Multiple Shoot Regeneration Response of Recalcitrant Cotton 
(Gossypium hirsutum L.) Cultivar CIM-443

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Abstract: Induction of multiple shoots in Gossypium hirsutum L. variety CIM-443 has been achieved by using
meristem and cotyledonal node as explants. Meristems of size 3-4 mm were excised from embryos isolated from
seeds while cotyledonal nodes were cut from seven days old seedling and the mean shoot number per explant
response for total of three experiments was maximum i.e., (6.72±0.79) and (4.92±0.67), respectively for both
explants in MS medium supplemented with benzy1-amino purine (BAP) 1.0 µg L⁻¹ + naphthalene acetic acid
(NAA) 0.05 mg L⁻¹. Shoot elongation was observed in MS medium amended with NAA 0.1 mg L⁻¹. The
percentage of shoots forming roots was maximum (79.16) in case of ½ MS supplemented with NAA 0.05 mg L⁻¹.
Rooted plantlets hardened in soil and normal boll formation observed.

Key words: Gossypium hirsutum Cv. CIM-443, micro-propagation, multiple shoot regeneration

INTRODUCTION

Cotton is the world’s most important fiber crop and most valuable oil seed crop. The crop is damaged by
many insects and pests thus making it an important candidate for genetic manipulation. In vitro regeneration
is necessary prerequisite for genetic manipulations but in cotton limited to only a few cultivars possibly due to fact
that tissue culture responses were genotype dependent.[9]. Cotton plants have proved to be difficult to manipulate in
tissue culture[9].

Many species of Gossypium hirsutum (L) grow into complete plants in simple MS basal medium by using
different explants especially embryos and meristematic nodes[14]. But for shoot multiplication purpose hormonal
manipulations are considered very critical[24]. Meristematic shoot tip and bud cultures derived plants are
phenotypically homogenous there by indicating genetic stability[25]. In this context there in need to develop a micropropagation protocol resulting in very little gross variability among regenerated plants. This paper reports a micropropagation protocol to produce genetically stable plants of high yielding and virus resistant Gossypium hirsutum variety CIM-443.

MATERIALS AND METHODS

Sterilization and explant preparation: Seeds of Gossypium hirsutum cultivar CIM-443 obtained from
Central Cotton Research Institute, Multan. These seeds were delinted with concentrated sulphuric acid @ 10.0 ml
for 100.0 g of seeds and subjected to five washings with distilled water containing few drops of tween-20. Under
aseptic conditions these seeds were surface sterilized with 0.1% mercuric chloride +0.1% SDS (sodium dodecyl
sulphate). The seeds were rinsed 5 times with autoclaved distilled water and incubated on soaked filter paper in
petriplate. All the processes were carried out under strict aseptic conditions and then seeds were incubated in dark
for 72 h at 37°C.

Mature embryos were isolated from incubated seeds after the given time interval and 3-4 mm epicotyl
(meristematic) portion was excised. These missetmens were subjected to designed media with the base in the medium
under aseptic conditions for induction of multiple shoots. Cotyledonal nodes of size 2-3 mm were detached from
seedlings that were grown for one week on MS basal medium[8] supplemented with B5 vitamins, glucose, 3.0%;
MgCl2, 0.075%; myo-inositol 0.01%; phytigel, 0.3% with out any amendment of phytohormones. After excision,
cotyledonal nodal explants were cultured on designated media with hypocotyl portion in the medium for induction
of multiple shoots.

Media for in vitro micropropagation and hardening of plants: Explants were subjected to media designated as
MSb, amended with B5 vitamins along variable concentrations of growth regulators in different combinations including: MSb, devoid of any growth
regulator; MS\(_{2}\) containing sole BAP (1.0-2.0 mg L\(^{-1}\)); MS\(_{3}\), supplemented with BAP (1.0-1.5 mg L\(^{-1}\)) and NAA (0.05-0.1 mg L\(^{-1}\)). Apical meristem and axillary node explants were cultured on these media compositions for induction of multiple shoots. In the multiplication stage, each explant expanded and micro shoots were produced from a basal mass. Percentage explant response was calculated by formula:

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\text{Total number of explants representing multiple shoot regeneration} = \frac{\text{Explant response (\%)} \times 100}{\text{Total number of explants cultured}}
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The word multiple shoot regeneration was used when the explant regenerated more than two shoots. Micro shoots of 2-3 cm size were cut from the explant and cultured on MS basal medium amended with giberellin acid (GA) 0.1 mg L\(^{-1}\) for rooting. Micro shoots when attained suitable length 5-6 cm in culture vessel then transferred to half strength MS basal medium amended with NAA (0.05-0.10 mg L\(^{-1}\)) for rooting. After three weeks rooted shoots were removed from the medium and washed thoroughly the root portion to remove the potential source of contamination before transferring to pot soil containing soil, sand and peat moss 1:1:1 ratio. Plants were covered with polythene bags and placed under green house conditions with 16 h photoperiod and 30±2°C temperature. Plants were uncovered on weekly basis till these were become compatible to natural environment.

