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Crossing maize with sorghum, *Tripsacum* and millet: the products and their level of development following pollination

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Summary. Maize was crossed with sorghum, *Tripsacum* and millet with the aim of introgressing desirable alien characteristics into maize. The products of crosses were analyzed as to their level of differentiation following pollination; their further development on artificial culture medium was compared. In spite of a stimulation rate close to 5%, no evidence of hybridization between maize and sorghum or millet could be obtained. The plants recovered proved to be of maternal origin. However, with an appreciable frequency, stimulation leading to hypertrophic growth of nucellar tissue was observed. This phenomenon is bound to pollination, never occurring in non-pollinated ears. In crosses involving *Tripsacum*, more than 140 true hybrids were isolated. The influence of the genotypes used as well as factors such as climatic conditions or *in vitro* techniques are discussed. Except for one haploid maize plant, all the plants recovered proved to be classical hybrids, most of them showing the expected complement of chromosomes from each parent (10+36 chromosomes), a few others being slightly hyperploid (2n=47 to 50 chromosomes). No non-classical hybrids constituted by a non-reduced female gamete and a reduced male gamete were obtained.

Key words: Maize – Sorghum – *Tripsacum* – Intergeneric hybridization – *In vitro* grain development

Introduction

CIMMYT is carrying out experimental work under several different climates to create interspecific hybrids. The objective of these hybrids is essentially to provide breeding material in which selection can be practiced for characteristics not present in maize, or for which little progress is being made within the existing germ base. These include changes in plant architecture, adaptation to problematic soils and climates, and improving resistance to diseases and parasites.

We are currently working with the introgression of *Tripsacum* genes to maize and attempting to use genes from sorghum and millet.

Tripsacum is a variable genus having a wide tolerance to soil conditions and resistance to most common maize diseases and insect pests. *Tripsacum* can be experimentally hybridized with maize (Mangelsdorf and Reeves 1931, 1939; Randolph 1950; Farquharson 1957; Anand and Leng 1964; Chaganti 1965; Galinat 1961, 1971; Petrov et al. 1971; De Wet et al. 1972a, 1972b; James 1979). CIMMYT has routinely used embryo culture to aid in the recovery of maize and *Tripsacum* hybrids (F_1's). The success obtained when crossing maize with *Tripsacum* led us to emphasize the different products obtained when maize is crossed to sorghum and millet.

The objective of this analysis was to better understand the products of the crosses and their level of development after pollination. We also studied the development in vitro of stimulations following pollination and the chromosome complement of the plants derived from these crosses.

Unless otherwise specified, the results presented in this paper are related to the research carried out during the 1982 A cycle, which corresponds to an irrigated crop at CIMMYT's Tlaltizapán experimental station at an altitude of 970 m.

Material and methods

Plant material

Several heterozygous gene pools of diploid maize (*Zea mays* L.) were used as female parents in crosses with three alien

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species (sorghum, millet and *Tripsacum*)[1]. The maize pools were used as male parents in some crosses with sorghum. A large sample of sorghum varieties [*Sorghum bicolor (L.) Moench*, 2n=20] was tested and one variety of pearl millet [*Pennisetum americanum (L.) Leeke*] was used. The sorghum × maize crosses employed male sterile genotypes (genetic or cytoplasmic), and in a few crosses the sorghum was manually emasculated. Eleven different clones of *Tripsacum dactyloïdes L.* (2n=4x=72) were used as male parents. The selection of parents took into account the results obtained in previous cycles. Most crosses were done during the 1982A cycle in Tlaltizapán. However, the results obtained from the germination of mature F_1 seed of maize × *Tripsacum* correspond to the 1981 harvest (cycle A and B). Furthermore, crosses involving sweet corn and those in which sorghum was emasculated were performed at CIMMYT's Poza Rica station, a lowland tropical environment (60 m).

Methods

1 Crossing techniques

Ear preparation before crossing, as well as pollination techniques, are the same as those previously described (Mangelsdorf 1974; James 1979a). However, we have used pollen of only one species for each cross, excluding pollen mixtures.

Pollinated ears were checked regularly and were harvested at different stages of maturity, varying from a few days to 2–3 weeks, depending on the species.

2 In vitro cultures

a Immature grains. Immature grains were individually collected from the ear, sterilized with commercial bleach (0.3% active chlorine), and rinsed three times with sterile water. They were then dissected in a laminar flow cabinet under a binocular microscope (7× to 30×, depending on grain size). The grain contents were cultivated in plastic Petri dishes (55 mm diameter) on artificial culture media solidified with agar (0.8%). After testing the effects of different culture media on the development of maize × maize and maize × *Tripsacum* embryos of different ages (Blaydes 1966; Murashige and Skoog 1962; Nitsch and Nitsch 1969; Taira and Larter 1978), we adopted a modified Blaydes' medium which was successful for the regeneration of embryos resulting from the culture of Triticale anthers (Bernard 1977). The sucrose concentration used was either 20 or 50 g/l, the latter being better adapted to the needs of very young embryos.

