J. Phytol. Res. 9 (2): 145-147, 1996

# CALLUS INDUCTION, SOMATIC EMBRYOID FORMATION AND PLANT REGENERATION IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

**G. RAJASEGAR*, S.R. SREE RANGASAMY, P. VENKATACHALAM** and G.R. RAO**
Department of Plant Science, School of Life Sciences,
Bharathidasan University, Tiruchirappalli-620 024, India.
*Department of Botany, National University of Singapore, Singapore-119260.
**Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, India.

High frequency of callus induction and somatic embryogenesis was observed on MS medium containing various concentrations and combinations of different growth regulators. Among the various explants, young leaf was found to be best for maximum frequency of callus induction on MS medim fortified with NAA (2.0 mg/1) in combination with 2 iP (3.0 mg/1). These calli developed embryoids on MS medium containing 2 iP and ABA (2.0 mg/1 each) with 10 mM glutamine. Embryoids formed here developed into plantlets and plant regeneration frequency was low.

**Keywords:** Callus culture; Cotton; Explants; Hormones; Plant regeneration; Somatic embryogenesis.

## Introduction

Tissue culture of cotton has been notoriously difficult[1]. With the first report of regeneration of cotton plants[2], progress in plant regeneration has not been achieved rapidly, because of the lengthy culture period[3]. The plant regeneration was achieved from hypocotyl explants of American Coker 201 and 315 cultivars. Recently, various types of embryoids were obtained and developed into fertile plants[4]. The objective of this investigation was to devise a tissue culture method for plant regeneration via somatic embryogenesis to our Indian cultivar MCU-7.

## Materials and methods

Delinted seeds were surface sterilized with 0.1% (W/V) mercuric chloride for 5 min and germinated on MS[5] basal medium under dark. Different explants viz., hypocotyl, cotyledon young leaf and immature embryo were used for callus initiation. The MS medium supplemented with two auxins (2, 4-D and NAA) along with three different cytokinins (Kin, 2 iP and BAP) were used.

Cut pieces of explants were placed in 25 x 150 mm culture tubes with 20 ml of the medium containing respective growth regulator combinations and incubated at 25±2 C with 16 hrs photoperiod in cool-white fluorescent light (1500 lux). Callusing percentage was calculated after 20 days. MS medium supplemented with different levels of auxins and cytokinins and other organic supplements like ABA and glutamine were used for regeneration study.

## Results and Discussion

*Callus induction and proliferation:* In the present study, different explants were used for callus initiation. NAA (2.0 mg/1) along with 2 iP (3.0 mg/1) was found to be the best combination for callus induction (Fig 1). However, the combination and dosage varied with explants.

The leaves had maximum callus induction in NAA (2.0 mg/1) along with 2 iP (3.0 mg/1). In hypocotyl, NAA (2.0 mg/1) in combination with Kin (0.5 mg/1) induced the maximum frequency of callus. The highest frequency of callus induction was observed on MS medium containing 2, 4-D and Kin

**Table 1.** Optimum dosage of auxin and cytokinin for maximum frequency of callus induction from different explants of cotton.

| Growth Hormones | EXPLANTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Hypocotyl | | Cotyledon | | Young leaf | | Immature embryo | |
| 2,4-D : Kin | 0.1 | 0.5 (75.3) | 0.1 | 0.1 (83.3) | 0.5 | 0.5 (80.3) | 0.1 | 1.0 (60.0) |
| 2,4-D : 2 iP | 0.1 | 2.0 (69.7) | 0.1 | 3.0 (75.3) | 0.5 | 2.0 (71.7) | 0.1 | 1.0 (62.0) |
| 2,4-D : BAP | 0.1 | 1.5 (65.3) | 0.1 | 1.5 (54.3) | 0.3 | 2.0 (66.0) | 0.1 | 2.0 (46.7) |
| NAA : Kin | 2.0 | 0.5 (82.7 | 1.0 | 0.5 (72.7) | 2.0 | 0.5 (78.0) | 2.0 | 0.5 (63.0) |
| NAA : 2 iP | 1.0 | 2.0 (71.7) | 1.5 | 2.0 (75.3) | 2.0 | 3.0 (85.7) | 0.5 | 2.0 (55.3) |
| NAA : BAP | 1.0 | 1.5 (52.7) | 1.5 | 1.0 (54.7) | 2.0 | 2.0 (68.7) | 1.0 | 1.0 (38.7) |

Figures in parenthesis are indicated percent of callus induction

(0.1 mg/1 each) for cotyledon explant whereas 2,4-D (0.1 mg/1) and Kin (1.0 mg/1) combination was found to be best for maximum frequency of callus induction for immature embryos.

The differential response of the explants towards callus induction reveals the influence of explants, growth regulators and their concentrations. In the present study, both auxins had a similar effect on callus induction. However, the two auxins had individual effect when used along with different cytokinins. This apparant difference in callus induction with different explants might be due to the presence of endogenous auxin.

When 2, 4-D was used as auxin along with 3 different cytokinins for callus induction, maximum frequency of callus induction was in cotyledon (83.3%) with 2, 4-D and BAP (0.3 and 2.0 mg/1, respectively). A dose of 2, 4-D and 2 iP (0.1 and 3.0 mg/1, respectively) was also the best combination for cotyledon (Table 1).

