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MICROPROPAGATION OF *GOSSYPIUM HIRSUTUM* VARIETY L.H.900

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Excised plant parts i.e. leaf petiole, hypocotyl, cotyledon, nodal segments of *G.hirsutum* when cultured on Murashige and Skoog's medium supplemented with IAA, NAA,2,4-D, Kinetin or BAP developed callus and multiple leafy shoots. These leafy shoots developed roots on rooting medium. Thus the plantlets were obtained in culture.

Keywords: Callus, *Gossypium hirsutum;*, Multiple leafy shoots; Murashige and Skoog's medium.

Introduction

Cotton is the most important fibre in the world. The fibre is obtained from the seed hairs (lint) of a few of the several species of *Gossypium* which are native to both old and the New World. Cotton is cultivated in several parts of Rajasthan like Shriganganagar, Kota, Udaipur and Banswara.

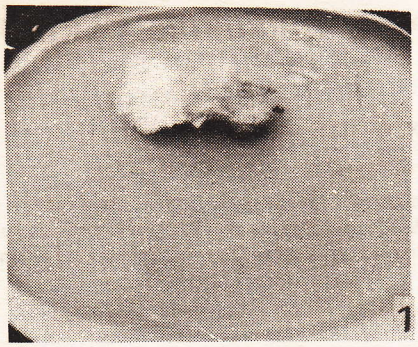
There are some reports on tissue culture of *Gossypium*, but members of *Gossypium* genus are severely limited in their regeneration *in vitro* from protoplast, callus or leaf tissue. *Gossypium hirsutum* can be regenerated from hypocotyl callus by asexual embryogenesis[1,3]. Shoot apex culture of *Gossypium* was first described by Morel[4,5] for claonal propagation and virus eradication. The present study deals with the growth and differentiation of cotton in tissue culture.

Material and Method

Seeds of *Gossypium hirsutum* variety L.H.900 were obtained from Shriganganagar. They were surface sterilized with 70% ethanol for 30 sec followed by treatment with 0.1% mercuric chloride for 3-5 min. The seeds were thoroughly washed with sterile distilled water for 4-5 minutes and aseptically germinated on basal M S medium[6]. Various explants like root, hypocotyl, cotyledon, cotyledonery node, leaf, stem, nodal segments were excised from 7 days old aseptically grown seedlings as well as from mature plants grown in nursery of Botany Department of Rajasthan University and were inoculated on M S medium containing 2% sucrose and 0.8% agar. Different growth regulators were added to the medium at various concentrations. The pH of the medium was adjusted to 5.8 before autoclaving. All the cultures were incubated at 25 ± 2°C under 18:6 hr light and dark period. Experiments were repeated at least twice to confirm the results.

Results and Discussions

Explants did not show callusing or rooting in the absence of growth regulators on M S medium. Callus was initiated from root, hypocotyl, cotyledon, stem and leaf segments within 14-25 days on the medium supplemented with different growth regulators at various concentrations (Table 1). Maximum callus growth was recorded on modified M S medium containing 2,4-D (0.2 mg/l) + K (0.2 mg/l). Auxins like IAA,



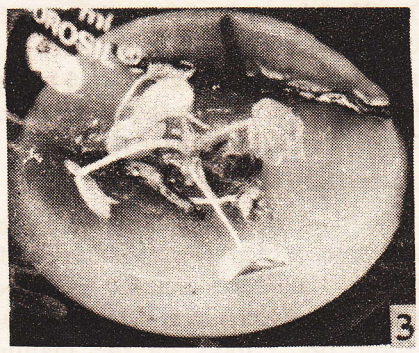


Fig. 1 Callus formation from petiole in *Gossypium hirsutum* Var.L.H.900;

Fig. 2. Differentiation of embryoid like structures in *Gossypium hirsutum* Var.L.H.900;

Fig. 3 Multiple leafy shoots from nodal explant in *Gossypium hirsutum* Var.L.H.900

NAA, IBA and 2,4-D alone were less favourable for callus initiation and its growth.