Depending on grain content and degree of development of the organs within the grains, one of the following methods was used:

1) When embryo size was 1 mm or more, it was placed on culture media, with the embryo axis turned up.

2) When embryo size was less than 1 mm, it was left in place on the endosperm and placed on the culture media, as described by James (1979a).

3) When the embryo was not visible, the endosperm was placed on media following dissection: either the surface appeared uniform, or a protuberance allowed us to guess the location of the embryo.

4) When we were unable to identify either an embryo or the endosperm after removing all the sheaths, grain content was recorded as a vesicle.

The Petri dishes were sealed with Parafilm, and later placed in a culture chamber (18–26 °C, photoperiod 14 h/day). The cultures were transferred regularly to Petri dishes containing fresh culture medium so as to stimulate their growth.

When seedlings developed 2–3 leaves and at least one root, they were transplanted to jiffy pots and kept for several days in the same culture chamber before being transferred to a greenhouse (22–30 °C, photoperiod 14 h/day). The weak or stunted plants showing poor growth were irrigated with alternating applications of water and a nutrient solution composed of macro- and micro-elements together with iron EDTA at the same concentration as the culture medium.

b Mature grains. Several different techniques were used to induce germination of mature grains harvested from crosses of maize × *Tripsacum* in 1981:

1) After surface sterilization the grains were placed on moist blotting paper to germinate. This was done with both intact grains and grains with pericarps removed.

2) Sterilization – Rinsing – Soaking between blotting paper sheets for 2–4 days, then Sterilization – Rinsing – Dissecting the pericarp. This was followed by either:

– putting the embryo attached to the endosperm on sterile blotting paper to germinate, or
– dissecting the embryo only and cultivating in a Petri dish, as in the case of immature grains.

The chromosome counts were done on the roots. These were taken either under sterile conditions within the culture medium when they had attained sufficient development, or some days after transferring the young plants into jiffy pots.

The roots were pretreated 3.5–5 h with 8-hydroxyquinoline at a concentration of 0.4 g/l and fixed with a mixture 3 : 1 of absolute alcohol-glacial acetic acid or 45% acetic acid containing 0.5% orceine. They were dissected and observed using phase contrast to obtain a chromosome count.

If further identification was required differential chromosome banding was performed (C-banding) (Fig. 1).

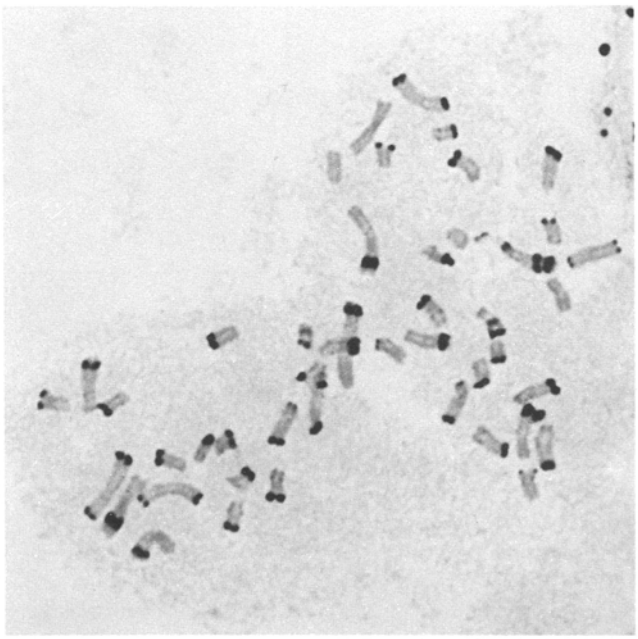

Fig.1. C-banded metaphase of a maize × *Tripsacum* F_1 hybrid with 46 chromosomes

1 Information on the nature and content of the maize pools may be obtained by writing to CIMMYT, Maize Programme

Results

Crosses with sorghum

The crosses carried out are presented in Table 1. In most cases, sorghum pollination of maize ears induced no visible effect on the maize ears. However, in 4.7% of the cases (220 ears involving 68 sorghum parents), some development was observed. This ranged from the stimulation of blister-like ovules to the formation of grains with normal aspect.

The control ears, which were prepared for crosses but not pollinated, showed no stimulation of the ovaries. The stimulation that leads to vesicle or grain formation appears to be closely related to the pollination. Some grains developed normally and could be readily identified 10 to 12 days after pollination. These grains were contaminants, larger than the other structures present on the ear. In some cases, they could also be differentiated on the basis of seed color. Most of the other structures degenerated quickly in the two weeks following pollination. It was not always possible, however, to distinguish contaminants on the basis of visible characteristics. In the early stages of seed development (before 8–10 days) only dissection permitted us to detect the presence of endosperm and/or an embryo in the grain. Moreover, we observed that some structures that were translucent at the beginning of their development sometimes acquired a pericarp and became opaque, thus imitating the appearance of the normal grain.

