Haploid Induction in Cotton: A Future Perspective

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ABSTRACT

The value of haploids in genetic analysis and plant breeding has long been known. This is more so in cotton, since the production of double haploids in large numbers could open the way for the development of partial interspecific hybrids between Gossypium hirsutum and G. barbadense. For the induction of haploids in cotton three approaches were applied, namely: anther culture, induction of parthenogenesis after pollination of cotton flowers with alien pollen, and semigamy. In vitro culture of anthers originated from 10 cotton varieties and a number of intraspecific and interspecific cotton hybrids resulted only in the production of callus with a number of haploid cells and very few embryoids. Haploid and double haploid plants were obtained in a relatively small number after pollination of a semigametic line with pollen from interspecific cotton hybrids. Pollination of cotton flowers with pollen from related species (Hibiscus syriacus, Abelmoschus esculentus, Malva sylvestris, Datura stramonium, Hibiscus cannabinus) and in vitro culture of young ovules resulted in the production of abnormal plantlets when A. esculentus and D. stramonium were the pollinators and of mature cotton plants when the pollinator was H. cannabinus.

Introduction

Haploids in cotton (*Gossypium hirsutum* L., *G. barbadense* L.) were first detected in 1920 when wild types of Pima cotton plants were obtained from twin seeds (Harland, 1940; Mahill *et al.*, 1984). So far, several alternative procedures have been proposed for the production of haploids in cotton. Turcotte and Feaster (1973) reported the production of haploids at a low frequency through the phenomenon of semigamy. Since then, several researchers have developed a number of semigametic lines (Barrow and Chaudhari, 1976). Androgenesis and gynogenesis, however, which are the two principal systems for the production of haploids in several crop species have not been developed in cotton. Barrow *et al.* (1978) were the first to apply anther culture in cotton but so far the progress has been limited to only in the production of friable emryogenic callus (Barrow *et al.* 1978; Turaev and Shamina, 1986). Furthermore, the study of gynogenesis is also limited. Beasley and Ting (1974) developed a system for *in vitro* fertilization able to support the development of ovules. In addition, Stewart and Hsu (1977) proposed a culture medium (SH) suitable for the growth of *in vitro* ovules originating from wide hybridization of cultivated cottons with diploid *Gossypium* species. Finally, Zhou *et al.* (1991) reported the production of haploid cotton plants at a low frequency through parthenogenesis when cotton flowers were pollinated with pollen from *Hibiscus cannabinus*.

This work was undertaken to investigate further the possibility of producing haploids in cotton using anther culture technique and an *in vitro* ovule culture system which exploits the phenomena of gynogenesis and parthenogenesis.

Materials and Methods

Cotton cultivars from the species *G. hirsutum* and *G. barbadense* and their F_1 hybrids were used for *in vitro* anther and ovule culture. In addition, 17 semigametic lines and 5 interspecific F_1 hybrids were used for the production of haploid plants via semigamy. The genetic material used are given in Table 1.

Anther culture. Flower buds were collected when the microspores were at the uninucleate stage. After sterilization of buds, anthers were dissected and cultured into three types (liquid, solid and bilayer) of two modified media, [B5 +2,4-D (0.1 mg/L) +GA_3 (0.5 mg/L) + KIN (1 mg/L) and [MS + NAA (0.2 mg/L) + BA (0.5 mg/L)]. Once the most effective media were defined, the effect of pretreatments on flower buds (cold treatment : 4°C for 48 hours, heat treatment: 42°C for 5 days) and starvation in anthers (no sucrose in the nutrient medium) were studied. All cultures were incubated in the dark at 28°C, and 30 days later the anther response was estimated. At this phase, the ploidy level of callus cells was also determined using MPVC analysis.

Semigamy. Seventeen semigametic lines were tested for their ability to produce haploids when they were pollinated with five F_1 interspecific cotton hybrids (Table 1). Doubled haploids (DH) were subsequently produced by the application of colchicine solution (0.2%) into soil. The ploidy level of the progeny plants was determined via the number of chloroplasts in guard cells (Chaundari and Barrow, 1975) and fluorometric analysis.