Callus was white/pale yellow, friable and nodulated and subcultured once in 20 days for maintenance on fresh medium containing 2,4-D (0.2 mg/l) + K (0.2 mg/l) (Fig.1). Explants cultured on medium containing cytokinins like BAP and kinetin showed relatively poor response in terms of callus initiation. The calli derived from different explants when subcultured on the medium supplemented with BAP (1-5mg/l) and NAA (0.5-2mg/l), the calli growth was resumed after 2-3 days and it became nodulated, green, hard and meristimetic nests were formed.

Embryoid like structures were differentiated when petiole derived callus on Modified MS + NAA (4mg/l) + K (1mg/l) and modified MS + 2,4-D(0.1mg/l) + K (0.1 mg/l) was subcultured on modified M S medium without any growth regulator (Fig.2).

Nodal segments of about 0.5-3 cm excised from 1 month old field grown plants showed varied responses to different growth regulators (Table 2). Regeneration of multiple leafy shoots took place on modified MS medium alone (Fig.3). On subculturing on modified MS + BAP (3.0mg/l) + activiated charcoal (0.3%), multiple leafy shoots increased in number but subsequently they began to die. On further subculturing on modified MS + BAP (5mg/l) multiple leafy shoots again resumed their growth and turned greenish again. Rooting was obtained when

Table 1. Response of auxins, cytokinins on callusing of different explants of *Gossypium hirsutum* variety L.H.900

Medium/growth regulators (mg/l)	Hypocotyl	Cotyledon	Stem	Leaf	Petiole
MS	NR	NR	NR	NR	NR
MS+IAA (1,2,5)	C++	C+	C+	C++	C++
MS+NAA (1,2,5)	C++	C+	C+	C++	C++
MS+IBA (1,2,5)	C+	C++	C++	C+	C+
MS+2,4-D(1,2,5)	C+++	C++	C+	C+	C+++
MS+K(0.2,0.04)	R+	R+	NR	NR	NR
MS+BAP(2,4,5)	C+++	C+	C+	C++	C+++
MS+NAA(0.7)+BAP(2.0)	GC++	GC++	GC+	GC++	GC++
MS+2,4-D(0.2)+K(0.2)	C++++	C+	C+	C+	C++++
MS+NAA(0.3)+K(1.5)	C++R+	NR	NR	NR	C+R+
MS+NAA(0.5)+K(0.5)	C++	C++	C+	NR	C++

Extent of callus growth: NR - No Response; C+ Callus very poor; C++ Poor; C+++ Good; C++++ Excellent; GC Green Callus

Table 2. Effect of growth regulators on nodal explants of mature plants (1 month old). (Sources Field grown plants)

Media + growth regulator (mg/l)	No. of nodal segments cultured	No.of nodal segments responded	Percent Response	Remarks
MS	20	15	75.00	Swelling
MS+BAP(0.1)	40	25	62.5	Slight callusing
MS+BAP(0.2)	40	20	50.0	Slow growth+callusing
MS+BAP(0.3)	40	18	45.0	Slight Callusing
MS+BAP(1.0)	40	15	37.5	Callusing
MS+BAP(3.0)	40	13	32.5	Callusing
MS+K(0.5)	40	14	35.00	NO Response
MS+K(0.1)	40	10	25.0	Callusing
MS+BAP(3.0)+ Activated charcoal	40	25	62.5	Multiple leafy shoots
MS+no hormone	40	30	75.00	Multiple leafy shoots
MS+activated charcoal (0.3%)	40	15	37.5	Multiple leafy shoots
MS+2,4-D(3)+BAP(1)	40	21	32.5	Callusing

multiple leafy shoots cultured on hormone free medium for 1 to 2 weeks were subcultured on the medium containing (0.3%) activated charcoal 2 to 3 weeks alternating with charcoal free medium until roots formed spontaneously. Hormone treatments tested to induce rooting in culture resulted in tissue mortality.Regeneration of *Gossypium hirsutum* and *Gossypium barbadense* from shoot apex has already been achieved[7].

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