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Research Note

In - ovulo embryo culture in interspecific crosses of *Gossypium*

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Abstract
The immature hybrid embryos of five back crosses viz., (MCU 5 x *G. armourianum*) MCU 5, (MCU 5 x *G. raimondii*) MCU 5, (MCU 12 x *G. raimondii*) MCU 12, (TCH 1569 x *G. armourianum*) TCH 1569,(TCH 1609 x *G. raimondii*) TCH 1609 were excised 8 days after pollination and were cultured in various concentrations of auxin and cytokinin. To prevent boll shedding in all the crosses, growth hormones NAA 100 mg/l and GA_3 50 mg/l was applied at the base of the pedicel for 8 days and then the ovules were excised and cultured on media to form calli. Out of different media combinations tried, maximum callus induction was observed in the medium containing IAA 2.0 mg/l, Kn 1.0 mg/l, CH 250 mg/l, AS 40 mg/l in all the crosses. Maximum response of callus induction was observed in the cross (MCU 5 x *G. raimondii*) MCU5.

Key words: *Gossypium*, Interspecificcross, in-ovuloculture, callus,induction

Cotton (*Gossypium spp.*) is an important commercial crop with a current global textile fibre market share of nearly 66 per cent and annual growth rate of 4 per cent. It is a large diverse and economically viable genus, which includes 50 diploid and tetraploid species indigenous to most of the tropical regions of the world (Fryxell *et al.*, 1992). However, only four are cultivated (*G. arboreum, G. herbacium, G. hirsutum* and *G. barbadene*) whereas the rest are wild ones. Some of the wild species are having useful characters like resistance to abiotic and biotic stresses; hence there is a lot of scope for its improvement through hybridization. The successful utilization of these wild species in breeding programmes is often limited by the operation of either pre-fertilization or post fertilization barriers in wide hybridization. Inspite of these difficulties, several workers have transferred desirable economic attributes from wild species to cultivated cotton through distant hybridization. The notable intergenomal transfers being the genes for lint length (Sapenfield, 1970), resistance to boll weevils (Lukefahr *et al.,* 1971), resistance to rust (Blank *et al.,* 1972), resistance to black arm disease (Knight, 1963), resistance to jassids and drought (Balasubramaniam, 1950), fibre strength (Richmond, 1950) and glandless character (Altman *et al.,* 1987). There has been much interest in the transfer of genes in crops through genetic manipulation of cells and tissue culture, and through somatic hybridization by the fusion of protoplast (Bajaj, 1994). The interspecific hybridization between tetraploid and diploid cottons was achieved by cultivation of excised embryos *in vitro* (Stewart and Hsu, 1977). Liu *et al* (1980) sought effective techniques of inducing somatic embryogenesis and plantlet regeneration to overcome the problem of incompatibility between cultivated and wild species of cotton.

Chromosome doubling in Asiatic diploid species can be used as a tool to overcome the incompatibility experienced in *G. arboreum* x *G. hirsutum* species which was reported to be difficult in many cases (Mehetre *et al*., 2003). Recently, some workers identified modification of MS media that might also be useful for improving the *G. arboreum* x *G.hirsutum* seedlings from cultured ovules (Wu *et al*., 2004; Sacks, 2008). A reliable, repeatable and easier *in vitro* technique for ovule/embryo culture is desirable and will greatly benefit the development of newer hybrid combinations. Tabar *et al*. (2014) reported successful generation and plant establishment of a triploid hybrid (3x= 39, AAD) between tetraploid, *G. hirsutum* (4n= 2x= 52, AADD) and diploid, *G.arboreum* (2n= 2x= 26, AA) by a simple method of *in vitro* culture of older (50-60 DAP) hybrid embryos excised from field pollinated seeds. Chen *et al*. (2015) used an embryo rescue technique to overcome the cross-incompatibility between these two parents, *G. hirsutum* (2n = 52, AADD) and *G. arboretum* for transferring favorable genes from *G. arboretum* into *G. hirsutum.* The results indicated that MSB2K supplemented with 0.5 mgl-1 kinetin and 250 mg-1 casein hydrolysate is an efficient initial medium for rescuing early (3 d after pollination) hybrid embryos. Eight putative hybrids were successfully obtained, which were further verified and characterized by cytology, molecular markers and morphological analysis. In the present study, the effect of growth regulators on callus induction in ovules of interspecific crosses has been studied.

G. hirsutum genotypes *viz.,* MCU 5, MCU 12, TCH 1569 and TCH 1609 and wild species, *G. armourianum* and *G. raimondii* (Jassid resistant donors) were used in the study. Crosses were effected between cultivated tetraploids and wild diploids. The triploids were again backcrossed with cultivated tetraploids. To prevent early boll shedding, growth regulators were applied @ NAA 100 mg/l and GA_3 50 mg/l for 8 days at the base

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of the pedicel. The bolls were excised at 8th day after pollination and washed in 70% ethanol and surface sterilized with 0.1 per cent mercuric chloride solution for 10 minutes followed by three times thorough washings in distilled water. The ovules were excised aseptically and cultured in Murashige and Skoog medium. The cultures were incubated in dark for 15 – 20 days after which they were maintained at 28± 2° C with a photoperiod of 16/8 h at 1000 Lux light intensity. The ovules were cultured on MS medium supplemented with various concentrations of Indole acetic acid, (IAA), Naphthalene acetic acid (NAA), kinetin (Kn), Casein hydrolysate (CH) and Adenine sulphate (AS).

