Short Communication

Production of Haploid Wheat Plants through Pollination of Sorghum Pollen

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Five genotypes of *Sorghum bicolor* were examined for the production of haploid plants in wheat (*Triticum aestivum* L. var. Fukuhokomugi) through intergeneric crosses followed by 2,4-D treatment. Embryos without endosperm were produced from all the combinations of wheat × sorghum crosses with a frequency ranging from 8.9% to 21.1%. There was no significant difference in embryo production among the sorghum genotypes. Haploid wheat plants were developed with a frequency ranging from 10.5% to 54.2% from the embryos by employing the embryo-culture-method. Sealing with tape of the stem of pollinated spikes on the points injected with 2,4-dichlorophenoxyacetic acid did not result in a significant increase of embryo induction. In conclusion, it was considered that sorghum was one of suitable pollen donors for the production of haploid wheat by the intergeneric crossing method in terms of year-round flowering.

KEY WORDS: *Triticum aestivum*, *Sorghum bicolor*, haploid, intergeneric cross.

Introduction

It has been reported that intergeneric pollination of wheat with the pollen of *Hordeum bulbosum*, *Zea mays*, *Pennisetum americanum* and *Z. mays* ssp. *mexicana* enabled to produce immature haploid embryos (BARCLAY 1975, LAURIE and BENNETT 1988a, AHMAD and COMEAU 1990, USHIYAMA *et al.* 1991). On the other hand, LAURIE and BENNETT (1988b) found fertilization of wheat and sorghum (*Sorghum bicolor*). Sorghum can continue to bloom throughout a year in a greenhouse without short-day treatment (ONO *et al.* 1989). Therefore we determined whether pollination with sorghum pollen followed by 2,4-D treatment enabled to produce haploid embryos in wheat.

Materials and Methods

A wheat variety "Fukuhokomugi" was used as a female parent. Water absorbed seeds were kept at 4°C for 11 days (seed vernalization). After vernalization, the plants were grown in a glasshouse from October 22 to November 22 (short daylength), then exposed to a 23 to 30°C and 16-hour daylength regime. Five genotypes of sorghum (Table 1) were used as pollen donors. The plants were grown in a greenhouse at temperatures ranging from 23 to 30°C.

Pollination and treatment with 2,4-dichlorophenoxyacetic acid (2,4-D) were applied according to the method of SUENAGA and NAKAJIMA (1989). Emasculated florets of Fukuhokomugi were pollinated with fresh sorghum pollen on one day after blooming. About ten to twenty minutes after pollination, 100 ppm of 2,4-D solution was injected into the uppermost internodes of the wheat stems with a plastic syringe (Terumo, 1 ml) and a needle (23G ×

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Table 1. Effect of sorghum genotype on embryo formation through wheat $\times$ sorghum crosses

Sorghum Genotype	No. of Florets Pollinated	No. of Embryos Obtained	No. of Plants Obtained
R 473	540	48 (8.9 ¹⁾)	25 (52.1, 4.6 ²⁾)
White seed	426	67 (15.7)	25 (37.3, 5.9)
PN-4106	562	106 (18.9)	38 (35.8, 6.8)
Combine kafir	90	19 (21.1)	2 (10.5, 2.2)
Combine kafir 602	132	24 (18.2)	13 (54.2, 9.8)

¹⁾ Frequency with percentage of embryos obtained from pollinated florets.

²⁾ Frequency with percentage of plants developed from embryos obtained and from pollinated florets, respectively.

1"). After injection of 2,4-D, some of the stems were immediately sealed with a tape (Teflon seal tape) over the injected points. Twelve-day-old embryos were excised under a microscope and cultured on a half strength MS basal medium (MURASHIGE and SKOOG 1962) supplemented with 20g/l sucrose and 8g/l agar at 25°C in the dark. The other procedures were the same as those described in the report by SUENAGA and NAKAJIMA (1989).

The chromosome number of the root tips of obtained plants was determined using the acetocarmine squash method.

Results and Discussion

Pollination of sorghum pollen on wheat stigmas followed by 2,4-D treatment resulted in the development of embryos and plants (Table 1). The embryos were found in enlarged ovaries lacking endosperm. All the genotypes of sorghum tested produced embryos with a frequency ranging from 8.9% to 21.1%. There was no significant difference in embryo production among the sorghum genotypes. The plants were developed with a frequency of 10.5% to 54.2% from the embryos obtained. Then, 2.2% to 9.8% of the wheat florets pollinated with sorghum produced potential haploid plants.

All the developed plants from the rescued embryos exhibited smaller spikes and sterile

Fig. 1. A spike of a developed plant
Left: Fukuhokomugi
Right: plant from wheat $\times$ sorghum crosses

Table 2. Effect of sealing after 2,4-D injection on embryo production

Treatment	No. of Florets Pollinated	No. of Embryos Obtained	% of Embryos Obtained
Sealed	63	6	9.5
Unsealed	66	7	10.6

Pollination was done with the pollen of PN-4106.

flowers (Fig. 1). The chromosome number of some of the developed plants was counted and found to be 21, which is equivalent to the haploid chromosome number of wheat.

After the injection of 2,4-D solution, part of the solution leaked out from the injected points. But sealing with tape of the stem on the injected points did not result in a significant increase of embryo induction (Table 2). These findings suggest that the slow leakage of 2,4-D from the stems after injection did not decrease embryo induction.

In our preliminary experiment (data are not shown), we observed that the pollen grains of sorghum germinated and the pollen tubes elongated on a wheat stigma. LAURIE and BENNETT (1988b) found that the zygotes fixed 25 to 27 h after pollination had 21 large wheat chromosomes and 10 much smaller sorghum chromosomes and that the sorghum chromosomes were rapidly eliminated and the embryos with three or more cells contained only 21 wheat chromosomes. In this study, we were able to rescue these abortive embryos by the 2,4-D treatment and the embryo culture technique and to obtain haploid plants.

Since sorghum blooms in all seasons in a greenhouse without short-day treatment and regrows from stump after cutting, fresh sorghum pollen is available whenever we need. In conclusion, it is considered that sorghum is one of the suitable pollen donors for the production of haploid wheat plants by using the intergeneric crossing method.

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ソルガム花粉を用いた属間交雑による小麦半数体の作出

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コムギとソルガムの属間交雑によりコムギ半数体の作出を試みた。フクホコムギに5系統のソルガム花粉を授粉し、直後に100 ppmの2, 4-Dを穂軸へ注入したところ、全ての組合せで胚が形成され、胚培養により植物体を得ることができた。交雑小花数当りの胚形成率は8.9%から21.1%であり、植物体獲得率は2.2%から9.8%であった。穂軸注入後の2, 4-Dの針穴からの漏れは、胚形成率に影響を及ぼさないことが明らかとなった。得られた植物体は不稔性を示し、染色体数を確認した個体では全て半数体であった。ソルガムは、周年開花を容易にさせられることから、属間交雑によりコムギの半数体を獲得するために有効な花粉親であると思われる。