In ovule - embryo culture. Flowers from ten cotton cultivars and five interspecific hybrids were pollinated with pollen from *H. syriacus, A. esculentus, H. cannabinus, D. stramonium* and *M. sylvestris* (Table 1). Young bolls were collected 3, 5 and 8 days after pollination (DAP). After ovary sterilization the ovules were dissected out and cultured on modified SH media [SH basal or SH + IAA (0.1 mg/L) + GA_3 (0.5 mg/L) + KIN (1mg/L)]. In addition, unfertilized and self-fertilized ovules of the same age were cultured as controls. All cultures were incubated in the dark at 28°C for 50-58 days. After this period, ovules were dissected and the isolated embryos were floated on the same liquid media. Four to six weeks later, germinated embryos were transferred to G1 [SH + GA_3 (0.1 mg/L) + KIN (0.5 mg/L)] or G2 [SH + KIN (1mg/L)] medium for another two weeks. Surviving plantlets were transferred to rooting media (RT) [MS + AC (0.3%) + NAA (1 mg/L) + BA (1 mg/L)]. When plantlets attained 4-5 leaves they were transplanted into a soil mixture (1: sand 1: peat 1: vermiculite) and covered with a plastic bag. The ploidy level of all surviving plants was determined cytologically via MPVC and fluorometric analysis.

Results and Discussion

Anther culture. The results of anther culture verified the observation that callus production is genotype dependent (Barrow *et al*, 1978; Turaev and Shamina, 1986). Anthers from F_1 interspecific hybrids gave better, well developed, friable callus. This callus contained the highest number of haploid nuclei (60-72%) as determined via MPVC analysis. In general, callus growth was better on solid than on liquid medium and the percentage of callogenesis ranged from 4 to 72.9%. Liquid medium, however, promoted microspore release into the medium. Finally, the pretreatments had a negative effect on callus production but in some cases (cold treatment) increased the quantity of microspores released. The most important observation was the presence of embryoids when anthers of Coker 310 and Acala - Sindos were cultured on solid B5 medium enriched with 2,4-D (0.1 mg/L), GA_3 (0.5 mg/L) and KIN (1 mg/L). Unfortunately, these embryoids did not grow further to regenerate plantlets.

Semigamy. Semigametic lines produced haploid and DH cotton plants. From 17 semigametic lines, only one (Sem-13) gave a satisfactory number of haploids (7.6%). When F_1 interspecific cotton hybrids were crossed with this semigametic line, carpodesis ranged from 48 to 62% compared to the control, while the percentage of haploid plants was 12.3%. It is important to note that some of the haploid seeds (42%) did not germinate when they were planted in the greenhouse. This could be due to dormancy (Barrow and Chaudhari, 1976). Artificial chromosome doubling using colchicine solution (0.2%) through irrigation of individual haploid plants was effective. This was confirmed through fluorometric analysis and chloroplast counts. On the basis of the DNA content, haploids, DH and an intermediate ploidy category of cotton plants were identified .

In ovule - embryo culture. Since Zhou *et al*. (1991) obtained parthenogenetic seed set (1.4-2.3%) after pollination of cotton flowers with pollen from *H. cannabinus*, one could assume that a larger number of unfertilized ovules could have been stimulated by the alien pollen for embryo development that were unable to reach maturity. Thus, the development of an *in vitro* culture protocol able to support the growth of young parthenogenetic embryos was needed.

The results of this study indicate that pollination of cotton flowers with pollen from alien species induces the development of ovules which could grow further under *in vitro* conditions. The stimulus of alien pollen on cotton stigma resulted in the maintenance of bolls on the plant for a longer period, as reported for a number of other crop species (Laurie and Bennett, 1988). However, the genotype of the female parent, the pollinator, and the age of the cotton flowers affected the response. Korina, Pima S5, Carnak and F_1 hybrids were the best female parents for intergeneric crosses. These cultivars gave a high percentage of carpodesis and produced viable ovules with all pollinators (Table 2). For most of the crosses, embryos and embryoids were found in the ovules after 45 - 58 days in culture. The only exceptions were crosses with *M. sylvestris*. The embryoids originating from crosses with *H. syriacus* died very early (42-48 days) and before their transfer into liquid germination medium. On the contrary, in *vitro* culture of ovules crossed with *A. esculentus*, *D. stramonium* and *H. cannabinus* yielded a number of viable plantlets. Pollination of cotton flowers with pollen from *A. esculentus* and *D. stramonium* led to the production of abnormal plantlets which grew very slowly and had a different ploidy level. Mature haploid and tetraploid plants were produced only after pollination with *H. cannabinus.* Perhaps the fertile tetraploid plants originated from haploids after spontaneous diploidization. This is supported by the observation of an additional peak at the haploid level in flow cytometric analysis of these plants (Figure 1). If this occurs, a new method which combines the pollination of cotton flowers with pollen from alien species and an *in ovulo*-embryo culture system will contribute towards the production of haploids and DH in cotton.