In the present investigation, young ovules (8th day after pollination) of various back crosses (MCU 5 x *G. armourianum*) MCU 5, (MCU 5 x *G. raimondii*) MCU 5, (MCU 12 x *G. raimondii*) MCU 12, (TCH 1569 x *G. armourianum*) TCH 1569, (TCH 1609 x *G. raimondii*) TCH 1609 were cultured on MS media containing various combinations and concentrations of IAA NAA, Kinetin, casein hydrolysate and Adenine sulphate as given in table 1. When the cultures were kept in dark for 15 days, the hybrid ovules enlarged initially. Taber *et al.*, 2014 reported i*n vitro* culture of ovules 5 days after pollination (DAP), failed to support the growth of viable embryos. Hybrid embryos 50 DAP, excised from field pollinated hybrid seeds, successfully germinated *in vitro*.

Gill and Bajaj (1984) also reported the embryo enlargement in interspecific hybrids of cotton when incubated the culture in dark for 15-25 days. The hybrid ovules cultured on various media supplemented with IAA, Kin, CH, AS, underwent profuse callusing in almost all the crosses. The ovules started to proliferate after 10 days and profuse callusing was seen on all the media. The callus was creamy white to greenish white, soft and friable. Maximum response of callus induction and proliferation was observed when MS media was supplemented with IAA (2.0 mg/l) + Kn (1.0 mg/l + CH 250 mg/l + Adenine sulphate 40 mg/l, and sucrose (3%). In this media combination, the callus appeared green and friable. In all the crosses, very low induction and proliferation of callus was observed in the basal MS media and high induction was observed in MS + IAA (2.0 mg/l + Kn 1.0 mg/l + CH 250 mg/l + AS (40 mg/l). The percentage of callus formation (proliferating ovules) ranged from 50.5% to 89.8% in the cross (MCU 5 x *G. armourianum*) MCU 5, maximum percentage of proliferating ovules was recorded in media containing IAA (2 .0 mg/l) + Kn 1.0 mg/l + CH 250 mg/l + AS (40 mg/l) and lower callus induction was observed in basal MS media.

Among all the crosses, the cross (MCU 5 x *G. raimondii*) MCU 5 recorded the highest average of proliferating ovules in the medium MS + IAA 2.0 mg/l + Kn 1.0 mg/l + CH 250 mg/l + AS 40 mg/l). In the cross, (MCU 12 x *G. raimondii*) MCU 12, the percentage of callus proliferation ranged from 50.5 – 75.8 percentages with an average of 65.6%. In the other two crosses, *viz.*, (TCH 1569 x *G. armourianum*) TCH 1509, (TCH 1609 x *G. raimondii*) TCH 1609, callus induction and proliferation was maximum when the media was supplemented with AS (40 mg/l).

In the basal MS media, ovules enlargement and proliferation was less and callus appeared creamy white. But when the media supplemented with IAA, Kn, CH and Adenine sulphate, ovules enlargement and proliferation was high and the callus appeared greenish white in colour and highly friable. The response of ovules among the different crosses for callus induction was different which indicated that callusing of ovules was under genetic control (Shoemaker *et al.*, 1986). The effect of phytohormones and CH has also been reported in ovule culture in *Gossypium* species (Gill and Bajaj, 1987). Chen *et al*., 2015 also reported MSB2K supplemented with 0.5 mgl-1 kinetin and 250 mg^{-1} casein hydrolysate is an efficient initial medium for rescuing early (3 d after pollination) hybrid embryos.

The formation of hybrid callus, which shows greater variations, can be utilized to generate environments for enhancing chromosome breakage and reunion events (Larkin and scowcroft, 1981). Such a hybrid callus may thus provide means for obtaining genetic exchange in interspecific hybrids. The plants from hybrid callus are likely to show an enhanced frequency of genetic variation. This is an area, which needs to be explored, and which may open wide vistas for future plant breeding programmes.

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Table 1. Callus induction in hybrid ovules in various media

	Media combination	No. of ovules cultured	No. of ovules proliferated	% of proliferated ovules
I	**MCU 5 x *G. armourianum*) MCU 5**			
1.	Basal MS	150	100	66.7
2.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l)	138	108	78.2
3.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l	142	106	74.7
4.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l + AS 40 mg/l	178	160	89.8
5.	MS + IAA (2.0mg/l) + Kn (0.2 mg/l) + CH 300 mg/l	158	100	63.3
II	**(MCU 5 x *G. raimondii*) MCU 5**			
1.	Basal MS	132	100	75.7
2.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l)	163	125	76.7
3.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l	142	112	78.9
4.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l + AS 40 mg/l	112	96	85.7
5.	MS + IAA (2.0mg/l) + Kn (0.2 mg/l) + CH 300 mg/l	105	90	85.7
III	**(MCU 12 x *G. raimondii*) MCU 12**			
1.	Basal MS	95	48	50.5
2.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l)	108	63	58.3
3.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l	125	90	72.0
4.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l + AS 40 mg/l	132	100	75.8
5.	MS + IAA (2.0mg/l) + Kn (0.2 mg/l) + CH 300 mg/l	98	70	71.4
IV.	**(TCH 1569 x *G. armourianum*) TCH 1569**			
1.	Basal MS	158	100	63.3
2.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l)	172	120	69.8
3.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l	165	100	60.6
4.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l + AS 40 mg/l	182	100	76.9
5.	MS + IAA (2.0mg/l) + Kn (0.2 mg/l) + CH 300 mg/l	138	140	72.5
V.	**(TCH 1609 x *G. raimondii*) TCH 1609**			
1.	Basal MS	148	85	57.4
2.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l)	156	100	64.1
3.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l	176	110	62.5
4.	MS + IAA (2.0 mg/l) + Kn (1.0 mg/l) + CH 250 mg/l + AS 40 mg/l	145	110	75.9
5.	MS + IAA (2.0mg/l) + Kn (0.2 mg/l) + CH 300 mg/l	165	115	69.7