1 Number of grains per ear

The number of *gynoecia* stimulated was generally low. One hundred and ninety-two ears from maize × sorghum were analyzed after discarding old ears with evident contamination. Most of the ears carried very few grains, from 1–5, with a mode equal to 1. However, some ears had a higher net quantity, between 30 and 40. This elevated the mean to 4.8 grains/ear (Fig. 2).

Table 1. Crosses carried out in 1981 (cycles A and B) and 1982 A cycle

Type of cross and cycle	No. of crosses made			No. of ears harvested (showing grain formation or stimulation)			Rate of ear stimulation = harvested ears × 100 / pollinated ears
	Tlaltizapán Station	Poza Rica Station	Total	Tlaltizapán Station	Poza Rica Station	Total	
Maize × sorghum 1982 A	4,478	199	4,677	188	32	220	4.70
Sorghum × maize 1982 A	255	24	279	10 (panicles)	4	14	5.02
Maize × millet 1982 A	1,008	–	1,008	45	–	45	4.46
Maize × *Tripsacum* 1982 A	408	–	408	79	–	79	19.36
1981 A and B	1,600	–	1,600	179	–	179	11.19
Total	7,749	223	7,972	501	36	537	

Table 2. Grain analysis in crosses involving sorghum

	No. of ears		No. of grains		Grain content (% of cultivated grains in brackets)			
	Pollinated	Harvested	Collected	Placed on medium	Endosperm with embryo	Endosperm-embryo not visible	Vesicle	Empty grain
Maize × sorghum	4,677	220	1,109	716	111 (15.5)	44 (6.2)	478 (66.8)	83 (11.6)
Sorghum × maize	279	14	180	165	130 (78.8)	30 (18.2)	5 (3.0)	0 (0)

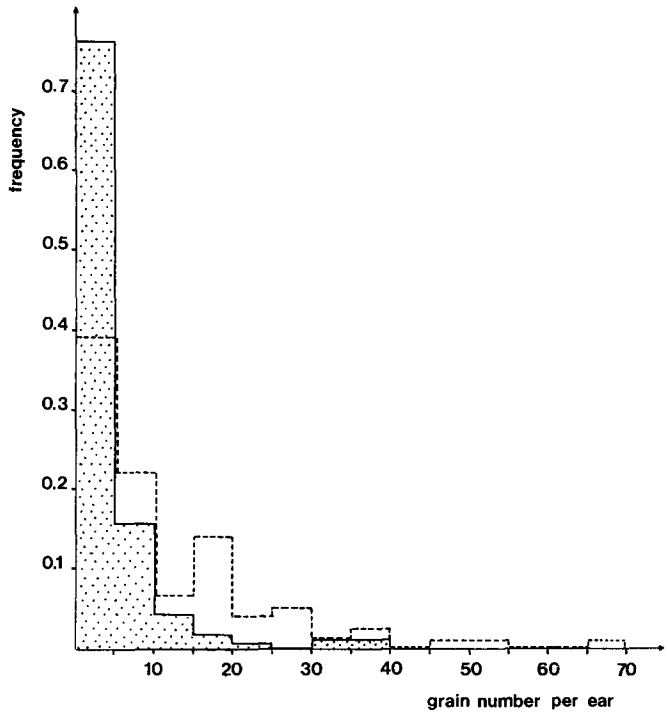

Fig. 2. Grain number per ear in maize×sorghum (———) and maize × *Tripsacum* (– – – –) crosses

2 Content analysis

The selected material was separated into 4 main categories during dissection under the dissecting microscope (Table 2):

a) normally constituted grains that presented an endosperm and an embryo; b) grains with only endosperm visible. Among these grains with an endosperm, the proportion showing a visible embryo regularly increased with age. Before 9 days it was almost impossible to identify the embryo using a dissecting microscope. At 9 days, the embryo was visible in 50% of the cases. From 10 days on, it was possible to distinguish the embryo in at least 80% of dissected grains; c) grains with neither a visible endosperm or visible embryo. Their aspect evolved with their age as follows:

– 6–7 to 11–12 days after pollination: grains appeared as turgid, translucent vesicles with an homogeneous content. The envelopes and the nucellus could be identified. The entire grain volume was occupied by the nucellus.

– 11–12 to 14–15 days after pollination: a central cavity appeared as a result of internal rupture of nucellar tissues. This phenomenon was frequently accompanied by a depression of the envelopes, giving the grain a dented aspect.

