Short Communication

Effect of kinetin on multiple shoot induction in cotton (*Gossypium hirsutum* L.) cv. NIAB-999

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Abstract

Multiple shoot induction was studied in upland cotton cv. NIAB-999. Cotyledonary nodes obtained from aseptically raised seedling were cultured on modified Murashige and Skoog medium (MS) 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 medium supplemented with 0.5 mgl^{-1} napthalene acetic acid (NAA) and 0.1 mgl^{-1} Kinetin.

Keywords: *Cotton; Cotyledonary nodes; Kinetin; Multiple shoots; Shoot tip.*

In vitro culture can be utilized for cotton genetic improvement but it requires presence of effective regeneration system which is highly genotypic specific in cotton (Trolinder and Xhixian, 1989). Cotton regeneration was first observed in *Gossypium hirsutum* cv. Coker 310 (Davidonis and 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 medium from somatic tissues of cotton plants (Shoemaker *et al*., 1986; Chen *et al*., 1987; Trolinder and Goudin, 1987; Zhang and Wang, 1989; Voo *et al*., 1991; Kolganova *et al*., 1992; Rauf and Rahman, 2005). Although efficiency of cotton regeneration has been improved but still there is a scope for improvement. The objective of the present study was to evaluate the effect of various concentrations of kinetin and explant type on multiple shoot induction and subsequent rooting in *Gossypium hirsutum* cv. NIAB-999.

The experiments were conducted in the Plant Tissue Culture Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Seeds were delinted with H_2SO_4 (15 ml H_2SO_4 for 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 medium to raise seedlings in aseptic conditions at 25 ± 2°C temperature. Three type of explants were used; Shoot apex with cotyledonary nodes; and 0.5 cm hypocotyl with out cotyledons, with both cotyledons and with single cotyledon attached. Seedlings were grown on MS basal salts (Murashige and Skoog, 1962). Explants were cultured on MSB medium prepared from MS macro and micro salts and vitamins concentration of B5 medium (Gamborg *et al.*, 1968), glucose 30%, solidified with 1.5 gl^{-1} Phytagel. 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 30 ml MSB medium supplemented with five doses (0.00, 0.10, 0.25, 0.50, 1.00 mgl^{-1}) of Kinetin.

For experiment on multiple shoot induction, 250 ml glass jars were used. There were 3 replicates of 15 jars per treatment accommodating single explant. Rooting experiment was carried in test tubes (18×150 mm)

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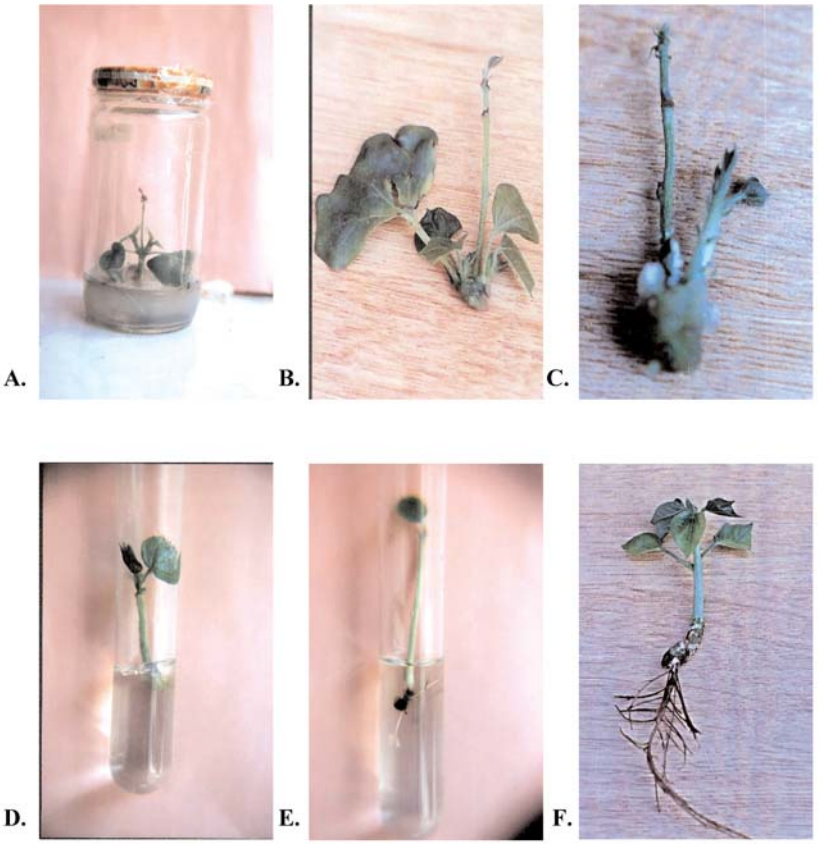
containing 10 ml medium. Elongated, 4-5 cm shoots were excised and cultured on MS basal medium with four concentrations of growth regulators; T1, MS medium (control); T2, MS + 0.5 mgl^{-1} NAA; T3, MS+ 0.1 mgl^{-1} Kinetin; and T4, MS+ 0.5 mgl^{-1} NAA + 0.1 mgl^{-1} kinetin. The medium was solidified with 1.5 gl^{-1} phytagel and pH was adjusted at 5.8 before autoclaving. The photoperiod was 16h and light intensity was 2500 lux. There were thirty tubes per treatment, each containing single explant. Rooted shoots were taken out, washed with tap water, planted in pots containing sterilized sand. Half strength MS medium was applied to moisten the sand and covered with polythene bag for hardening. Pots were placed under 2500 lux light intensity with 16h photoperiod for 10 days. 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 sand and peat moss (50:50) in the greenhouse.

