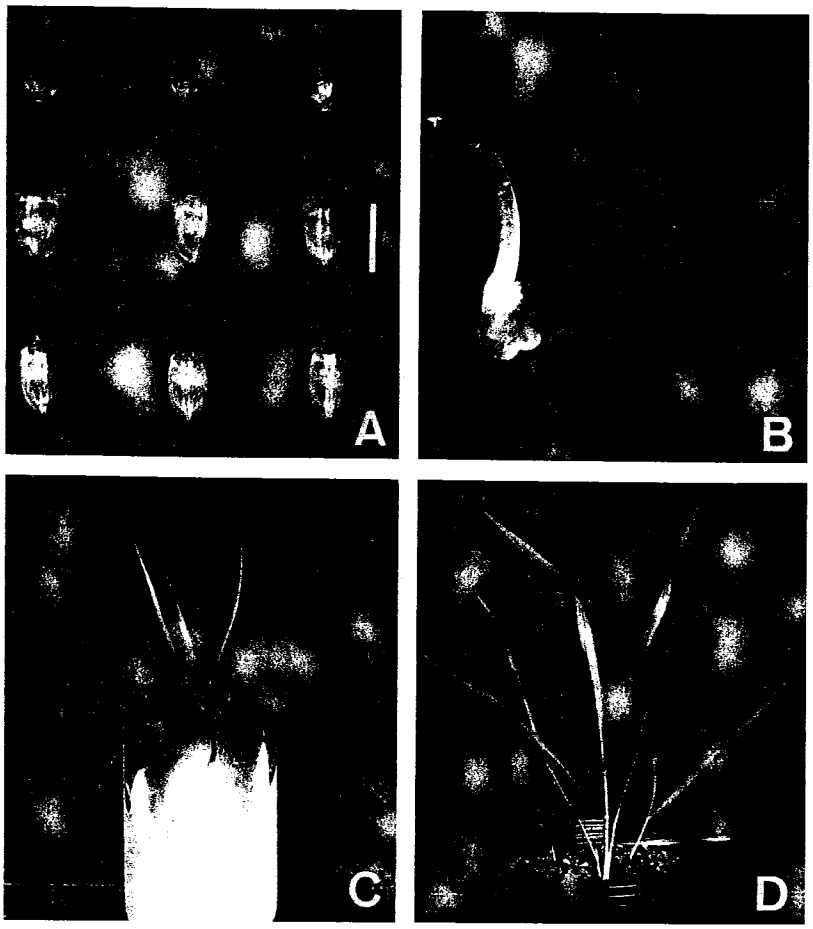

Fig.1. Plant regeneration through embryo culture in Fukuho-komugi. **A**: Caryopses 2 weeks after pollination. Upper row: 2,4-D not applied; middle:Treatment A; lower:Treatment B.Bar represents 5mm. **B**: Germinating embryo on a half strength MS medium 1 week after inoculation. **C**: Plantlet in a test tube 2 weeks after transfer. **D**: Plant in soil.

Of the wheat varieties used in this study, Haruhikari was non-crossable with H.bulbosum (Inagaki 1986). Like the other varieties, this variety showed crossability with maize in Treatment A(Table 1). This finding indicates the absence of a correlation between the crossability of wheat with H.bulbosum and maize, which is consistent with the result of Laurie and Bennett(1988). The frequency of embryo formation in Treatment A varied from 8.3% to 14.6% among the wheat varieties(Table 1), but this variation was not significant statistically. Results from pollination with the maize inbred and F1 were pooled for each wheat variety, because they gave almost the same frequencies of embryo formation(10.5% and 13.3%, respectively). Since a small range of wheat varieties were used in this study, it is essential to screen a wider range of

Table 1. Frequencies of embryo and plant formation in 4 Japanese wheat varieties through wheat x maize crosses.

Wheat Variety	2,4-D Treatment[a]	No.of Florets Pollinated	No.of Embryos Obtained	No.of Embryos Cultured	No.of Plants
Haruhikari	A	62	7(11.3)[b]	7	2(28.6)[c]
Shirogane-komugi	A	48	4(8.3)	0	———
	B	114	3(2.6)	0	———
Asakaze-komugi	A	48	7(14.6)	7	5(71.4)
	B	98	0(0.0)	0	
Fukuho-komugi	A	156	19(12.2)	14	7(50.0)
	B	94	0(0.0)	0	
Total	A	314	37(11.8)	28	14(50.0)
	B	306	3(1.0)	0	

*A619 and A116xA619 were used as pollinators. [a] A:100ppm 2,4-D was injected immediately and 1 day after pollination. B: 100ppm 2,4-D was injected 1 and 2 days before pollination. [b] Figures in parentheses indicate the percentage of embryo formation(No.of embryos obtained/No.of florets pollinated). [c] Figures in parentheses indicate the percentage of plant formation(No.of plants regenerated/No.of embryos cultured).

wheat varieties for crossability with maize.