– after 14–15 days: the grain content that had not completely degenerated lost its turgor, and acquired the aspect of viscous jelly. This content did not fill the entire cavity and was interpreted as the nucellar tissue in the process of dehydrating.

d) Empty grains, reduced to their envelopes. On the grains placed on medium, 66.8% had neither endosperm nor embryo. The normal type grains represent 15.5%.

Grain content varied according to pollination date, showing slight variations in the frequency of vesicles, and the presence of an obvious peak for the frequency of grains with albumen with or without a visible embryo (Fig. 3).

3 Evolution of different types of grains in culture

Table 3 summarizes the number of grains of each type placed on culture media, their development in vitro, and the number of plants obtained and transferred to the greenhouse. In general, the rate of plant recovery increased with the level of grain differentiation prior to dissection.

In the case of grains with an embryo, a good number of embryos were noted as possible contaminants due to their typical aspect and large size (more than 2 mm when dissected). About 90% of these grains developed in vitro and in more than 80% of the cases, plants were obtained. Most were typical of maize and developed into seedlings, as did the checks (maize × maize), within only a few days. Thirty-seven plants of the 92 produced exhibited retarded or perturbed development (leaves rolled, chlorotic or colored with

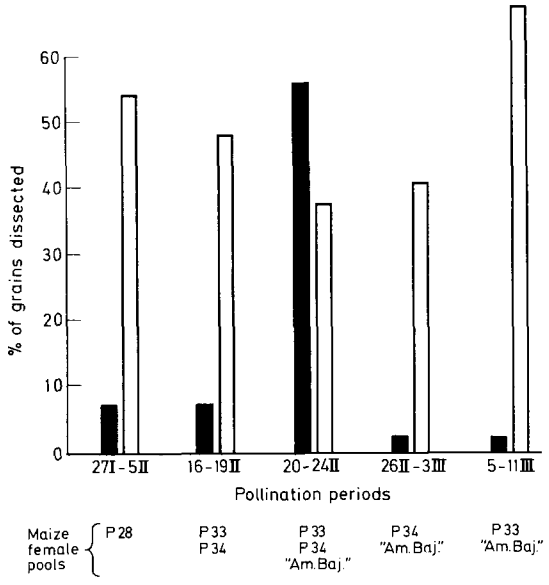

Fig. 3. Effect of pollination period on the level of grain differentiation (presence of embryo ■; nucellar stimulation □)

Table 3. Maize × sorghum cross : organ differentiation and plant recovery in relation to grain content

Grain content content		No. of grains cultivated in vitro	No. of grains showing differentation (% of cultivated grains in parenthesis)	No. of plants recovered	Plants transferred to the greenhouse	Plants grown to maturity
Embryo	Placed on medium	83	78 (93.9)	69 (83.1)	21	16
Embryo	Left on endosperm	28	25 (89.3)	23 (82.1)	16	10
Endosperm	Small protuberance visible	29	19 (65.5)	15 (51.7)	9	6
Endosperm	Smooth surface	15	0	0	0	0
	Vesicle	478	11 (2.3)	5 (1.0)	3	2
	Total	633	133 (21.0)	112 (17.7)	49	34

anthocyanines, or slow root development) and were grown to maturity for further study.

The structures presenting an apparent protuberance on the endosperm surface developed in 65% of the cases. Those in which the endosperm exhibited a smooth surface never developed irrespective of age (9–18 days). It is possible that the artificial conditions did not permit the germination of any embryos present, especially in the case of very young grains (9–13 days).

In most cases (98.96%), the grains noted as vesicles (neither embryo or endosperm visible when dissected) showed no sign of differentiation on culture media. Some vesicles presented a temporary green coloration of the non-excised part of their pericarp, followed by necrosis. Only 5 of the structures classified in this group had developed plants.

Three of those came from the same ear (8 days old), from which 20 very small grains were taken out of 100 grains present. This ear was thought to be the possible result of a contamination, because of the large number of grains present. The other 17 grains in culture did not develop and the three that gave rise to plants grew very slowly. Only one plant survived and was transferred to soil, but did not reach maturity. The other two structures came from grains taken from 9 and 11 day-old ears, respectively. One developed an endosperm and then an embryo that matured as normal maize.

Three vesicles were cultured from another ear, and after 9 days of cultivation a plant was recovered, which reached maturity and was normal. Some other grains showed unusual structures, described below. One, 12 days old, had an endosperm of an abnormal consistency (in the process of degeneration) and a normal embryo. The plant that resulted from this was very weak, chlorotic, and slow-growing. It produced only three roots and did not survive being transferred to soil; its karyotype analysis disclosed 20 maize chromosomes. This plant was possibly not a maize × maize hybrid but rather the result of apomixis or diploid parthenogenesis. An absence of progeny makes it impossible to choose between these possibilities. A second one, just 8 days old, had a shrivelled endosperm and a true but tiny embryo which produced a normal maize plant. A third grain contained two small, separated, white masses, one of which was hollow. These did not occupy the whole grain cavity and aborted quickly.

