Comparison of Polyhaploid Production Frequencies in Crosses of Hexaploid Wheat with Maize, Pearl Millet and Sorghum¹⁾

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¹⁾ Contribution from the collaborative research project with the Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan.

Summary

Frequencies of polyhaploid production were compared among three hexaploid wheat varieties ($2n = 6x = 42$, Chinese Spring, Norin 61 and Siete Cerros) pollinated with maize, pearl millet and sorghum lines. All wheat varieties produced embryos in crosses with maize lines at frequencies ranging from 12.6 to 26.8 %. In crosses with pearl millet lines, the embryo formation frequencies were affected by both the wheat varieties and pearl millet lines. In crosses with sorghum lines, the frequencies were highly dependent on the wheat varieties and Siete Cerros produced no embryos. Embryos were obtained from Chinese Spring and Norin 61 in crosses with all lines of maize, pearl millet and sorghum. The embryos from crosses with maize were larger, but developed into plants at lower frequencies than those from crosses with pearl millet and sorghum. Most of the 377 plants cytologically examined were wheat polyhaploids that carried a complement of $3x = 21$ chromosomes. It is inferred that maize-mediated polyhaploid production is more stable than those mediated by pearl millet and sorghum because of less genotypic effects on embryo formation.

Key Words : *Triticum aestivum*, *Zea mays*, *Sorghum bicolor*, *Pennisetum glaucum*, haploid, embryo culture, intergeneric cross.

Introduction

Intergeneric crosses of hexaploid wheat (*Triticum aestivum* L.) with maize (*Zea mays* L.) result in the formation of globular embryos (Zenkter and Nitzsche 1984) following preferential elimination of maize chromosomes from hybrid zygotes (Laurie and Bennett 1986), even when wheat varieties not crossable with *Hordeum bulbosum* L. are utilized in crosses (Laurie and Bennett 1987). Due to enhancing effect of 2,4-dichlorophenoxyacetic acid (2,4-D) applied to wheat plant at pollination time, the globular embryos develop to immature embryos capable of regenerating polyhaploid wheat plants (Suenaga and Nakajima 1989, Inagaki and Tahir 1990). The maize technique is hence used as a replacement for the *H. bulbosum* technique (Inagaki 1990) for wheat polyhaploid production (Laurie and Reymondie 1991).

However, polyhaploid production frequencies are about 10 % of the wheat florets pollinated (Riera-Lizarazu *et al.* 1992), warranting further procedural development on crossing and embryo rescue.

Recent cytological evidence indicates fertilization of wheat egg cells with pearl millet (*Pennisetum glaucum* (L.) R. Br.) and sorghum (*Sorghum bicolor* (L.) Moench) pollen. These hybridization events resulted in the preferential elimination of the chromosomes of both pollen parents from hybrid zygotes, by a process similar to that observed in crosses with maize (Laurie and Bennett 1988, Laurie 1989, Ahmad and Comeau 1990). These facts suggest that pearl millet and sorghum are potential pollen sources for wheat polyhaploid production. This paper presents a comparison of polyhaploid production frequencies in hexaploid wheat crossed with maize, pearl millet and sorghum, together with embryo formation and plant regeneration from crosses with three different pollen sources.

Materials and Methods

Plant materials

Three hexaploid wheat varieties ($2n = 6x = 42$), Chinese Spring, Norin 61 and Siete Cerros, were used as female parents. Pollen parents were two lines each of maize ($2n = 2x = 20$), pearl millet ($2n = 2x = 14$) and sorghum ($2n = 2x = 20$). Two maize lines contained one open-pollinated line and one F₁ hybrid line. All of the pearl millet and sorghum lines were inbreds. Wheat plants were grown in potted soil in a temperature-controlled greenhouse at 25/15 °C (max./min.) and natural photoperiods (12 - 14 hour). Maize plants came from the maize breeding fields at El Batán, CIMMYT, Mexico. Pearl millet and sorghum lines were obtained from an ICRISAT (International Crops Research Institute for the Semi-arid Tropics, India) project and planted in potted soil under natural field conditions of 25/10 °C (max./min.) and 12 - 14 hour photoperiods.

