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Direct Pollination of *Zea mays* Ovules in vitro with *Z. mays, Z. mexicana* and *Sorghum bicolor* Pollen

S. Dhaliwal and P.J. King
H. Friedrich Miescher-Institut, Basel (Switzerland)

Summary. Sorghum (*Sorghum bicolor*) pollen tubes penetrated and grew in corn (*Zea mays*) styles. The limited length of the sorghum pollen tubes (3-5 mm) and the absence of stigmatic hairs on the basal (5-10 mm) section of the corn styles prevented effective pollination in vivo and in vitro. Normal fertilisation occurred after in vitro pollination of exposed corn ovules with either corn or teosinte (*Zea mexicana*) pollen. Six per cent of corn ovules pollinated directly with sorghum pollen responded by rapid, massive growth of nucellar tissue.

Key words: Direct ovule pollination in vitro – Embryo and endosperm development in vitro – Hyperplastic nucellar growth – In vitro pollination – Sorghum – Zea

Introduction

Sexual hybrids involving various genera of *Gramineae: Saccharum* × *Sorghum* (de Wet et al. 1976), *Zea* × *Tripsacum* (Stalker et al. 1977) and *Triticum* × *Agropyron* (Sears 1972) have been successfully used for transferring genetic information from one genus to the other. Similarly, *Zea* × *Sorghum* crosses would allow transfer of particular genetic information in both directions and might also offer the possibility of obtaining monoploids following chromosome elimination as in *Triticum* × *Hordeum* (Barclay 1975). Reciprocal crosses involving several cultivars each of corn and sorghum, including diploids and tetraploids, were unsuccessful (Mock and Loescher 1973), mainly because pollen of either species failed to germinate on the stigma of the other. CIMMYT (1977) reported a supposed corn × sorghum hybrid among many thousands of crosses but critical cytological evidence is still lacking. Contamination with corn pollen was one of the serious problems reported by several workers in field and greenhouse crosses. In this paper, we report a further examination of pollen tube behaviour in reciprocal crosses between *Zea* and *Sorghum*, and the application of a technique of direct ovule pollination in vitro which may assist in the recovery of a corn × sorghum hybrid.

Materials and Methods

Pollen tubes were stained for callose with aniline blue (Kho and Baer 1968) and examined with a Zeiss IVFI epifluorescence unit, where pollen and pollen tubes fluoresced greenish-yellow. The usual practices for avoiding uncontrolled pollination, non-receptive stigmas and non-viable pollen, were strictly observed; corn and sorghum pollen could easily be distinguished on the basis of size difference.

For in vitro pollinations, corn cobs, bagged prior to the emergence of the silks were detached at appropriate times and the protruding silks immediately cut. All husks were removed except the last one, which was thoroughly moistened with a cotton swab dipped in 95% ethanol. Subsequent operations, i.e. removal of the last husk, cutting silks to a length of ca. 1-2 cm, cutting the cob into transverse and radial sectors each with 4-6 ovules and transferring the sections into petri-dishes (Fig. 1), were carried out under sterile conditions. Freshly dehiscing anthers of corn or sorghum were carried over the centrally arranged silks (Fig. 1) and teased with a dissecting needle to disseminate the pollen over the silks. No segment of the anther was allowed to touch or fall into the petri-dish. Eighty per cent of the petri-dishes remained sterile during the operations. The basic medium was a modified Murashige and Skoog medium (Green and Phillips 1975) with 5% (w/v) sucrose. The petri-dishes were incubated in the dark at 28°C.

The method used for direct ovule pollination (Fig. 3) was similar in principle to that used in successful ovule pollination of dicotyledons (Zůbková and Sladký 1975). However, it was not possible to completely separate corn ovules from the ovary walls. Instead, the silks were removed entirely by a cut made a little below their junction with the ovary walls (Fig. 3), thereby exposing the translucent nucellar tissue of the ovules. Corn, teosinte or sorghum pollen was lodged over the exposed ovules as described above. In all the experiments corresponding unpollinated controls were established. Besides the modified MS medium used for in vitro pollination, other media, i.e. B^{II} (Norstog 1973), modified MS medium with casein hydrolysate (400 mg/l) and modified MS medium with GA_3 (10 mg/l), were used to allow the culture of

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Table I. Description and source of the corn, teosinte and sorghum cultivars used in various crossing experiments in vivo and in vitro

Description	Source	Chromosome no.
Corn		
G4507 hybrid	Funk Seed International, USA	20
G4444 hybrid	,,	20
G4747 hybrid	,,	20
A B Pl. (4 n)	Dr. R.J. Lambert, University of Illinois, Urbana, USA	40
gl (4 n)	,,	40
B73	,,	20
Teosinte		
'Chalco'	Dr. G.J. Waines, University of California, Riverside, CA, USA	20
'El Salado'	,,	20
Sorghum		
'Martin' (male sterile)	Dr. O.J. Webster, University of Arizona, Tucson, Arizona, USA	20
'CK 60' (male sterile)	,,	20
'Hegari' (day neutral)	,,	20
'T 7078'	,,	20
'Plainsman'	,,	20
'Caprock'	,,	20
'4n Bulk'	Dr. W.M. Ross, University of Nebraska, Lincoln, USA	40
'4x Rio'	Dr. J.R. Harlan, Crop Evolution Lab., University of Illinois, Urbana, USA	40

ovules after direct pollination. All media contained 5% sucrose and agar (0.75% w/v).