Four other grains, 15 to 18 days old, did not show embryos and presented an endosperm with an abnormal color or granular aspect, sometimes attached to a mucous tissue that was interpreted as the remains of nucellus not digested by the albumen in the process of abortion.

Forty-nine plants were transferred to the greenhouse, 34 of which reached maturity. The morphological characteristics observed (pilosity, waxiness, and floral structure), together with the chromosome complements of these plants, showed that all were normal maize. In addition, these plants did not include aneuploids or individuals with B chromosomes.

Table 4. Maize × *Tripsacum* cross : results obtained from immature grains

Ear age (days following pollination)	No. of ears harvested	No. of grains collected	No. of grains per ear	Grain content at the time of dissection			Final no. of grains having exhibited an embryo[a] (%)	No. of embryos showing organogenesis (%)	No. of plants recovered (% of embryo no. in parenthesis)	No. of plants grown to maturity
				(1) Endosperm with embryo (%)	(2) Endosperm without visible embryo (%)	(3)=(1)+(2) At least an endosperm (%)				
6–14	18	315	17.5	52 (16.5)	227 (72.1)	279 (88.6)	86 (27.3)	23 (26.7)	4 (4.7)	2
15–21	44	522	11.9	277 (53.1)	164 (31.4)	441 (84.5)	301 (57.7)	84 (27.9)	17 (5.7)	13
22–26	16	146	9.1	115 (78.8)	9 (6.2)	124 (84.9)	115 (78.8)	7 (6.1)	2 (1.7)	1
Total	78	983	12.6	444 (45.2)	400 (40.7)	844 (85.9)	502 (51.1)	114 (22.7)	23 (14.6)	16

[a] Includes embryos appearing after in vitro culture

Reciprocal cross. Out of 279 sorghum panicles pollinated by maize, 14 were collected showing seed stimulation. The dissected grains gave rise to maternal plants only which were characterized by an absence of pilosity, the presence of wax in the young plants, and typical sorghum inflorescences when mature.

Crosses with Tripsacum

1 Immature grains (cycle 1982 A, Tlaltizapán)

Four hundred and eight crosses were made, representing 19 combinations. Only forty ears (9.8%) showed no stimulation and were identical to the unpollinated controls. On another 289 ears, grains formed and degenerated rapidly. Seventy-nine ears were collected (19.4%) at different stages of maturity and their grains were dissected and cultivated in vitro.

a Number of grains per ear. The number of grains showed an important genotypical and individual variation. The mean was three times that of the maize × sorghum crosses (Fig. 2). In the latter, 61% had 1–3 grains and 79% 1–6 grains. In the case of *Tripsacum*, 61% had 1–10 grains, and 82% had 1–20 grains. The mean for all genotypes was 12.44 grains/ear. Important differences were observed depending on the *Tripsacum* clones used as males. These differences ranged from 6–30 grains on the average. The crosses made with 'Maize Pool 33' as the female had on the average 2.5 grains per ear more than the crosses made with 'Amarillo Bajío'. However, these differences did not influence appreciably the number of plants obtained with the different parents used.

The number of grains per ear varied also with the stage of maturity as defined by days from the pollination to harvest, with 17.5 grains/ear for ears two weeks old or less, 11.9 for those 2–3 weeks old, and 9.1 in ears aged more than 3 weeks. This decrease illustrates the in vivo abortion rate of developing grains. The grains that became completely flat or necrotic were not collected.

b Grain description and content analysis. Collected grains were regularly shaped and of a size intermediate between those from maize × sorghum and those from normal maize at the same age.

Content analysis (Table 4) shows the presence of a well-shaped endosperm in 86% of the grains dissected; 51% of them proved to have an embryo as well. We did not find grains with an embryo and without endosperm. Eighty-eight per cent of the embryos observed were visible at the time of culture. The remaining 12% appeared during in vitro culture.

The grains without endosperm (14%) were mostly empty with the exception of 11 (1.1%) that contained nucellar tissue. Up to 14 days after pollination the grains had a turgid white or slightly translucent endosperm and a small underdeveloped embryo attached to a long slender suspensor. Most of these embryos were cultivated in place on their endosperm, except for those larger than 1–2 mm. In comparison, maize embryos taken from ears cultivated under the same conditions were visible around 9–10 days, and were well developed at 14 days.