In the differential performance of 2, 4-D no callus induction was observed using cotyledon explant[1]. This contradicts to the present results where maximum callus induction (83.3%) with cotyledon was recorded with 2, 4-D as auxin (Table 1).

When NAA was used as auxin along with 3 different cytokinins, NAA with 2 iP combination induced highest frequency of callus induction (85.7%) at (2.0 and 3.0 mg/1 respectively) for leaf tissue, whereas in hypocotyl, NAA (2.0 mg/1) and Kin (0.5mg/1) combination found to be best for maximum frequency of callus induction. NAA and BAP (2.0 mg/1 each) combination was also found to be better for maximum frequency of callusing in young leaves. Trolinder and Xhixian[6] also reported that the NAA and 2 iP combination found to be the best combination for callusing in cotton.

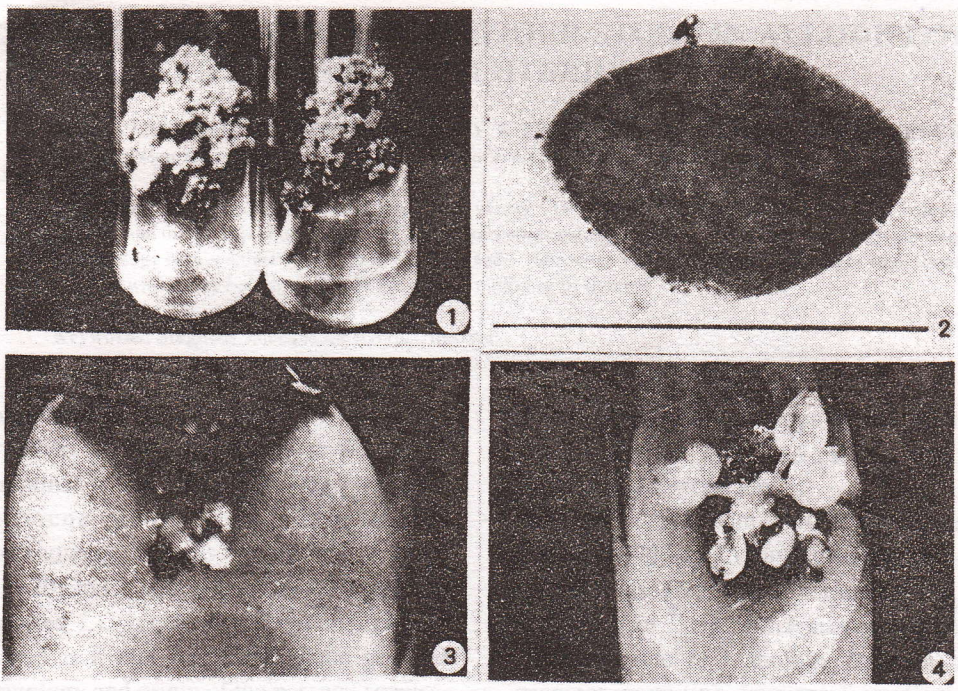

Fig. 1-4 : Efficient callus induction; (2) Heart shaped embryoid; (3) Plantlet development; (4) Well developed plantlet.

*Regeneration:* In the present study, MS medium supplemented with 2 iP and, ABA (1, 2 and 3 mg/1 each), glutamine (5 to 20 mM) along with organic additives were tried (Data not shown). Addition of ABA and 2 iP (2.0 mg/1 each) with 10 mM glutamine resulted in the embryoid formation (Fig 2). MS medium supplemented with ABA (5 µm) produced six types of somatic embryos in cotton[4].

Increased concentration of $KNO_3$ and addition of $MgCl_2$ was not found to be significant for embryoid formation. High frequency of somatic embryos was obtained with the addition of 2x. $KNO_3$ and $MgCl_2$[1,3,7,8].

*Plant recovery:* In the present study, embryos were transferred to MS medium with the elemination $NH_4NO_3$ but with 2x $KNO_3$ and $MgCl_2$ (750mg/1), solidified in 2% (W/V) gelrite. Embryoids developed into leaf structures and developed into small plantlets (Fig. 3 &4). Cousin *et al.*[9] used the same medium for germinating Australian cultivar siokera 1-3 with less frequency.

## References


1. Trolinder N L and Goodin J A 1988, *Plant Cell Tissue and Organ Culture* **12** 31
2. Davidonis G H and Hamilton R H 1983, *Plant Science Letter* **32** 89
3. Shoemaker R C, Couche L J and Galbranth D W 1986, *Plant Cell Reports* **5** 178
4. Voo K S, Rugh C L and Kamalay J C 1991, *In vitro Cellular Developmental Biology* **27** 117
5. Murashige T and Skoog F 1962, *Physiologia Plantarum* **15** 473
6. Trolinder N L and Xhixian C 1989, *Plant Cell Reports* **8** 133
7. Gawel N J, Rao A P and Robacher C D 1986, *Plant Cell Reports* **5** 201
8. Troinder N L and Goodin J A 1988, *Plant Cell Tissue and Organ Culture* **12** 43
9. Cousins Y L, Lyon B R and Llewellyn 1991, *Australian Journal of Plant Physiology* **18** 481