Results and Discussion

Pollen germination and pollen tube penetration was examined in reciprocal crosses between the corn, teosinte and sorghum lines given in Table 1. Corn pollen germinated but did not penetrate sorghum stigmas, irrespective of the ploidy level of the corn or sorghum cultivars used. The pollen tubes were usually much shorter than their normal length of several centimeters when on corn silks. Sorghum pollen, however, either alone or in mixed pollinations, germinated very rapidly and the pollen tubes penetrated corn silks within 0.5 h of pollination. Although some tubes burst, branched or misorientated in the silks or stigmatic hairs, many grew normally but with a maximum length of 3-5 mm. This occurred with all varieties used. These latter observations do not agree with those of Mock and Loescher (1973) who, whilst using a similar selection of cultivars, reported the failure of sorghum tubes to penetrate corn styles. In our experiments, corn silks were receptive to sorghum pollen, and pollen tubes penetrated throughout their length, except at the basal 5-10 mm section, probably due to the absence of stigmatic hairs in this region (sorghum and corn pollen tubes invariably penetrated corn silks via stigmatic hairs). These observations suggested that fertilisation in corn × sorghum crosses might be possible if sorghum varieties were used in which pollen tube growth exceeded 10 mm on shortened corn silks, or if sorghum pollen was applied directly to corn ovules in vitro.

Pollen tube behaviour was also observed in vitro, after pollination of silks of detached corn ovaries under sterile conditions, by a modification of the method first described by Sladký and Havel (1976; see also Gengenbach 1977) (Figs. 1, 2). Corn × corn pollinations (5 experiments each with ca. 200 ovaries) produced up to 50% of developing seeds (Fig. 2). No seed developed after application of sorghum pollen to corn styles (3 experiments each with ca. 250 ovaries). The absence of any response in corn (♀) × sorghum (♂) pollinations in vitro, even on greatly shortened silks, appeared (like the in vivo pollinations) to be due to the absence of stigmatic hairs on the basal 5-10 mm section of corn silks.

To reduce the distance between the point of sorghum pollen tube penetration and the micropylar end of the

