INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2004/06–4–704–707
http://www.ijab.org

In Vitro Regeneration and Multiple Shoot Induction in Upland Cotton (*Gossypium hirsutum* L.)

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ABSTRACT

Multiple shoot induction was studied in upland cotton. Cotyledonary nodes obtained from aseptically raised seedling were cultured on modified Murashige and Skoog media supplemented with different doses of Kinetin. Cotyledonary nodes produced maximum number of shoots (3.43 shoots/explant) when cultured on MS medium supplemented with 0.25 mgL^{-1} Kinetin. Highest percentage (93.3 %) of root development and root length (5.85 cm) was obtained when shoots were cultured on MS media supplemented with 0.5 mgL^{-1} NAA and 0.1 mgL^{-1} Kinetin.

Key Words: Cotton; Cotyledonary nodes; Caulinar apex; Multiple shoots

INTRODUCTION

Cotton is an excellent source of textile fiber and is cultivated in many countries. Because of its high economic value considerable attention has been paid to improve cotton plant by conventional plant breeding methods. However genetic improvement of cotton through conventional means is limited due to many factors like absence of necessary variation, especially against the pests and diseases causing major threats to cotton plant. Plant Tissue Culture provides an alternative mean of improvement. *In vitro* culture can also be utilized for cotton genetic and physiological development and improvement but it requires the availability of effective regeneration system through somatic embryogenesis which is quite difficult in cotton. Cotton regeneration was first observed in *Gossypium hirsutum* cv. Coker 310 (Davidonis & Hamilton, 1983) since then major work has been carried out for the development of protocol for an efficient regeneration system in cotton. Several scientists have successfully produced somatic embryoids and multiple shoots using various methods and media from somatic tissues of cotton plants (Shoemaker *et al*. 1986; Chen *et al*., 1987; Trolinder & Goudin, 1987; Zhang & Wang, 1989; Voo *et al*., 1991; Kolganova *et al*., 1992). Although efficiency of cotton regeneration have been significantly improved but some difficulties still remains. Regeneration in cotton is limited to few cotton cultivars (Trolinder & Xhixian, 1989). In order to use different techniques of biotechnology, broad range of genotypes must be responsive to the regeneration. The purpose of this research was to study the effect of various concentrations of kinetin on multiple shoot induction.

MATERIALS AND METHODS

The present research was conducted in Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad-Pakistan.

Seed material. Seeds of Cotton Cultivar NIAB-999 were obtained from Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad.

Preparation of plant material. Seeds were delinted using H_2SO_4 @ 15 ml per 100 g of seeds. Seeds were disinfected with 0.1% $HgCl_2$ for 20 minutes followed by 70% ethanol for 10 minutes. Sterilized seeds were rinsed with double distilled water for 2-3 times and cultured on MS media to raise seedlings in aseptic condition at 25 ± 2 °C for 72 h.

Explants comprised. Shoot apex along with cotyledonary nodes; along with 0.5 cm hypocotyl with out cotyledons, with both cotyledons and with single cotyledon attached.

Media composition. Seedlings were grown on MS basal salts (Murashige & Skoog, 1962). Explants were cultured on MS macro and micro salts, vitamins of B5 medium (Gamborg *et al.*, 1968), glucose 30%, solidified on Phytagel 1.4 gmL^{-1}. The medium pH was adjusted to 5.8 before autoclaving. Effects of various growth regulators on shoot induction were observed. Explants were embedded in 250 ml glass bottles containing 50 ml MS medium supplemented with following doses of Kinetin: K1= MS media (Control); K2 = MS+0.10 mg L^{-1} kinetin; K3 = MS+0.25 mg L^{-1} kinetin; K4 = MS+0.50 mg L^{-1} kinetin; K5. MS+1.00 mg L^{-1} kinetin

Rooting of shoots. Elongated shoots (4-5 cm) were excised and cultured on MS basal media with following concentrations of growth hormones.

T1 = MS media (control); T2 = MS+0.5 mg L^{-1} NAA (Napthaleneacteic Acid); T3 = MS+0.1 mg L^{-1} Kinetin; T4 = MS+0.5 mg L^{-1} NAA +0.1 mg L^{-1} Kinetin

Hardening of plants. Rooted shoots were taken out, washed with tap water, planted in pots having sterilized sand. Half strength MS medium was applied to moisten sand and covered with polythene bags. Pots were placed under 2500 lux light at 16 h photoperiod for one weak. After 3-4 days holes were made in polythene bag to gradually expose them to external environment. After 10 days they were transferred to larger pots containing 50% sand and 50% peat moss and transferred to green house.

RESULTS AND DISCUSSION

Disinfection of seeds through delinting with concentrated H_2SO_4 and then by 0.1 $HgCl_2$ for 20 minutes followed by 10 minutes with ethanol proved successful (Saeed *et al.,* 2004). Germination was observed after 48 h and within 3 days produced well developed root system with expanded cotyledon. The germination of cotton seeds on agar has also been observed by Shoemaker *et al.* (1986) and Zhang (1994).

