Euphytica 36 (1987) 625–630

HYBRIDIZATION BETWEEN DIPLOID (*GOSSYPIUM ARBOREUM*) AND TETRAPLOID (*GOSSYPIUM HIRSUTUM*) COTTON THROUGH OVULE CULTURE

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Received 13 August 1986

INDEX WORDS

Gossypium arboreum, Gossypium hirsutum, cotton, ovule culture, interspecific hybridization.

SUMMARY

With in vitro culture of ovules, interspecific hybrids have been obtained in an otherwise incompatible cross between a diploid (*Gossypium arboreum*) and a tetraploid (*G. hirsutum*) cultivated cotton. The early abortion of the embryo was prevented by repeated treatment of the flowers, immediately after pollination with a solution of gibberellic acid and naphthalene acetic acid. The ovules excised three days after pollination and cultured in a liquid medium underwent profuse proliferation, whereas on an agar-solidified medium supplemented with casein hydrolysate, indoleacetic acid and kinetin they germinated to form hybrid plants.

INTRODUCTION

Gossypium hirsutum (2n = 52) and *G. arboreum* (2n = 26) are the two species which occupy a major portion of the area under cotton in India. *G. hirsutum* types generally yield more, possess longer fibre of superior quality, and are resistant to diseases such as *Fusarium* wilt, whereas *G. arboreum* can better tolerate environmental stresses and is comparatively resistant to various insect pests. Efforts to combine the desirable characters of these two species have not been encouraging due to abortion of the hybrid embryo/endosperm in the early stages of seed development (WEAVER, 1957; PUNDIR, 1972; STEWART & HSU, 1978). Thus in our ongoing cotton improvement program, tissue-culture methods (BAJAJ, 1986) have been incorporated with a view to increase genetic variation (BAJAJ & GILL, 1985). In an earlier communication the use of embryo and ovule culture, for breeding interspecific hybrids between various diploid cotton species was reported (GILL & BAJAJ, 1984). The present article deals with the hybridization between a tetraploid *Gossypium hirsutum* and a diploid *G. arboreum*.

MATERIALS AND METHODS

Cultivars of *Gossypium hirsutum* (F414 and LH 372) and *G. arboreum* (G27 and LD133) were grown in the Experimental Fields of the Punjab Agricultural University at Ludhiana. Reciprocal crosses between these species were made in the field. A mixture of growth regulator solution (gibberellic acid 50 mg/l + naphthalene acetic acid

100 mg/l) was applied in cotton wool at the base of the pedicle of the pollinated flowers on the day of pollination and the day after pollination. The developing bolls (cross pollinated as well as selfed) were excised three days after pollination (DAP), sterilized by dipping them in 70% ethyl alcohol and subjected to flaming. The ovules were scooped out of the bolls aseptically in a laminar flow chamber and cultured on agar solidified MS medium (MURASHIGE & SKOOG, 1962) or in liquid SH medium (STEWART & HSU, 1978) with or without growth regulators. The cultures were transferred to fresh medium every four weeks. The germinating ovules were transferred to a medium containing half the concentration of MS salts and 1% sucrose. The culture conditions and other details were as provided earlier (GILL & BAJAJ, 1984).

RESULTS AND DISCUSSION

Effect of growth regulators. In both the parents there was normal development of the bolls, however after interspecific pollination, the flowers generally abcised within three days. The application of a solution of gibberellic acid (50 mg/l) and naphthalene acetic acid (100 mg/l) to the flowers immediately after cross pollination and the following day considerably enhanced their retention (Table 1).

In the controls (untreated) the flower retention with three days of pollination was only 6.4 to 17.6% in *G. hirsutum* × *G. arboreum*. When *G. arboreum* was used as the female parent, all the bolls were shed after 3 days. The process of shedding started on the second day after pollination, and by the third day the bolls were either completely shed or they fell with slight disturbance. However, with the application of growth regulators, depending on the cultivar, the retention varied between 52.9% – 79% in *G. hirsutum* × *G. arboreum*, and 43.7 – 60.7% in the reciprocal crosses.

