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Short Communication

Immature ovule embryo culture — A tool to multiply wild cotton (*Gossypium* L.) germplasm

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Germplasm collection and maintenance is the ideal to obtain seed for all species but seed is further frequently incompletely ripened, inviable/ not found. Supplemental use of *in vitro* techniques of cotton germplasm conservation have been proposed in order to maximize efforts to preserve our genetic resources of cotton germplasm [1-2].

The present investigation was carried out with an aim to (1) determine appropriate conditions for growing young immature ovules of *Gossypium* spp. through *in vitro* culture techniques, (2) utilize this information for multiplication of plant of different species, of which multiplication through seed is rather difficult; and (3) to make available wild species and entire gene pool of genes of *Gossypium* to cotton geneticist and breeders for their utilization.

The flower buds of eighteen wild species of *Gossypium* (Table 1), which were expected to open following morning, were selfed and tagged.

The tagged flowers were harvested 7 days after flowering (DAF) and brought to the laboratory. Bracteoles and sepal were removed and ovaries were soaked for few seconds in disinfectant, followed by washing in sterilized double distilled water. After that, the ovaries (developing bolls) opened and the ovules were removed under aseptic conditions. These ovules were transferred to culture tubes containing Murashige and Skoog [3] nutrient medium + agar solidified (0.7 %) supplemented with various concentrations and combinations of Casein Hydrolysate (CH), indol acetic acid (IAA) and kinetin (KIN). At least 18-30 ovules of each species were cultured and kept in controlled growth environment at 32^{0}C in complete darkness for 21 days and then exposed to light (3000 lux). The germinated ovules were allowed to grow up to 2-3-leaf stage. After that they were transferred to pots containing soilrite under green house condition.

The data given in Table 1. indicated that highest (75.63 %) germination of ovules was recorded the culture medium MS supplemented with MS+1.5 mg/l IAA +0.5 mg/l Kinetin + Casein Hydrolysate 250 mg/l (M_1) in followed (47.8%) by medium supplemented with 2.0 mg/l IAA +2.0 mg/l Kinetin + 1.0 mg/l NAA + Casein Hydrolysate 250 mg/l (M_5). Further, it is revealed that 7 day old immature ovules of *G. trilobum*, *G. davidsonii G. lobatum* and *G. sturtianum* (Fig. 1); *G. bickii* (Fig. 3) gave better response for proliferation (Fig. 2) and direct germination and production seedlings. However germination of the ovules of *G. aridum*, *G. gossypioides*, *G. longicalyx* and *G. barbasonum* was

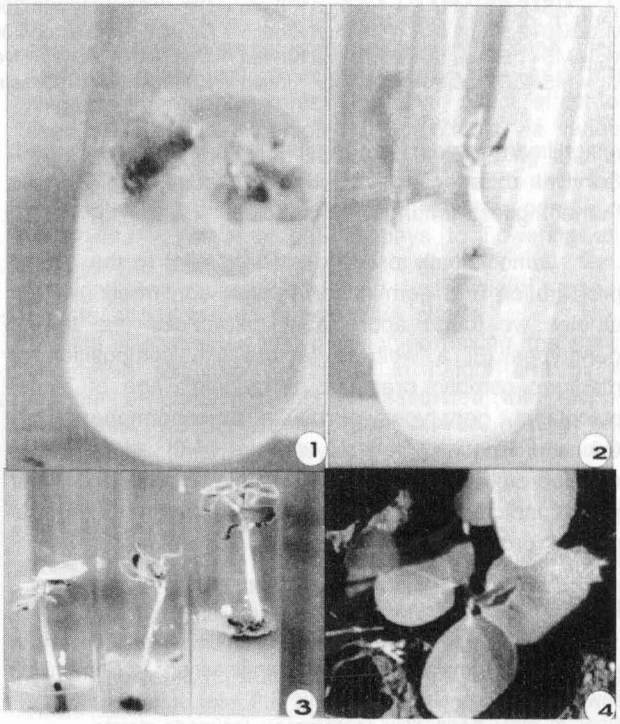

Fig. 1-4. Formation of callus in *G. gossypioides* on culture medium M_3, (MS+2.5 mg/l IAA +1.0 mg/l Kinetin + Casein Hydrolysate 200 mg/1); 2-3. Germination of ovules of *G. sturtianum* and *G. bickii* on medium M_1 (MS+1.5mg/l IAA +0.5 mg/l Kinetin + Casein Hydrolysate 250 mg/1); 4. Survival of seedlings of *G. sturtianum*