Larger endosperms were evident at 2–3 weeks. These were irregularly shaped and cream colored, with

Table 5. Maize × *Tripsacum* cross : results obtained from mature seeds (embryos dissected and cultivated on artificial medium)

Maize pools (female parent) / *Tripsacum* clones (male parent)	Pool 33			Pool 34			"Amarillo Bajio"			Total		
	Grain no.	Embryo no.	Plant no.	Grain no.	Embryo no.	Plant no.	Grain no.	Embryo no.	Plant no.	Grain no.	Embryo no.	Plant no.
T 7116	267	96	1	32	12	6				299	108	5
T 7117				22	4	–	56	13	1	78	17	1
T 7127				88	7	–	32	3	–	120	10	–
T 7138	131	43	–				145	24	–	276	67	–
T 7139	9	5	–	7	2	–	1	1	–	17	8	–
T 7158	45	21	13							45	21	13
T 7203	28	1	1 (maize)	36	17	1				64	18	2
T.65.1234	37	14	13	104	72	30	150	128	29	291	214	72
Total	517	180	28	289	114	37	384	169	30	1,190	463	95

a depression at the embryo level. Surprisingly, most of the embryos seemed not to have developed. Some had even lost contact with the endosperm and were "floating" in the empty space at the extreme of their suspensor. Others attained a size of 2 mm and acquired a triangular shape (stage coleoptilar). Finally, some grains were small and dense and had an endosperm and a large embryo closely associated. The embryo was comparable to a maize embryo in size and shape.

After the three weeks, the endosperm grew increasingly yellow and shrivelled. The embryo was still frequently visible at this stage but appeared to have stopped development and dried up in most cases.

c Development in in vitro culture. Embryos taken from grains of up to 14 days had developed poorly in cultivation. The use of R5 media (50 g/l of sucrose instead of 20 g/l) together with regular transfer onto fresh media had a favorable effect on the organogenesis. The embryos of less than 1 mm showed no development. The most advanced (26.7%) developed leaves or rough shapes of leaves with or without roots. Four plants had been obtained, of which two survived to the greenhouse stage (4.65% of the embryos).

Embryos from 2–3 week-old ears were the most productive: 28% showed a beginning of organogenesis, and 5.6% gave rise to normal seedlings. All 17 plants isolated came from ears harvested from 15–19 days after pollination. Each harvest day within this favourable period provided at least one plant. Only 6.1% of the embryos from ears more than 3 weeks old developed with 1.74% reaching the seedling stage. The maize × *Tripsacum* embryos were slower to develop at all stages than the check maize embryos, especially with respect to the root system.

d Cytology. Chromosome counts were made as soon as possible to determine the incidence of chromosome loss during the first stages of the plant development previously reported (James 1979 a). A root was aseptically taken from the culture media, and sometimes from a seedling transferred to a "Jiffy pot". The counts gave the following results: two plants had 48 chromosomes and came from the same male parent (T. 7158) with two different female parents. One plant was a maize haploid with 10 chromosomes, and 20 other plants had the expected number of 46 chromosomes (36 from *Tripsacum* and 10 from maize).

All plants except the maize haploid were typical interspecific hybrids. No "non-classical" hybrids (James 1979 a, b) or hypoploid plants were obtained.

2 Mature grain

Samples were collected during cycles 1981 A and B from 1,600 crosses, 179 of which produced mature grains. The seeds were small, shrivelled, irregularly shaped, and heavily infected by fungi and bacteria spores.

In a first batch, resulting from crosses involving *Tripsacum* clone T 65.1234, 27 plants were recovered from uncoated grains and allowed to germinate between blotting paper sheets. Except for one maize plant, they all were normal hybrids with the expected chromosome number of 46. In a second batch, embryos were excised and plated on artificial medium. Table 5 summarizes the results obtained for each hybrid combination. With *Tripsacum* male parent T 65.1234: 73.5% of the grains dissected had an embryo, 33% of which produced plants. The other genotypes tested had an average of 27.70% of grains with embryos; only 8.84% produced plants. In addition, the proportion of grain

with embryos and the development of plants from these embryos was superior during cycle B (rainy season), probably because of better *Tripsacum* pollen production.

A large number of seeds exhibited a depression of the endosperm where the embryo was normally positioned. This suggests that the embryo had formed but degenerated during the maturing process. Observations carried out on immature grains supported this hypothesis.

A total of 95 plants were isolated, of which 13 died before reaching maturity. Chromosome counts were made on 51 plants at an early stage. One plant was a typical maize, with 20 chromosomes. The others exhibited classical hybrid morphology (James 1979a), with chromosome numbers ranging from 46–50, as follows: 41 had 46 chromosomes, 5 had 48 chromosomes, and 4 had 47, 49 or 50 chromosomes. With the exception of one plant, *Tripsacum* clone T 7158 was the male parent of those plants containing 48 chromosomes.

Crosses with millet

One thousand and eight maize ears were pollinated by millet; 45 were collected; 287 grains were taken and dissected. Most of them were comparable to those from the maize × sorghum cross showing nucellar tissue becoming gelatinous with age.