Explants containing shoot apices along with both cotyledons attached and 0.5cm hypocotyls produced multiple shoots more efficiently (Fig. 1A). Explants containing shoot apex with single cotyledon were also able to produce multiple shoots but with relatively less efficiency (Fig. 1B). Non-regenerable green callus was observed in explant without cotyledon (Fig. 1C) which may have reduced its efficiency to produce multiple shoots. Relative efficiency of explant for regeneration has been reported by various scientists (Zhang *et al*. 2001, Sakhanokho *et al.* 2001) suggesting that type of explant greatly influence their ability to regenerate. Single shoots were observed in all treatments including control, indicating that single shoot may be obtained without any growth hormone. Induction of multiple shoots however is affected by the concentration of cytokinin. These results are in accordance with the finding of Jorge *et al.* (1998) who found that cytokinin is directly responsible for reprogramming the embryonic apical meristem axes of cotton towards the multiplication of buds.

Highest average multiple shoots developed on 0.25 mgL^{-1} kinetin (Table I). Further increase in shoot number was not observed with increasing concentrations of cytokinin, which showed that optimum level of growth hormone concentration is required for multiple shoot induction. Jorge *et al*. (1998) found that higher concentration of growth hormone yields fewer shoots. The effect of single growth hormone was studied by Hemphil *et al*. (1998) in which they observed best development of shoots on MS media containing 0.3 µM BA. Multiple shoots elongated within same media. Agarwal *et al*. (1997) obtained highest number of shoots by culturing cotyledonary nodes devoid of apical meristem in MS basal medium supplemented with 6-benzylaminopurine (BAP) and Kinetin 2.5 mg L^{-1} each. However, shoot could not elongate in same media. Elongation of shoots was observed in liquid media or in MS media solidified with agar. This could be due to the difference in the concentration of growth hormone and the genotype.

On an average shoots elongated up to 4-5 cm with in 30 days (Fig. 1D). Shoot thickness was similar to the primary shoots. This improved growth of shoots may be due to larger amount of medium (50 ml/ bottle). The positive influence of larger culture vessel has also been documented

Table I. Effect of cytokinin on shoot induction in cotyledonary nodes of cotton cultivar N-999 after 30 days of culture

Treatments+Medium	Phytohormone Kintein mgL^{-1}	Cotyledonary Node With Single Cotyledon Explant Shoots/ response explant (%) (Means ± SD)	With Both Cotyledon Explant Shoots/ response explant (%) (Means ± SD)	Without Cotyledon Explant Shoots/ response explant (%) (Means ± SD)
K1 MSB	0.00	80.00 1.00 ± 0.40	91.70 1.00 ± 0.40	61.00 0.90 ± 0.30
K2 MSB	0.10	100.00 1.33 ± 0.40	100.00 1.60 ± 0.40	100.00 1.90 ± 0.40
K3 MSB	0.25	100.00 2.61 ± 0.70	100.00 3.43 ± 0.32	91.17 2.21 ± 0.83
K4 MSB	0.50	85.00 2.07 ± 0.44	82.00 2.62 ± 0.66	71.23 1.94 ± 0.41
K5 MSB	1.00	60.00 1.60 ± 0.90	67.00 2.08 ± 0.49	43.00 1.53 ± 0.54

Table II. Effect of NAA and Kinetin on rooting of shoots after 30 days of culture

Treatments	Medium	Growth Hormone (mgL^{1})NAA Kinetin	Number of shoots Evaluated for rooting	Number of shoots rooted	Rooting(%)	Root length (cm ± SD)
T_1	MSB	0.00 0.00	30	21	70.00	3.85 ± 0.50
T_2	MSB	0.50 0.00	30	25	83.33	4.60 ± 0.54
T_3	MSB	0.00 0.10	30	23	76.66	4.02 ± 0.52
T_4	MSB	0.50 0.10	30	28	93.33	5.85 ± 0.93

1A. Induction of multiple shoots from cotyledonary node with both cotyledons

1B. Induction of multiple shoots from cotyledonary node with single cotyledon

1C. Multiple shoots from cotyledonary node without cotyledon with green callus at the base

1D. Excised shoot inoculated for rooting

1E. Rooted shoot

1F. Complete Plantlet of cotton Cultivar NIAB-999.

explant response was obtained when both cytokinin and auxin was used (Fig. 1F). These results find support from Saeed *et al.* (1997). They observed best development of roots on medium containing 2.68 mM NAA and 0.46 mM kinetin. Rooting length and percentage were highest with 0.5 NAA mgL^{-1} and 0.1 mgL^{-1} Kinetin (Table II). Presence of 0.5 mgL^{-1} NAA improved root length of the plantlet as compared to control and T_3 (Table II). Gupta *et al.* (1997) also observed rooting by culturing isolated shoots on MS basal salts supplemented with NAA. Explant response for rooting was higher than control in the presence of 0.1 mgL^{-1} kinetin, which showed that presence of low cytokinin enhances root formation (Table II). Overall shoot response was high toward rooting. This may be due to the use of kinetin as a growth hormone for multiple shoots induction (Table I). Effect of types of cytokinin used for *in vitro* shoot proliferation on the subsequent rooting of shoots was studied by Bennett *et al.* (1994) they found that shoots from the multiplication medium containing kinetin produced more roots and remained healthy for a longer period on the rooting medium as compared to shoots taken from multiplication medium containing BAP.

ACKNOWLEDGEMENT

We are grateful to Mr. Manzoor Ahmad Manj, SSO, Cotton Division, Nuclear Institute of Agriculture and Biology (NIAB), Jhang Road, Faisalabad-Pakistan for providing seed cotton of cultivar NIAB 999.

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(Received 10 April 2004; Accepted 20 June 2004)