Short Communication

Hybridization between *Gossypium herbaceum* and *Gossypium stocksii* through embryo rescue

GILL MANJEET S, YPS BAJAJ¹

Punjab Agricultural University, Ludhiana, India

ABSTRACT

Nutrient media and culture conditions have been defined for ovules 3 and 5 d after pollination and embryos of *Gossypium herbaceum* respectively. The technique was then used to produce interspecific hybrids between a cultivated *Gossypium herbaceum* and a wild species. *G. stocksii*. The hybrid plants were transferred to field and they exhibited most of the characters of the pollen parent i.e. *G. stocksii*.

Key words: *Cotton, biotechnology, embryo culture, wide hybridization.*

INTRODUCTION

Gossypium herbaceum in one of the two 'A' genome diploid cultivated species of cotton. It is grown in some parts of central India, especially as a rainfed crop. *G. stocksii* is a wild species belonging to 'E' genome and is found in West Asia. This species has a very good root system and is tolerant to drought[1]. Thus, the hybridization between *G. herbaceum* and *G. stocksii* is desired to develop drought-tolerant varieties in *G. herbaceum*. The attempts to produce interspecific hybrids by the conventional methods are often unsuccessful due to the formation of underdeveloped abortive seeds. However, with the advances made in the field of embryo-rescue in the oilseed crops[2], now it is possible to produce hybrids between *G. herbaceum* and *G. stocksii*.

1. Corresponding: Professor YPS Bajaj, Lady Irwin College, Sikandra Road, New Delhi 110001, India.

Hybridization between *G. herbaceum* and *G. stocksii* through embryo rescue

Earlier we reported interspecific hybridization in various *Gossypium species*[3-5], this work has now been succeseefully extended to *G. herbaceum* x *G. stockii*.

MATERIALS AND METHODS

The plants of *Gossypium herbaceum* cv. SM 132 and *G. stocksii* were grown in the field by following the recommended agronomic practices. *G. herbaceum* flowers were pollinated wih pollen of *G. stocksii*. A mixture of growth regulators (100 mg/l NAA and 50 mg/l GA) was applied to the base of the pedicel of the cross-pollinated flowers. The developing bools were harvested 3 d after pollinationn (DAP) for ovule culture and 15 DAP for embryo culture. At first, various combinations and concentrations of IAA, kinetin(KT), casein hydrolysate (CH) and coconut water (CW) in MS medium[6] were tested for the culture of ovules and embryos of *G. herbaceum*. The level of sucrose was kept at 3% or 6%. Subsequently the hybrid embryos and ovules were cultured on the most suitable medium. The cultured embryos were kept in complete darkness for the first 15 d of culture, and then transferred to a well-illuminated room with 16 h light. The ovule-derived plants were transferred to 1/2 MS + 1% sucrose medium for further development, and then transferred to pots at 2-3 leaf stage. These plants were transferred to field in April (normal time for sowing cotton). Observations on various morphological characters were recorded in the field.

RESULTS AND DISCUSSION

The observations and the data on culture of ovules and embryos of *G. herbaceum*, and *G. herbaceum* x *G. stocksii* are presented in Tab 1-2 and Fig 1. The observations recorded on the interspecific hybrids are given in Fig 2.

Ovule culture

For ovule culture, the developing bolls (self-pollinated or cross-pollinated) were harvested 3DAP and cultured on MS medium containing various combination and concentrations of IAA, KT, CH and CW (Tab 1). It was observed that some of

Tab 1. Effect of different culture media on germination of cultured ovules (3 d after pollination)

MS media (mg/l)				Ovules that produce seedlings (%)	
				<i>G. herbaceum</i>	<i>G. herbaceum</i> x <i>G. stocksii</i>
1	Basal			7.9	6.7
2	CW 7%			18.0	NC
	CH	IAA	KT		
3	500	-	-	18.8	14.9
4	250	0.5	0.2	16.6	NC
5	250	0.5	0.5	12.5	NC
6	250	1.0	0.2	22.5	13.3
7	250	1.0	0.5	19.4	NC
8	250	1.5	0.5	25.0	22.2

NC= not cultured

G. herbaceum x *G. stocksii*

Fig 1. Plantlet formation from hybrid embryos of *G. herbaceum* x *G. stocksii*, and their transfer to pots and the field.

- A. Embryo (15 DAP) after 35 d of culture on MS + IAA (1.5 mg/l) + KT (0.5 mg/l) + CH (250 mg/l), showing good root and shoot formation.
- B. Hybrid plant 45 d after transfer into the pot.
- C. Hybrid plant after 3 months of transfer to the field; note the bushy nature of the plant.
- D. Hybrid plant at flowering stage after 5 months of transfer to the field.