Four seedlings were obtained, but they were identified either as maize (2 plants) or maize × *Tripsacum* (2 plants with 46 chromosomes) as the result of contaminant pollen. One 14 day old ear produced three grains which contained endosperm but no embryo. These endosperms did not develop in in vitro culture. Another ear, 17 days-old, produced 70 grains, of which 60 were dissected. Their contents were remarkably homogeneous. All contained endosperm which was underdeveloped in relation to the age of the ear. This endosperm was located in the basal part of the grain, with the opposite part (silk side) full of gelatinous tissue, probably nucellar remains. Embryos were not visible at the time of dissection. About ten embryos developed on the endosperm after some days of in vitro culture. They were placed in contact with the media when they reached 0.5 mm, however none manifested organogenesis.

Discussion

1 Maize × sorghum

In spite of the large number of crosses performed and the fact that the stimulation rate was 4.7%, we did not observe any evidence of hybridization between maize and sorghum. The plants obtained corresponded to typical maternal plant phenotype and cytology. Most of these plants probably came from non-controlled pollination, with contaminating pollen from neighbouring breeding material. Support for this hypothesis is that most of these grains were obtained during a very short period, while the stimulation rate for nucellus showed little variation during the growth period. Further, most of the plants isolated came from the youngest grains, making it difficult to distinguish between possible hybrids and contaminants at the time of plating. However, it is possible that some of these maize plants had an apomictic origin, reproducing the maternal genotype, or resulted from parthenogenesis, following the development of an oosphere (doubled but not fertilized). Other grains, lacking an embryo but showing a degenerating endosperm, may have come from fertilization of maize polar nuclei by sorghum pollen, as sorghum pollen does indeed germinate on maize silks (Dhaliwal and King 1978; Reger and James 1982).

However, the most frequent type corresponds to a stimulation of diploid maternal cells which responded by a massive growth of nucellar tissue without any sign of fertilization. Dhaliwal and King (1978) have made the same observation on excised ovules in vitro after direct pollination by sorghum pollen. The non-pollinated ovules did not show any modification of their initial condition.

Thus, it seems established, from two different sources, that the pollinated ears exhibited stimulation and that the control ears did not. This stimulation may have been caused by a hormonal or enzymatic substance released from burst pollen tubes.

2 Maize × Tripsacum

From the population of collected ears that carried grains from 5–7 days old, it was possible to estimate that the percentage of fertilization was 51%, with 86% containing at least an endosperm. The percentage of single fertilization was 35% and involved endosperm fertilization only.

The relatively small number of plants obtained in comparison to the number of embryos in culture was due to the difficulty of saving the young embryos (less than 11–12 days old) either plated alone or left in culture on their endosperm. The stage of development at the time of embryo culture was apparently important, as the 15–19 day-old embryos gave rise to 17 of the 23 plants obtained in cycle 1982A. Four hundred and eight crosses were made with a success rate of 5.6%. This result was not obtained under optimal conditions if we consider the low pollen production during this cycle (dry season) and the fact that the most easily

crossable parent, T. 65.1234, was used only once, since an adequate number of hybrids involving this clone had been produced previously at CIMMYT (CIMMYT reports 1976–1977 and 1978–1979). Results show the male parent T. 7116 as noticeably better in terms of hybrid embryo production (10 hybrid plants from 93 immature grains, equal to 10.8%). Recovery of hybrids from mature seed with this parent was much lower (2.3%), illustrating the benefit of embryo culture for hybrid recovery.

3 Maize × millet

The response observed after crossing maize × millet is comparable to that observed for maize × sorghum, except for one ear collected 17 days after pollination. This ear was probably not contaminated by maize, as it showed insufficient development for 17 days. These grains could be the result of a successful cross between maize and millet, although it is surprising that only one ear should have this amount of stimulation. We did not observe as high a rate of grains possessing an endosperm on other ears.

There are clearly similarities and differences between the 3 types of crosses. The results reported here essentially consist of grain production or stimulations of maternal tissue as the result of a pollination, controlled or not. The controls (unpollinated ears) did not exhibit any comparable stimulation.

Following pollination, the number of modified ovules and the type of development varied considerably with the species used. A noticeable modification in maize × sorghum was hypertrophied nucellar tissues growth. With maize × *Tripsacum,* approximately 20% of the ears produced grains, of which 86% contained at least endosperm. The differences detected concerning the level of grain differentiation may explain why seed collapse takes place earlier and is more sudden in maize × sorghum than in maize × *Tripsacum* crosses.