Culture of ovules of the two parents. The response of *G. hirsutum* and *G. arboreum* ovules cultured on MS medium containing various combinations and concentrations of indoleacetic acid (IAA), kinetin (kin), casein hydrolysate (CH) and coconut water (CW) is shown in Table 2. It was observed that some of the self pollinated ovules

Table 1. Effect of growth regulators (GA_3 50 mg/l + NAA 100 mg/l) on boll retention in interspecific crosses in *Gossypium hirsutum* × *G. arboreum* and their reciprocals.

Cross	Treated			Control		
	number of flowers crossed	number of bolls shed	% retention	number of flowers crossed	number of bolls shed	% retention
G. hirsutum × *G. arboreum*						
F 414 × G 27	71	20	71.8	36	30	16.7%
F 414 × LD 133	62	13	79.0	15	14	6.6%
LH 372 × G 27	56	24	57.1	31	29	6.4%
LH 372 × LD 133	34	16	52.9	17	14	17.6%
G. arboreum × *G. hirsutum*						
G 27 × F 414	28	11	60.7	11	11	0
LD 133 × F 414	16	9	43.7	17	17	0

Table 2. Percentage of ovules giving seedlings and the callus growth response of excised ovules (3 days after pollination) of *Gossypium arboreum* and *G. hirsutum* cultured on various media (figures in parenthesis indicate the number of ovules cultured).

Medium (mg/l)	*G. arboreum* germination (%)	Callus[1] development	*G. hirsutum* germination (%)	Callus development
MS basal	17.6 (125)	+	8.3 (96)	–
MS + CH 250	29.2 (96)	+	22.2 (99)	–
MS + CW 7%	21.2 (80)	+	25.4 (110)	–
MS + CH 250 + IAA 0.5 + kin 0.2	22.2 (108)	+	23.8 (122)	–
MS + CH 250 + IAA 0.5 + kin 0.5	23.7 (93)	+	25.0 (120)	–
MS + CH 250 + IAA 1 + kin 0.2	28.1 (121)	+	44.4 (133)	++
MS + CH 250 + IAA 1 + kin 0.5	20.3 (103)	+++	24.1 (108)	++
MS + CH 250 + IAA 1.5 + kin 0.5	37.5 (112)	+++	18.8 (117)	++

– no callus; + little callus; ++ good callus; +++ profuse callus.

of both the parent species germinated and produced seedlings even on simple MS basal medium, however, the germination was considerably enhanced when the medium was supplemented with either CH or CW alone or in combination with IAA and kin. On almost all the media, the ovules started to proliferate within a week of culture. There was profuse callusing in *G. arboreum,* this callus was soft, friable and pinkish. *G. hirsutum* callus was slow-growing, compact and off-white. Maximum callus was produced on media containing IAA (1 and 1.5 mg/l), kin (0.5 mg/l) and CH (250 mg/l). The ovules kept growing along with the mass of callus, and these could be seen either as lying on the callus surface or embedded in it. Germination of ovules started after about 50 days in *G. arboreum* and 60 days in *G. hirsutum*. At the time of germination, the size of *G. arboreum* ovules was smaller than those developed in situ, whereas *G. hirsutum* ovules attained size almost equal to the in vivo developed ones. Seedlings of *G. hirsutum* obtained from ovule culture were also greener and more vigorous than those of *G. arboreum*. In most of the cases the cotyledons were underdeveloped, absent or malformed. The best response was observed on MS + CH (250 mg/l) + IAA (1.5 mg/l) + kin (0.5 mg/l) and on MS + CH (250 mg/l) + IAA (1 mg/l) + kin (0.2 mg/l) in *G. arboreum* and *G. hirsutum* respectively.

Table 3. Percentage germination and callus growth response of hybrid ovules cultured on various media (data based on 100 ovules cultured in each experiment).