Crossing procedures

Wheat spikes were enclosed in polyethylene bags to maintain humidity after emasculation. At the predicted time of wheat anthesis, emasculated spikes were pollinated with freshly collected pollen of maize, pearl millet and sorghum. The polyethylene bags were then replaced with glassine paper bags. On the two consecutive days

after pollination, the upper nodes of the wheat culms with the pollinated spikes were needle-injected with a solution (100 mg/l) of 2,4-D (Inagaki and Tahir 1990). Five to 17 wheat spikes were used for each cross combination. Variances of embryo formation frequencies were statistically analyzed after transforming all data for individual spikes to arc-sine.

Embryo rescue

At 16–18 days after pollination, embryos were aseptically excised from the seeds set on the wheat florets, and transferred onto a half strength Murashige and Skoog (1962) medium supplemented with sucrose (20 g/l), gelrite (2 g/l), indole-3-acetic acid (0.2 mg/l) and 6-benzylaminopurine (0.25 mg/l) in 25 ml glass tubes. The embryos were then incubated under the conditions of 20 °C and 24-hour photoperiods (ca. 2000 lux fluorescent light intensity). Embryos randomly selected were measured for length with a micrometer.

Cytological examination

After four weeks of incubation, all the plants that developed shoots and roots were transferred to potted soil, and grown in a greenhouse for further four weeks. Each plant was sampled for root-tips and examined mitotically according to the method of Mujeeb-Kazi and Miranda (1985).

Results

Embryo formation

Most of the wheat florets pollinated with maize, pearl millet and sorghum produced plump seeds. In all cross combinations, the seeds set were filled with an aqueous solution, instead of solid endosperm that were found in selfed seeds (Fig. 1 a), but did not always contain embryos. Table 1 gives the frequencies of embryo formation in crosses of wheat with maize, pearl millet and sorghum. Three wheat varieties produced embryos in crosses with two lines of maize with frequencies between 12.6 and 26.8 %. Statistical analyses in Table 2

indicate that frequency differences in the maize cross were significant only for wheat varieties. In crosses with pearl millet, embryo formation frequencies were highly varied from 0.8 to 39.4 %. Significant frequency differences existed for both wheat varieties and pearl millet lines. In crosses with sorghum, Norin 61 had the highest frequency (41.1 and 42.1 %) of embryo formation. Siete Cerros produced no visible embryos. The frequency differences in sorghum crosses were significant only for wheat varieties.

Plant regeneration

Embryos varied greatly in size and shape among crosses with maize, pearl millet and sorghum (Fig. 1 b). Table 3

Table 2. Analysis of variance for embryo formation frequencies in crosses of hexaploid wheat with maize, pearl millet and sorghum

Source of variance	d. f.	Mean square		
		Wheat x maize	Wheat x pearl millet	Wheat x sorghum
Wheat variety (A)	2	610.68*	1747.98**	4044.26**
Pollen line (B)	1	18.54	1544.91**	0.09
(A) × (B)	2	40.02	28.86	2.05
Residuals	24	88.08	84.53	131.23

* and **: Significant at the probability level of 5 % and 1 %, respectively.

Table 1. Percent frequencies of embryo formation in crosses of hexaploid wheat varieties with maize, pearl millet and sorghum lines

Pollen source	Wheat variety		
	Chinese Spring	Norin 61	Siete Cerros
(Maize)			
Population-93 A	26.0	22.4	12.6
Hybrid-33	18.4	26.8	13.1
(Pearl millet)			
NEC-7006	39.4	37.6	13.6
NEC-7268	23.3	15.6	0.8
(Sorghum)			
ICSR-LM-90166	22.1	42.1	0.0
Toluca-1	20.4	41.1	0.0

Five wheat spikes (ca. 125 florets) were used for each cross combination.

Fig. 1. Seeds (a) and embryos (b) obtained from hexaploid wheat variety Chinese Spring self-pollinated, crossed with maize, pearl millet and sorghum (from left to right) at 17 days after pollination. Bars indicate 5 mm.