Table 1. Response of immature ovules of different wild diploid (2n = 2x = 26) *Gossypium* species grown on different culture media

Sr. No.	*Gossypium* speices	Origin	Haploid genome	Types of media used for ovule culture				
				M_1 G	M_2 G	M_3 G	M_4 G	M_5 G
1.	*G. trilobum*	Mexico	$D_8$3	85.95	54.94	63.43	50.77	62.03
2.	*G. klotschianum*	Galapagos	$D_{3\text{-}K}$	85.95	39.23	30.00	35.06	47.87
3.	*G. thurberi*	Mex. Ariz	D_1	85.95	54.94	39.23	45.00	54.94
4.	*G. australe*	Australia	C_2	85.95	45.00	26.56	40.97	46.14
5.	*G. anomalum*	Africa	B_1	68.03	50.77	50.77	54.94	40.97
6.	*G. triphyllum*	Africa	B_2	85.95	40.98	50.77	45.00	85.94
7.	*G. sturtianum*	Australia	C_1	85.95	60.00	63.43	63.44	66.42
8.	*G. davidsonii*	Mexico	$D_{3\text{-}d}$	85.95	50.77	60.00	60.00	63.64
9.	*G. stocksii*	Arabia	E_1	85.95	35.06	60.00	60.00	60.00
10.	*G. somalense*	Africa	E_2	85.95	35.06	35.06	35.06	43.28
11.	*G. bickii*	Australia	$C_4$3	85.95	45.00	30.00	45.00	49.60
12.	*G. aridum*	Mexico	D_4	54.93	26.57	32.58	04.05	33.21
13.	*G. gossypioides*	Mexico	D_6	35.06	04.05	04.05	04.05	17.46
14.	*G. raimondii*	Peru	D_5	85.95	38.06	04.05	60.00	43.85
15.	*G. capitis -viridis*	Cape verde Is	$B_4$3	85.95	49.02	40.97	04.05	45.00
16.	*G. lobatum*	Mexico	D_7	85.95	63.43	85.95	60.00	69.73
17.	*G. longicalyx*	Africa	F_1	45.00	04.05	04.05	04.05	17.46
18.	*G. barbosanum*	Cape verde Is	B_3	40.98	04.05	04.05	04.05	15.34
	General mean			75.63	38.94	38.05	37.52	47.81
	S.E. ±			04.30	04.37	05.66	05.41	04.45

G = Germination percentages of ovules.
M_1 = MS+1.5 mg/l IAA + 0.5 mg/l Kinetin + Casein Hydrolysate 250 mg/l
M_2 = MS+2.0 mg/l IAA + 1.0 mg/l Kinetin + Casein Hydrolysate 300 mg/l
M_3 = MS+2.5 mg/l IAA + 1.0 mg/l Kinetin + Casein Hydrolysate 200 mg/l
M_4 = MS+1.5 mg/l IAA + 1.0 mg/l Kinetin + 1.0/l NAA + Casein Hydrolysate 275 mg/l
M_5 = MS+2.0 mg/l IAA + 2.0 mg/l Kinetin + 1.0 mg/1 NAA + Casein Hydrolysate 250 mg/l

insignificantly poor amongst all the species tested. Survival of seedlings of *G. sturtianum* (Fig. 3) was found highest amongst all species.

Genotypically oriented response [4] to the ovules proliferation and germination is also confirmed . In our studies we found appreciable differences due to the genotypes [5] a well as factors like composition of medium; osmotic pressure of medium; age of ovule; cytoplasm, genotypes of ovule; phytohormones, IAA, GA and kinetins. Several workers [4-6] attempted to develop embryo ovule culture technique in cotton. Their results indicated that phytohormones play important role on the growth of the ovule.

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