Regarding the number of hybrid plants obtained, our results differ from those obtained between 1975 and 1980 (James 1981), where the hybrid isolation rate for both types of crosses was comparable: 36 plants on 32,000 maize × *Tripsacum* crosses (0.11%) and 25 plants on 30,000 maize × sorghum crosses (0.08%). In contrast, in our experiments, no hybrid plant was obtained after 4,956 crosses with sorghum, whereas with *Tripsacum,* 142 plants have been identified as hybrids from 2,008 crosses, representing an average success rate of 7.07%.

Our results represent considerable progress in maize × *Tripsacum* crosses, multiplying the success rate by 64. Several factors can explain this progress: 1) we obtained a higher rate of ovule development and a greater number of grains per ear. According to James (1979a), 80% of the ears stimulated has from 1–3 grains. On our material, 82% of the ears showed between 1 and 20 grains. This may be due to slight changes in the pollination technique: 2) with dry grains, removing the pericarp prior to germination (Mangelsdorf 1974) allowed us to increase the number of plants obtained; 3) with immature material, the use of Petri dishes and systematic embryo replanting on new media with increased sugar levels for small embryos contributed to the recovery of a larger number of embryos than in the past.

Failure to isolate maize × sorghum hybrids raises a question as to the feasibility of fertilization in maize × sorghum crosses. After pollinating maize in vitro and in vivo with sorghum; Daliwal and King (1978) observed that pollen germination was only through the stigmatic hairs. This germination does not take place at the basal portion of the style lacking stigmatic hairs, 5–10 mm long. The length of the pollen tubes did not exceed 3–5 mm; hence ovule fertilization appeared to be impossible. For the same cross, Reger and James (1982) observed the pollen germinated at the stigma level, in the style region without stigmatic hairs and even in that of the ovule at the level of micropyle. They estimated that pollen tube length could reach 15 mm, and concluded that the delivery of the sorghum male gamete to the maize female gamete was physically possible.

Another point to be discussed concerns the genomic constitution of the hybrids produced: James (1978, 1979b, 1981) stated that 100% of the hybrids produced from the maize × sorghum cross were "non-classical", constituted by a non-reduced female gamete and a reduced male gamete, with the hybrid progressively losing the paternal chromosomes during its development. The same phenomenon was observed for approximately a third of maize × *Tripsacum* hybrids (11 out of 36).

Many authors have noted the formation of non-reduced gametes, which generally follows a backcross of an F_1 interspecific hybrid (Mujeeb-Kazi and Rodriguez 1983; Islam and Shephard 1980). In some cases, the unexpected numbers have been explained (Jewell and Mujeeb-Kazi 1982). However, non-reduced gametes have been very seldom mentioned to explain F_1 chromosome number. Apart from James (1978, 1979a, b, 1981), De Wet et al. (1976) have reported the work of Singh (1954) who estimated that "both the cytologically reduced as well as unreduced gametes of sugarcane may function sexually to produce hybrids with sorghum". But the living F_1 hybrid *Saccharum* × sorghum was not studied.

After crossing 79 barley spikes with rye pollen, Fedak and Nakamura (1981) obtained a plant with 21 chromosomes in most of its cells and concluded that the female parent provided a non-reduced gamete.

Islam and Shepherd (1980) noted a slightly different case of an interspecific hybrid with an unexpected number of chromosomes. The progenies they obtained from a wheat (cv. 'Chinese Spring') × barley (cv. 'Betzes') cross had one "classic" hybrid ($n_1 + n_2 = 28$), and 19 plants with chromosome numbers spaced between 21 and 35. The 35 chromosome plant of this series was interpreted as the result of the union of a normal female gamete of 21 and a non-reduced male gamete of 14 chromosomes. The reciprocal cross led to normal F_1's of 28

chromosomes. Using the same cultivars, Fedak (1980) obtained normal hybrids from this cross, irrespective of which species was the female parent.

Conclusion

With few exceptions, all our plants from the maize × *Tripsacum* cross corresponded to classic hybrids; most had 46 chromosomes. Some plants were slightly hyperploid (2n = 47 to 50 chromosomes). In spite of the systematic utilization of in vitro culture for the 1982 A cycle and the high number of plants obtained and counted in comparison to the preceding years, we could obtain no evidence in support of non-classical hybrids with maize. In addition, the backcross of a normal maize × *Tripsacum* F_1 produced a plant with 2n maize complement and n *Tripsacum* complement (56 chromosomes). This plant is perennial and *Tripsacum*-like and has not been observed to exhibit chromosome loss during the plant cycle.

Our conclusion for the maize × sorghum cross is that pollen substances are capable of inducing stimulation of the maternal tissues, leading to nucellus' hypertrophy and possibly, in some cases, to apomixis.

We have now completed over 15,000 maize × sorghum crosses (Jewell, unpublished), and no evidence has been obtained to suggest that hybridization has occurred. The sexual combination of maize and millet has been equally unpromising. Other means of combining these species are currently being evaluated while work continues with the progeny of maize × *Tripsacum*.

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