Table 3. Plant regeneration from the embryos obtained in crosses of hexaploid wheat varieties with maize, pearl millet and sorghum

Wheat variety	Pollen source	Embryo length (mm)	No. of embryos incubated	No. of plants regenerated (%)
Chinese Spring	Maize	1.32	57	23 (40.4)
	Pearl millet	0.91	186	83 (44.6)
	Sorghum	0.96	79	50 (63.3)
Norin 61	Maize	1.45	96	40 (41.7)
	Pearl millet	1.17	152	93 (61.2)
	Sorghum	1.15	188	106 (56.4)
Siete Cerros	Maize	Not measured	31	16 (51.6)
	Pearl millet	Not measured	18	13 (72.2)

shows the embryo length at the time of incubation and the plant regeneration frequencies. Embryos from crosses with maize were larger than those with pearl millet and sorghum. In contrast, plant regeneration frequencies in crosses with maize were lower (40.4 and 41.7 %) than those in crosses with pearl millet and sorghum (44.6-63.3 %). Some of the embryos that developed only roots ceased to develop further and failed to regenerate plants. These embryos also formed calli from the scutellum tissue.

Chromosome number of regenerated plants

A total of 424 plants were obtained from the crosses of three wheat varieties with maize, pearl millet and sorghum. Some of the plants could not be examined cytologically due to their lethality or poor growth in pots. Table 4 shows classification of the 377 plants identified for their somatic chromosome number. Out of them, 373 plants were wheat euphaploids that carried a complement of 21 chromosomes. Two plants were aneuphaploids with 20 and 22 chromosomes. The other two plants were diploids with 42 chromosomes. These chromosome complements are shown in Fig 2a-2d. None of the plants examined carried small chromosomes from either maize, pearl millet or sorghum.

Discussion

Genotypic effects on embryo formation

The first step in polyhaploid production of hexaploid wheat is embryo formation from the intergeneric crosses. Embryo formation frequencies in crosses with maize were influenced by wheat varieties used, although embryos were obtained from all cross combinations of three wheat varieties with two maize lines. Inagaki and Tahir (1990) using four wheat varieties and eight maize lines reported significant differences of embryo formation frequencies only among wheat varieties. Embryo formation frequencies were stable across the maize lines.

In crosses with pearl millet, significant differences of embryo formation frequencies were found between pearl millet lines, as well as between the wheat varieties. In one cross combination, a wheat variety, Siete Cerros,

Table 4. Chromosome number of the plants regenerated from crosses of hexaploid wheat varieties with maize, pearl millet and sorghum

Wheat variety	Pollen source	No. of plants examined	Chromosome number			
			20	21	22	42
Chinese Spring	Maize	21	0	21	0	0
	Pearl millet	81	0	81	0	0
	Sorghum	47	0	46	0	1
Norin 61	Maize	36	0	36	0	0
	Pearl millet	93	0	92	0	1
	Sorghum	85	1	83	1	0
Siete Cerros	Maize	2	0	2	0	0
	Pearl millet	12	0	12	0	0
Total		377	1	373	1	2

Fig. 2. Complements of somatic chromosomes of the plants obtained from crosses of hexaploid wheat variety Norin 61 with pearl millet and sorghum. (a) $3x=20$ from the sorghum cross, (b) $3x=21$ from the pearl millet cross, (c) $3x=22$ from the sorghum cross, (d) $6x=42$ from the pearl millet cross. Bars indicate $10\ \mu\text{m}$.

expressed a very low frequency (0.8 %), too poor for efficient polyhaploid production. Pearl millet is a cross-pollinated crop, and highly heterogeneous in nature (Kumar and Andrews 1993). It may be advantageous to select favorable lines from populations of pearl millet, that can produce embryos at high and stable frequencies with diverse varieties of wheat.