**RESULTS**

Meristem and cotyledonary node explants were subjected to various tissue culture media designed as MS\(_{2,11}\) (Table 1). MS\(_{2}\) medium is devoid of any plant phytohormone and served as control for other media containing different concentrations of cytokinins (BAP, kinetin) and auxins (NAA). MS basal medium did not support the induction of multiple shoots however response of both explants was found to be 100% (Table 1). In the MS basal medium as the concentration of sole BAP gradually increased from 1.0-2.0 mg L\(^{-1}\) in media (MS\(_{1}\)) then rate of multiple shoot response was also increased and found to be maximum i.e., (2.75 shoots per explant) for 2.0 mg L\(^{-1}\) concentration of BAP in case of meristem while (3.08 shoots per explant) for 1.5 mg L\(^{-1}\) concentration of BAP when cotyledonary node was used as explant (Table 1). Multiple shoot response was further increased for both explants when kinetin was amended along BAP in media (MS\(_{S,1}\)) (Table 1) in balance concentration from 1.0-2.0 mg L\(^{-1}\). Maximum shoot multiplication response (3.87 shoots per meristem explant) (Fig. 1A) was induced by 1.5 mg L\(^{-1}\) concentration of both cytokinin however this was further increased to 4.09 shoots per cotyledonary explant (Fig. 1B) when both cytokinins were added in 1.0 mg L\(^{-1}\) concentration. At low concentration of both cytokinins BAP (1.0 mg L\(^{-1}\)) and kinetin (0.5 mg L\(^{-1}\)) in case of MS, medium, multiple shoot response for meristem and cotyledonary node explant was found to be 3.32 and 3.88%, respectively (Fig. 1A). Explant response in MS, medium was 100% for both explants however this explant response was declined to 60 and 76% for meristem and cotyledonary node explants when both cytokinins in MS, medium were used in high concentration i.e 2.0 mg L\(^{-1}\).

Among all the tested media maximum shoot multiplication response (6.72 and 4.92 per meristem and cotyledonary explant, respectively) was observed in MS\(_{2}\) medium where combination of BAP (1.0 mg L\(^{-1}\)) and NAA (0.05 mg L\(^{-1}\)) was used (Table 1). In this medium both explants response was 100%. No further increase in multiple shoot response per explant and percentage explant response could be observed with an increase in concentrations of BAP and NAA in media (MS, and MS\(_{3}\)) (Table 1). The multiple shoots obtained on various shoot multiplication media were transferred to shoot elongation medium containing MS basal medium supplemented with giberellin acid (0.1 mg L\(^{-1}\)) (Fig. 1B).

The micro shoots of 4-5 cm length were then transferred to different rooting media containing different concentrations of auxins and half strength MS basal medium (½ MS) (Table 2, Fig. 1C). ½ MS supplemented...
with NAA (0.05 mg L$^{-1}$) showed maximum rooting (79.16%) however further increase in concentration of NAA (1.0 mg L$^{-1}$) decreased the rooting (45.83%) as well as number of rooting shoots from 31.66 to 18.33 (Table 2). MS alone resulted in 70.83% rooting and number of shoots rooted on this medium were found to be 28.33 (Table 2).

Rooted shoots were transferred to pots containing soil, sand and peat moss (1:1:1) under greenhouse condition. Survival rate of plants in soil was 80% after two months. The survived plants were successfully established in soil (Fig. 1D) and normal boll formation was observed (Fig. 1E) in all the established plants.
DISCUSSION

The results of present study describe a simple micropropagation procedure of recalcitrant cotton (*Gossypium hirsutum* L.) cultivar CIM-443 which despite of high yielding is also virus resistant. Cotyledonal nodes and meristem explants were subjected to various tissue culture media amended with different concentrations of cytokinins and auxins. Hormonal amendments are necessary because developmental capacity of meristem is due to subjacent leaf and stem tissues while for cotyledonal node growth reduction in attributed to detached apical meristem and cotyledons. It is therefore unlikely that the meristem and cotyledonal node explants have sufficient cytokinin to support growth and development with out hormonal amendment in culture media. The significant role of phytohormones was also documented by other workers in regeneration studies of various species of cotton including *Gossypium herbaceum*[^9^], *G. hirsutum*[^10^]. Cytokinins played a predominant role in multiple shoot regeneration. The effect is even more pronounced when two types of cytokinins (BAP and kinetin) were used in combination. Thus synergistic effect of two cytokinins leads to enhanced shoot regeneration as reported by Jayasree *et al.*[^11^] in potato. However, correct concentration of combination of growth regulators is necessary because unfavourable concentration may inhibit the growth of cellular mass as reported by Moore[^12^].

Multiple shoot regeneration response for both meristem and cotyledonal node explants was increased tremendously when BAP (1.0 mg L⁻¹) was used along NAA (0.05 mg L⁻¹). However, further increase in concentration of cytokinin and auxin resulted in no any further increase in shoot multiplication response from 6.72 shoots per meristem explant and 4.92 shoots per cotyledonal node explant. This is possibly due to the reason that normal development of somatic tissue required a fine temporal and spatial regulation of cell division, enlargement and differentiation that could be achieved by correct concentration of cytokinin to auxin as also reported by Ammiurato[^13^]. High multiple shoot regeneration response is also due to the reason that supplementation of cytokinins during the histodifferentiation phase can be compensated for the detrimental effects of auxins on the meristematic tissue development. This observation was in agreement with Merkle[^14^] who proposed the same fact in Magnoliaceae.

In conclusion present study developed a simple and efficient condition for micropropagation that may results in production of genetically stable plants by using meristems and cotyledonal node as explants. As establishment of definite regeneration condition is necessary requisitie for genetic improvement of plants therefore this procedure may be helpful for genetic improvement of *Gossypium hirsutum* cultivar CIM-443 with very low gross abnormalities.

REFERENCES