Media (mg/l)	*G. hirsutum* cv. F414 × *G. arboreum* cv. G27		*G. arboreum* cv. G27 × *G. hirsutum* cv. F414	
	germination %	callus[1]	germination %	callus
MS + CH 250 + IAA 1 + kin 0.2	27.5	+	10.7	++
MS + CH 250 + IAA 1.5 + kin 0.5	14.3	+	34.7	+++
SH (basal)	0	–	0	–
SH + IAA 0.5	0	+++[2]	0	++
SH + IAA 1	0	+++[2]	0	++

[1] – no callus; + little callus; ++ good callus; +++ profuse callus.
[2] Fibres were also formed in these cultures.

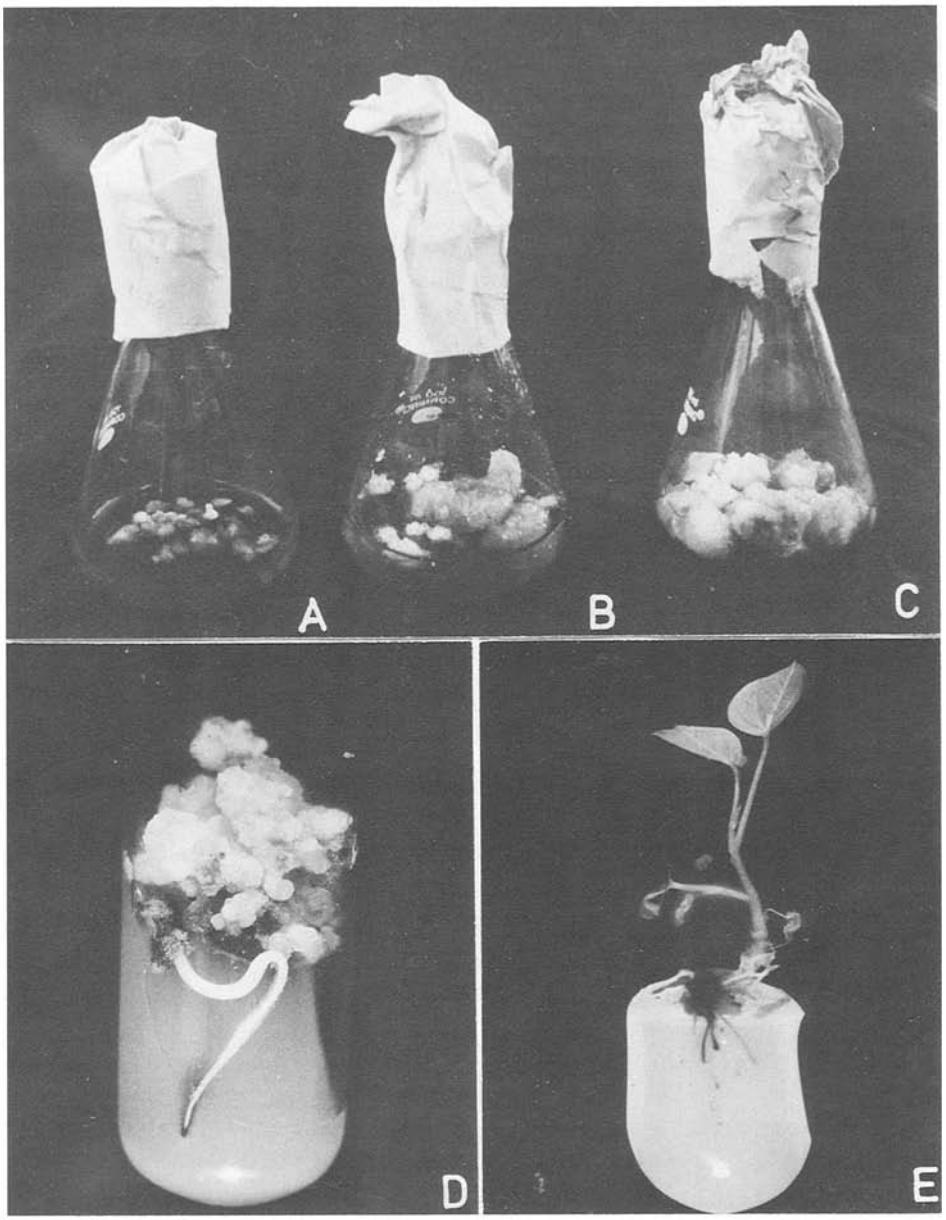

Fig. 1. In vitro growth of hybrid ovules of *Gossypium hirsutum* × *G. arboreum*, and *G. arboreum* × *G. hirsutum* cultured 3 days after pollination on various liquid media containing IAA (0.5 mg/l). B) Same, after 21 days of culture; note callus formation in some of the ovules. C) Same, after 28 days of culture showing profuse proliferation. D) *G. arboreum* × *G. hirsutum* ovule (3 DAP) after 55 days of culture on agar-solidified MS + IAA (1.5 mg/l) + kin (0.5 mg/l) + CH (250 mg/l) showing germination as well as callus. E) 12-week-old culture showing a seedling derived from 3 DAP ovule of *G. hirsutum* × *G. arboreum* cultured on MS + IAA (1 mg/l) + kin (0.2 mg/l) + CH 250 mg/l) after transfer to half strength MS + 1% sucrose.

Culture of hybrid ovules. The hybrid ovules were cultured on MS agar as well as on SH liquid medium (Table 3, Fig. 1). On basal SH liquid medium there was no visible growth or germination, the ovules gradually sank to the bottom and turned brown. When IAA (0.5 or 1 mg/l) was added to this medium, there was profuse callusing (Fig. 1 A–C), but no germination even after four months of culture.

On MS agar medium, the parental as well as the hybrid ovules underwent callusing (Fig. 1D) and their reponses were similar. However, the germination of hybrid ovules was slower in comparison to their respective parents, and no germination was observed in the absence of IAA and kin, whereas the parents ovules germinated even on basal medium. *G. hirsutum* × *G. arboreum* ovules produced more vigorous seedlings than the reciprocal hybrid.