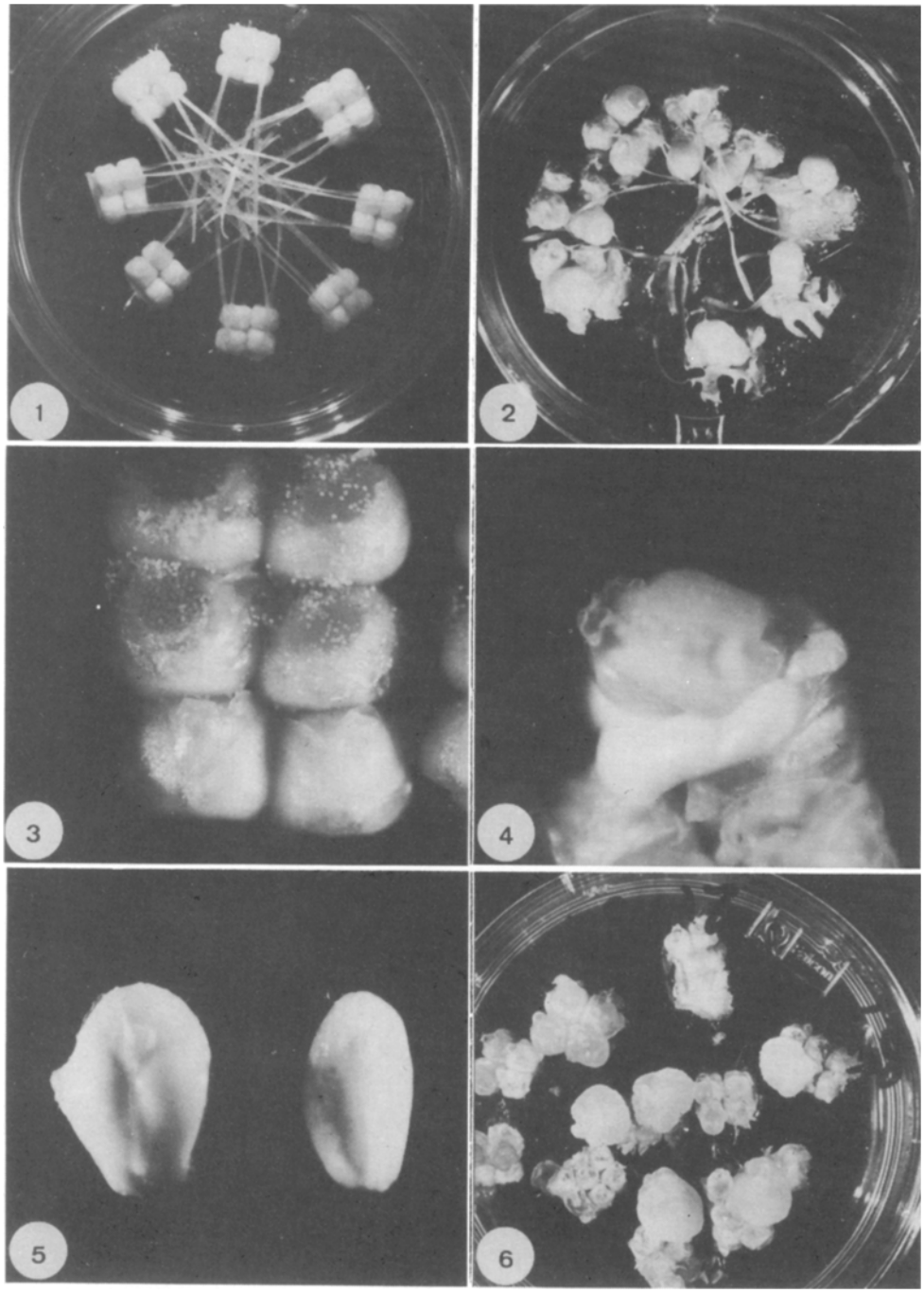

Fig. 1. In vitro pollination of corn with corn and sorghum pollen. X 1.7

Fig. 2. Seeds developing after corn X corn in vitro pollination. X 1.7

Fig. 3. Direct ovule pollination. The lowermost ovule pair has intact silk bases; in the two upper pairs the ovules are exposed and pollinated. X 90

Fig. 4. Completely developed corn embryo and endosperm surrounded by folded-back ovary wall after direct ovule pollination with corn pollen. X 10

Fig. 5. Completely developed embryos from direct pollination of corn ovules with teosinte pollen. X 25

Fig. 6. Hyperplastic nucellar growth after direct pollination of corn ovules with sorghum pollen. X 1.7

corn ovules, direct pollination, without any intervention of the stylar tissue, was attempted in vitro (Fig. 3). Direct ovule pollination of corn × corn (Fig. 4) and corn × teosinte (Fig. 5) was successful and resulted in about 5% of well developed but naked embryos with endosperm. (Corn × corn: 5 experiments with a total of ca. 300 ovules; corn × teosinte: 2 experiments with a total of ca. 150 ovules. Embryos were recovered from experiments using both B^{II} and MS plus GA_3 media). During ovule development, the cut ovary wall turned milky white at the rims and folded back in the maturing 'seed' (Fig. 4). The ovary wall of unfertilized ovules and control ovules did not grow and eventually became brown. Sorghum pollen was applied directly to corn ovules and 12 out of 200 ovules pollinated showed a rapid, massive growth of nucellar tissue (Fig. 6). This was never seen in unpollinated controls. There was no indication of embryo or endosperm development. The nucellar response was observed after direct pollinations of two corn hybrids (G4444 and G4747W, Funk Seeds Int.) with a diploid sorghum (Caprock). There was no response in a further 200 pollinations involving a tetraploid sorghum. Ca. 100 control ovules were cultured without pollination.

In summary, our observations show that a barrier to the growth and penetration of corn pollen exists on sorghum styles, but that normal growth and penetration of sorghum pollen can occur on corn styles. Fertilisation of corn by sorghum is prevented in the first instance by the limited growth of sorghum pollen tubes and the absence of stigmatic hairs on the basal 5-10 mm section of corn silks. For these reasons, pollination in vitro of intact or shortened corn silks with sorghum pollen and culture of the pollinated ovaries was unsuccessful. Direct ovule pollination of corn × corn and corn × teosinte led to successful fertilisation. Six per cent of the corn ovules pollinated directly with sorghum pollen responded by massive growth of nucellar tissues. This response has not been reported after in vitro ovule pollination of dicots but was demonstrated to be a post-fertilisation event on several wide crosses in vivo (Raghavan 1977). Further evidence for fertilisation of corn by sorghum is now required. It appears that direct pollination of exposed corn ovules with sorghum pollen in vitro is more likely to allow fertilisation and subsequent recovery of hybrid embryos.

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Dr. Harcharan S. Dhaliwal
Dr. Patrick J. King
Friedrich-Miescher-Institut
P.O. Box 273
CH-4002 Basel
(Switzerland)