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Table 1. The genetic material and strategies used for haploid production in cotton.

G. hirsutum	F_1 interspecific hybrids		Species used as pollinators
4 S	Sindos x Pima	***Ant***	***H**ybiscus **s**yriacus*
Sindos	4 S x Coker	***Ant, Ovul***	
Acala Sindos	4 S x Pima	***Ant***	***H**ybiscus **c**annabinus*
Korina	Coker x 4 S	***Ant***	
Coker 310	Coker x Menoufi	***Ovul***	***A**belmoschus **e**sculentum*
	B 403 x Acala Sindos	***Ovul***	
G. barbadense	Menoufi x Coker	***Ovul***	***D**atura **s**tramonium*
	Menoufi x Acala Sindos	***Sem, Ovul***	
Pima S5	Carnak x Coker	***Sem***	***M**alva **s**ylvestris*
Carnak	Carnak x Stoneville	***Sem, Ovul***	
Giza	Carnak x 4 S	***Ovul***	
Menoufi	Pima x Stoneville	***Sem, Ovul***	
B 403	Pima x Sindos	***Ant***	
	Pima x 4 S	***Ant, Sem***	

***An**t : anther culture, **Sem** : semigamy, **Ovul** : ovule culture*

Table 2. Number of crosses, carpodesis (%) and *in vitro* response of ovules collected after pollination of cotton flowers with alien pollen.

Cross Combination	No. of Crosses	(%) carpodesis	No. of ovules cultured	% of viable ovules (30d)	No. of Embryos	No. of plantlets
4S x H.s	48	48,2	460	22,7	1	0

4S x H.e	50	55,7	300	20,5	2	0
Korina x H.e	50	57,3	212	58,5	12	5
Korina x H.c	45	62	450	47,5	8	3
Coker x H.s	45	40,2	158	53	3	0
Coker x H.c	45	52,3	258	58	5	0
Pima x H.s	35	39,5	260	60,8	9	3
Pima x H.e	40	35,8	305	63,8	5	2
Pima x H.c	40	48,2	375	65,2	12	4
Pima x D.st	35	44,7	255	64	3	1
Giza x H.s	50	45,8	395	53	3	2
Carnak x H.e	42	54,6	255	69,5	12	6
Carnak x H.c	40	57,5	310	65,2	14	5
Menoufi x D.st	40	38,8	280	38,5	1	1
Menoufi x H.c	40	45,5	312	42,5	4	1
(Menoufi x Coker) x H.e	30	58,3	330	40,2	1	1
(Menoufi x Coker) x H.c	30	55,7	320	45,7	2	2
(Menoufi x Acala Sind) x H.e	25	50,8	300	42	1	1
(Pima x Stoneville) x H.e	30	60,2	380	50,4	5	4
(Pima x Stoneville) x H.c	30	50,4	320	45,2	2	2
(Carnak x 4S) x H.e	30	59,8	350	40,8	1	1
(Carnak x 4S) x H.c	30	58,5	330	38,5	8	6
(B403 x Acala Sind) x H.e	25	52,4	285	32,5	5	3
(B403 x Acala Sind) x H.c	25	47,9	255	35,7	4	3
UNFERTILIZED flowers	50	12,2	120	7,8	1	0
SELFFERTILIZED flowers	50	84,8	450	90,2	115	45

Figure 1. Flow cytometric analysis of leaf, in a plant obtained after pollination of cotton flowers with pollen from (a) *H. cannabinus* and (b) a tetraploid cotton plant used as control.