The length of the developed caryopses in Treatments A and B ranged from 3mm to 5mm at 2 weeks after pollination (Fig.1.A), and the caryopses were normally filled with water. The morphological features of the caryopses were not different between the treatments. Most of the caryopses of the florets on the upper part of the spikes were shrunken at the time of sampling, and no embryos developed.

No normal endosperm development was observed, but endosperm-like or aborted endosperm tissue was found in some of the caryopses in Treatment A.

The length of the dissected embryos ranged from 0.5mm to 2mm. Larger embryos tended to show better germination after culturing. Of the 28 embryos cultured,21 displayed rooting, shooting or both within 1-2 weeks(Fig.1.B). After transfer into test tubes, 14 of the germinated embryos grew further and developed normal roots and shoots(Fig.1.C). Once they showed normal rooting and shooting, they were able to survive after transfer into soil (Fig.1.D).

Two regenerated plants of Asakaze-komugi and 4 of Fukuho-komugi were randomly sampled, and the chromosome number of the root tips was determined. All 6 plants showed 21 chromosomes, which is equivalent to the haploid chromosome number of wheat(Fig.2).

At the 3- to 5-tiller stage, 14 regenerated plants were treated with colchicine. All the plants survived after the treatment and headed. It took about five months from crossing with maize to heading. Twelve plants (85.7%) of the colchicine-treated plants produced florets setting seeds. The number of seeds per plant ranged from 23 to 110 among the plants.

Experiment 2.

The frequencies of embryo formation among the 4 maize F1 hybrids used as pollinators varied significantly from 18.0% to 31.9%(Table 2). The difference in the average frequency between Experiment 1 and 2 is considered to be partially due to differences in the maize genotypes. When using the H.bulbosum technique, the genotype of H.bulbosum also affected embryo formation(Inagaki 1986, Sitch and Snape 1986). To obtain a higher efficiency of embryo formation after wheat x maize crosses, it is necessary to evaluate the maize genotypes from various origins.

On the other hand, since the frequency of embryo formation in the common cross between Fukuho-komugi and A116xA619 was increased from 11.1% in Experiment 1 to 18.0% in Experiment 2, it is considered that the bagging procedure may have also affected embryo formation. Flag leaves were bagged together with spikes in Experiment 2, whereas only spikes were covered with pollination bags in Experiment 1. Desiccation like in Experiment 1 was not observed in Experiment 2, and the caryopses of the upper florets kept developing presumably due to the fact that the humidity around the spikes was improved by transpiration from the flag leaves. Furthermore, it may be necessary to investigate the effect of the material of the pollination bags, which has been demonstrated to affect the seed set and quality in barley inter- and intra-specific crosses(Pickering 1982).

Fig.2. Root tip chromosomes of a regenerated Fukuho-komugi plant, showing 21 chromosomes, equivalent to the number of haploid wheat chromosomes.

Of the 73 embryo cultured, 61(83.6%) developed into plants. The increase of the frequency of plant formation compared with that of 50.0% in Experiment 1 may be due to the difference in the time of culture after pollination. After the colchicine treatment, all the plants survived and are in the process of heading at present.

When this technique is applied to practical breeding, the efficiency of haploid production from any genotype is very important. To increase the efficiency, we developed a method more convenient than that of Laurie and Bennett(1988), who regenerated haploid plants through spikelet culture and subsequent embryo culture. Spikelets 2 days after pollination were cultured on 2,4-D-

Table 2. Effect of maize genotype on embryo formation through wheat x maize crosses.

Maize Genotype	No.of Florets Pollinated	No.of Embryos Obtained	No.of Embryos Cultured	No.of Plants
A116 x A619	178	32(18.0)[a]	0	———
B14 x CI64	238	76(31.9)	51	40(78.4)[b]
B73 x A188	92	19(20.7)	13	13(100.0)
Oh545 x A34	74	19(25.7)	9	8(88.9)
Total	582	146(25.1)	73	61(83.6)

* Fukuho-komugi was used as female parent. 100ppm 2,4-D was injected immediately and 1 day after pollination.
[a] Figures in parentheses indicate the percentage of embryo formation(No.of embryos obtained / No.of florets pollinated). [b] Figures in parentheses indicate the percentage of plant formation plants regenerated / No.of embryos cultured).

supplemented medium for 3 weeks, and the dissected embryos were further cultured for plant formation. In the current method the spikelet culture was omitted, and, instead, the 2,4-D solution was injected into wheat internodes. Caryopses were allowed to develop on wheat plants. Moreover, the crossing procedure reported by Laurie and Bennett (1988) consisted of 2 steps; half of the florets within a spike were selfed, and the other half was emasculated and pollinated with maize pollen. In the current method, all the florets emasculated were pollinated with maize pollen, and selfing was not applied. The average frequencies of embryo and plant formation in Experiment 2 were 25.1% and 83.6%, which were higher than those of 20.6% and 68.1% reported by Laurie and Bennett(1988). Therefore, it appears that the simplified method is more efficient than that of Laurie and Bennett(1988), although different wheat and maize materials were used.

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