In crosses with sorghum, distinct frequency differences were observed among the wheat varieties. Norin 61 produced embryos at high frequencies, whereas Siete Cerros produced no embryos in crosses with two sorghum lines. Ohkawa *et al.* (1992) obtained embryos with frequencies ranging from 8.9 to 21.1 % in crosses of Fukuhokomugi with five sorghum lines. Fukuhokomugi is closely related to Norin 61 in its genealogical pedigree (Fukunaga and Inagaki 1985). Both of them are also highly crossable with *H. bulbosum* and thus amenable to polyhaploid production (Inagaki 1986). Although genetic analyses were not performed in this study, it is supposed that some genetical factor(s), like the crossability gene (*Kr*) observed in wheat x *H. bulbosum* crosses (Snape *et al.* 1979, Falk and Kasha 1983), controls the differences of embryo formation frequencies between Siete Cerros and Norin 61 in crosses with sorghum. It is thus suggested that sorghum is not a suitable pollen source for efficient polyhaploid production in hexaploid wheat because of genotypic barriers to embryo formation.

Efficiency of haploid production

The second step in polyhaploid production is plant regeneration from immature embryos through embryo rescue. Both wheat varieties, Chinese Spring and Norin 61, pollinated with maize developed embryos more rapidly than those pollinated with pearl millet and sorghum. However, plant regeneration from the maize cross occurred at lower frequencies than observed in pearl millet and sorghum crosses. By the time of embryo incubation, all chromosomes of the pollen parents were expected to be eliminated during the development of hybrid zygotes to embryos (Laurie and Bennett 1986, 1987, 1988, Laurie 1989). The effects of pollen chromosomes might be negligible on plant regeneration in the culture medium. Among the embryos from the maize cross, relatively smaller embryos tended to develop into plants quickly, although stringent data on the relationship between embryo length and plant regeneration was not collected. The developmental stage of embryos at the time of excision will be critical with respect to plant regeneration. This expectation is strongly supported by the results from the wheat x *H. bulbosum* cross (Inagaki 1986) and wheat x pearl millet cross (Inagaki and Bohorova 1995). Embryo rescue at a suitable stage of embryo development results in a high frequency (85.0 %) of plant regeneration in crosses of wheat F₁ hybrids with *H. bulbosum* (Chung and Inagaki 1986).

Results on cytological identification of the 377 plants regenerated from embryos indicated that most of them were wheat euphaploids with a complement of 21 chromosomes. Two aneuphaploid plants with 20 and 22 chromosomes are consequence of chromosomal aberrations that may occur during paternal chromosome elimination. The two diploid plants could be a consequence of spontaneous chromosome doubling. None of chromosomes of the pollen parents, which are smaller than those of

wheat (Laurie and Bennett 1986, 1987, 1988, Laurie 1989), were observed in the regenerated plants. However, retention of paternal chromosomes was rarely detected at the proembryo stage in crosses with maize and sorghum (Laurie and Bennett 1986, 1988), at the tillering stage of plants from crosses with pearl millet (Ahmad and Comeau 1990), and at the stage of doubled haploid plants derived from crosses with *H. bulbosum* (Inagaki 1987). These facts do not eliminate the possibility of translocating chromosome segments of pollen parents to the wheat genome through somatic associations. For advocates of such a phenomenon, further examinations will be however needed.

In comparison of the pollen sources used for crosses, the polyhaploid production of hexaploid wheat mediated by the pearl millet and sorghum crosses is less stable in terms of embryo formation than that by the maize cross. The unstable production of polyhaploids is not practical. It is required for use in genetical analyses and breeding purposes to produce a sufficient number of polyhaploids from any hexaploid wheat genotype. It has been so far reported that diverse genotypes of hexaploid wheat were amenable to maize-mediated polyhaploid production (Inagaki and Tahir 1990, Laurie and Reymondie 1991, Riera-Lizarazu *et al.* 1992). Some species related to maize, teosinte (*Z. mays* L. spp. *mexicana*) and eastern gamagrass (*Tripsacum dactyloides* (L.) L.), may be promising pollen sources (Ushiyama *et al.* 1991, Riera-Lizarazu and Mujeeb-Kazi 1993), but need to be further investigated for the genotypic effects on polyhaploid production frequency.

Acknowledgement

The authors would like to thank Mr. Gene Hettel, CIM-MYT for his editorial review of the manuscript.

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