The seedlings from the in vitro germinated ovules were transferred to a fresh medium (half strength MS with 1% sucrose) and placed in an illuminated room where they continued further growth (Fig. 1E). The seedlings from *G. arboreum* × *G. hirsutum* crosses had a lower survival rate (40%) than the seedlings from the reciprocal crosses. The cytological examination showed a triploid chromosome number ($2n = 3x = 39$) in the root tips of hybrid plantlets.

In an earlier study (GILL & BAJAJ, 1984) hybrids were obtained between two diploids species (*Gossypium arboreum* × *G. anomalum, G. herbaceum* × *G. stocksii*) through the culture of excised embryos. In crosses between a diploid (*G. arboreum*) and tetraploid (*G. hirsutum*) cotton the embryos abort at a very early stage, though some hybrids were obtained by STEWART & HSU (1978) through culturing ovules in a liquid medium. In our experience liquid medium had the disadvantage that many of the ovules sank to the bottom and produced callus. In the present study therefore ovules retained on the plants by the application of growth hormones and grown to a stage when the embryo was few celled, could be cultured on agar-solidified medium to form hybrids. On transfer to the soil most of the parent plants survived whereas the hybrids had a tendency to wither. The ovule-derived parent plants grew well and flowered normally in the field. However, the hybrids were sterile, and produced a few flowers which were shed later. The pollen from triploid plants (hybrids) was used to pollinate *G. hirsutum,* and *G. arboreum* flowers and a few shrivelled seeds were obtained. Attempts to restore fertility of triploids through chromosome doubling are made for their incorporation into cotton breeding program.

ACKNOWLEDGEMENTS

Financial assistance from the Indian Council of Agricultural Research is gratefully acknowledged.

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