Fig 2. Comparison of leaves, flowers and bracts in the cross *G. herbaceum* x *G. stocksii*.
A. Leaves of *G. herbaceum*, hybrid and *G. stocksii* (L to R). Note the difference in shape and size of the lobes and the pedicel length
B. Flowers of *G. herbaceum*, hybrid and *G. stocksii* (L to R); note the difference in size.
C. Bract of *G. herbaceum*, hybrid and *G. stocksii* (L to R); note the difference in shape and size.

the ovules germinated to produce seedlings even on MS basal medium. However the germination percentage was considerably enhanced when the medium was supplemented with either CH or CW alone, or in combination with IAA and KT. On almost all the media, the ovules started to proliferate to form callus within one week of culture. The callus was friable and creamy-white. Maximum callus was produced on media containing 1 and 1.5 mg/l IAA along with 0.5 mg/l KT and 250 mg/l CH. Along with the mass of callus, the ovules kept growing and these could be seen either as lying on the callus surface or as embedded in the callus. Germination of ovules started after about 50 d of culture. At the time of germination, the size of ovules was smaller than the *in vivo* developed ovules. The seedlings were also weak with malformed cotyledons. The best germination percentage was obtained on MS + 250 mg/l CH + 1.5 mg/l IAA + 0.5 mg/l KT. the seedlings developed roots and leaves after transfer to 1/2 MS medium.

The hybrid ovules were cultured on the four most responsive media. The callus formation from hybrid ovules was similar to that in *G. herbaceum*. The germination of hybrid ovules was slower than in *G. herbaceum*, however their behaviour with respect to germination percentage on different media was almost similar to *G. herbaceum*.

Embryo culture

The self pollinated embryos were cultured at 15 DAP stage on MS medium supplemented with various concentrations and combinations of IAA, KT, CH, CW and sucrose (Tab 2). The best response without any callus formation was observed on MS + 1.5 mg/l IAA + 0.5 mg/l KT + 250 mg/l CH + 3% sucrose. On this medium, seedlings with first pair of leaves were obtained in 35 d. The hybrid embryos were cultured on the best medium. Each cross-pollinated boll contained 3-8 viable embryos. The growth response of the hybrid embryos was similar to the *G. herbaceum* embryos (Fig 1 A-D).

The ovule-, or embryo-derived plants were transferred to pots at 2-3 leaf stage with well developed roots. There was 80-90% survival of potted plants.

Tab 2. Growth response of *G. herbaceum* embryos (15 DAP) cultured on various media

	MS media (mg/l)				Callus formation (%)	Plantlet formation (%)
	CW (%)	CH	IAA	KT		
1	6	-	1.0	0.2	30	10
2	6	-	2.0	0.2	33	0
3*	7	-	1.5	-	85.7	0
4	15	-	1.0	-	28	18
5	15	250	1.0	-	42	28
6	-	250	1.5	0.5	0	54
7*	-	250	1.5	0.5	0	22
8	-	500	1.5	0.5	35.3	17.6

* Sucros 6%, others 3%.

Hybridization between *G. herbaceum* and *G. stocksii* through embryo rescue

Field observations of hybrid plants

For comparison the two parents were grown along with the hybrid plants in the field and observations for various morphological characters were recorded in the field (Fig 2). In most of the characters the hybrid plants resembled the pollen parent *G. stocksii*. The hybrids were late-flowering, perennial, self-sterile, and showed semi-creeping habit.

ACKNOWLEDGEMENT

This paper is part of the Ph. D thesis submitted by the first author to the Punjab Agricultural University. Appreciation is expressed to the Bhabha Atomic Research Commission, and the Indian Council of Agricultural Research for financial assistance.

REFERENCES

- [1] Sikka SM, AB Joshi. Breeding. In: Cotton in India- A monograph. Pub1 Indian Central Cotton Committee, Bombay 1960:137-335.
- [2] Bajaj YPS. Wide hybridization in legumes and oilseed crops through embryo, ovule, and ovary culture. In: YPS Bajaj, ed. Biotechnology in agriculture and forestry, vol 10. Legumes and oilseedcrops I. Springer Verlag, Heidelberg Berlin New York 1990: 3-37.
- [3] Gill MS, YPS Bajaj. Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 1984; **33**:305-11.
- [4] Gill MS, YPS Bajaj. Hybridization between diploid (*Gossypium arboreum*) and tetraploid (*gossypium hirsutum*) cotton through ovule culture. Euphytica 1987; **36**:625-30.
- [5] Gill MS, YPS Bajaj. *In vitro* hybridization between *Gossypium arboreum* and *G. anomalum* and their backcross progeny. Plant Tissue Cult 1991; **1**(2):101-8.
- [6] Murashige T, F Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phyiol Plant 1962; **15**: 473-97.

Received 20-6-1995

Revised 23-1-1996

Accepted 12-3-1996