Disinfection of seed 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. Within 3 days seedlings produced root system with expanded cotyledon. The germination of cotton seeds has also been obtained on agar (Shoemaker *et al*., 1986 and Zhang, 1994). Explants containing shoot apices along with both cotyledons attached and 0.5 cm 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). 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 and Sakhanokho *et al*., 2001) suggesting that the type of explant greatly influence their ability to regenerate. Single shoots were observed in all treatments

Figure 1. Multiple shoot induction in *Gossypium hirsutum* cv. NIAB-999.



A. Induction of multiple shoots from cotyledonary node with both cotyledons
B. Induction of multiple shoots from cotyledonary node with single cotyledon
C. Multiple shoots from cotyledonary node without cotyledon with green callus at the base.
D. Excised shoot inoculated for rooting
E. Rooted shoot
F. Complete Plantlet of cotton Cultivar NIAB-999.

Table 1. Effect of kinetin on shoot induction in cotyledonary nodes of cotton cultivar NIAB-999 after 30 days of culture.

Treatments+ Medium		Kinetin (mgl^{-1})	Cotyledonary Node					
			With Single Cotyledon		With Both Cotyledon		Without Cotyledon	
			Explant response (%)	Shoots/ explant (means±SE)	Explant response (%)	Shoots/ explant (means±SE)	Explant response (%)	Shoots/ explant (mean ±SE)
K1	MSB	0.00	80	1.0±0.4	91.7	1.0±0.4	61.0	0.9±0.3
K2	MSB	0.10	100	1.33±0.4	100	1.6±0.4	100	1.9±0.4
K3	MSB	0.25	100	2.61±0.7	100	3.43±0.32	91.17	2.21±0.83
K4	MSB	0.50	85	2.07±0.44	82	2.62±0.66	71.23	1.94±0.41
K5	MSB	1.00	60	1.60±0.9	67	2.08±0.49	43	1.53±0.54

Table 2. Effect of NAA and Kinetin on rooting of shoots after 30 days of culture.

Treatments	Medium	Growth regulator (mgl^{-1})		Number of shoots evaluated for rooting	Number of shoots rooted	Rooting (%)	Root Length cm±SE
		NAA	Kinetin				
T_1	MSB	0.00	0.00	30	21	70.00	3.85±0.50
T_2	MSB	0.5	0.00	30	25	83.33	4.60±0.54
T_3	MSB	0.0	0.10	30	23	76.66	4.023±0.52
T_4	MSB	0.5	0.10	30	28	93.33	5.85±0.93

including control, indicating that single shoot may be obtained without any growth regulator. Induction of multiple shoots was however affected by the concentration of cytokinin; Cytokinin is directly responsible for reprogramming apical meristem axes of cotton towards the multiplication of buds (Jorge *et al*., 1998).

Highest average multiple shoots developed on 0.25 mgl^{-1} kinetin (Table 1). Further increase in the number of shoot was not observed with increase in the concentration of cytokinin. Jorge *et al*. (1998) also observed fewer shoots with higher dose of growth regulators. Hemphil *et al*. (1998) observed best development of shoots on MS medium containing 0.3 µM BA. Multiple shoots elongated within the same medium. Similarly, Agarwal *et al*., (1997) obtained multiple shoots by culturing cotyledonary nodes devoid of apical meristem in MS basal medium supplemented with 2.5 mg/l each of 6-benzylaminopurine (BAP) and Kinetin. However they could not be able to elongate shoots within the same medium.

On an average, shoots elongated up to 4-5 cm with in 30 days (Fig. 1D). Shoots were thick similar to the primary shoot. This improved growth of shoots may be due to larger amount of medium 50 ml/ bottle. The positive influence of larger culture vessel is also well established (McClellend and Smith, 1990). Highest root length and explant response was obtained when both cytokinin and auxin were 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 2). Presence of 0.5 mgl^{-1} NAA improved root length of the plantlet as compared to the control and T_3 (Table 2). 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 the control in the presence of 0.1 mgl^{-1} kinetin, which showed that presence of low cytokinin enhances root formation (Table 2). Overall shoot response toward rooting was high, contrary to the earlier observation of Gould *et al*., (1991). This may be the effect of kinetin as a growth regulator for multiple shoots induction (Table 1). Effect of the type 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.

Established method for multiple shoot induction may be useful for genetic transformation using shoot apex in crop species like cotton where regeneration responses are highly genotypic specific. Moreover, multiple shoot induction can be used in combinations with the conventional breeding programmes for rapid multiplication of specific combinations e.g. inter-species hybrids where seed setting is low and large numbers of plants are required for field evaluation.

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