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INITIATION OF CALLUS AND PLANT REGENERATION IN COTTON (*Gossypium hirsutum L.*)

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ABSTRACT

The hypocotyl explants of MDU 9 cultured on Murashige and Skoog medium supplemented with kinetin (1.0 mg/lit.) + IAA (1.5 mg/lit.) formed callus in 20 days. The calli on sub culture to media containing benzyl amino purine (2.0 mg/lit.) developed shoots. Multiple shoots were formed from each callus mass. These on further sub culture with reduced level of benzyl aminopurine developed well. Root initiation and growth was promoted by lowering the sucrose concentration and supplementing the culture media with NAA (3.0 mg/lit.).

A basic pre-requisite for the application of cell culture techniques to crop improvement is the ability to regenerate from callus. Plant regeneration can be achieved through organogenesis or through somatic embryogenesis. *Gossypium* germplasm contains much genotypic variation for callus initiation, proliferation, morphology and regeneration capacity (Shoemaker *et al.*, 1986). In cotton, plant regeneration has been achieved in specific callus lines in an unusually long time of 2 years (Davidonis and Hamilton, 1983). Somatic embryoids were differentiated from hypocotyl tissue of *G. klotzschianum* (Price and Smith, 1979) after 3-4 weeks of culture in liquid medium containing glutamine. Embryogenesis from stem and petiole section in *G.klotzschianum* (Finer and Smith, 1984) and regenerations from leaf discs are reported (Gawel *et al.*, 1986). The present study reports the induction of callus from hypocotyl tissue and regeneration of cotton plants.

MATERIALS AND METHODS

Seedlings of MCU 9 were aseptically grown in 1/2 strength Murashige and Skoog medium in darkness at 30° C for 7 days and then transferred to a culture room at a temperature of 25+2°C and a relative humidity of 65-70% and light intensity of 2000 lux and subjected to a light and dark cycle of 14/10 hours a day. Hypocotyl explants from 10-14 day old seedlings were cultured on MS medium supplemented with (1)kinetin (1.0 mg/lit) +IAA (1.5 mg/lit.), (2) NAA (1.0 mg/lit.) + kinetin (1.0 mg lit.) + adenine (40 mg/lit.) (Rani and Bhojwani, 1976), (3) IAA (2.0 mg/lit.) + kinetin (1.0 mg/lit.) and (4) LS medium supplemented with NAA (2.0 mg/lit.) + kinetin (1.0 mg/lit.) (Davidonis and Hamilton, 1983). Glucose 3 per cent was used instead of sucrose in the above media. Callus initiated in 20 days. Calli proliferated in another 15 days. These were transferred to MS + kinetin (1.0 mg/lit.) +IAA (3.0 mg/lit.) + glucose (3%). Transfers were made at 7 day intervals for 3-4 transfers.

After this series of transfers, calli were placed on to media containing sucrose 3 per cent instead of glucose. Calli were observed weekly for embryogenic potential.

Embryogenic calli on sub culture to MS medium with BAP(2.0 mg/lit.)produced multiple shoots. Further growth of the multiple shoots were enhanced by lowering the concentration of BAP. The multiple shoots were then separated and subcultured to media supplemented with NAA (3.0 mg/lit.) and reduced level of sucrose (1.5%). High root differentiation was noted. The plantlets were than transferred to mist chamber for hardening and then to glass house.

RESULTS AND DISCUSSION

Media 1 was better for callus induction (Table 1). The texture of the calli ranged from very hard compact to watery and friable. Browning was observed in all cases. Root proliferation was also observed. The results are in line with the findings of Shoemaker *et al.* (1986). Embryogenic callus was first observed as small sectors of a pale grey, compact callus emerging from a soft yellowish to brown friable callus. When subcultured to MS +BAP (2.0 mg/lit.). the embryogenic callus produced shoots. Less than 40 per cent of the embryogenic calli produced multiple shoots (Table 1).

Table 1. Callus induction and regenetation percentage in different media.

	Med.1	Med.2	Med.3	Med.4
No. of days for C.I.	20	24	27	30
No. of days for C.P. from the day of callus induction	15	20	23	27
C.I. %	60	55	57	51
Multiple shoot initiation %	37	30	28	27
Regeneration %	36	29	25	23

C.I. : Callus initiation
C.P. : Callus proliferation
Med.1 : MS + kinetin (1.0 mg/l) + IAA (1.5 mg/l)
Med.2 : MS + NAA (1.0 mg/l) + kinetin (1.0 mg/l) + adenine (40.0 mg/l)
Med.3 : MS + IAA (2.0 mg/l) + kinetin (1.0 mg/l)
Med.4 : LS + NAA (2.0 mg/l) + kinetin (1.0 mg/l)

Plant regeneration from callus derived somatic embryoids has been previously reported from cotton c.v. Coker -30 (Davidonis and Hamilton, 1983). However, these were reported to have developed only after two years in culture. In contrast, the regeneration reported by Shoemaker *et al.* (1986) is simple straight forward and rapid and conducive to most cotton improvement programmes, requiring plant regeneration from tissue culture. The regeneration of cotton plant form callus reported here is also very simple. Embryogenic calli can be obtained within 4- 6 weeks of the initial explant and can be found directly on MS medium with kinetin (1.0 mg/lit.) + IAA (1.5 mg/lit.) and regeneration of multiple shoots can be obtained within 6-8 weeks on MS medium with BAP (2.0 mg/lit.) and rooting of these shoots could be obtained within four weeks on MS + NAA (3.0 mg/lit.) + sucrose (1.5%).

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ALLELOPATHIC IMPACT OF COLOCASIA ON CROP PLANTS

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ABSTRACT

The *in vivo* allelopathic effect of above ground portion of colocasia extract was assessed on germination, TDMP, VI and growth of rice, maize, ragi, cumbu, green gram, black gram, red gram, sesamum, sunflower, groundnut, sorghum and cotton at 10 and 30 DAS. The colocasia treatment drastically and significantly reduced, the germination, DMP and VI of rice, ragi, cumbu, black gram and goundnut. However, the other crops could resist the adverse allelopathic effect caused by colocasia, and higher DMP and VI recorded in the rest of the crops might be reflected in higher crop productivity. The adverse effect was possibly due to the leaching out of few allelochemicals which hamper the crop growth and performance.

Information on the allelopathic nature of many plant species have accumulated over the years as interactions between plants in an ecosystem become more thoroughly studied. Under field conditions the deleterious effect of an allelopathic plant agent upon another plant's growth and yield may be facilitated by exudates (Rice, 1974), leachates from decomposing residues and residues incorporated in the growing medium (Garcia and Anderson, 1984). Though the information on the allelopathic effects of many weed species is available, relatively less has been done on the allelopathic nature of some annual crops, in the tropics.

Patterson (1981) pointed out that allelopathic substances are released into the soil during decomposition of crop residues. In taro *Colocasia esculenta*, it has been observed by Rice (1974) in a field continuously planted to the crop succeeding