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

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Abstract

Cotyledonary nodes of Upland cotton (*Gossypium hirsutum* L.) produced maximum number of shoots (3.43 shoots/explant) when cultured on MS supplemented with 0.25 mg/l Kn. Highest percentage (93.3) of root development and root length (5.85 cm) was obtained when shoots were cultured on MS supplemented with 0.5 mg/l NAA and 0.1 mg/l Kn.

Introduction

Cotton is an excellent source of textile fiber and 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, specially resistance against pests and diseases. Plant tissue culture techniques provide an alternative means of improvement obtaining somaclones, induced variants, somatic hybridization and doubled haploids to develop inbred lines or for introducing genes of interest against insects and different diseases through genetic engineering (Zhang and Zhao 1997). However, successful application of *in vitro* methodologies is mainly dependent on a reliable and reproducible regeneration system like somatic embryogenesis which is quite difficult in cotton. 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 media from somatic tissues of cotton plants (Shoemaker et al. 1986; Chen et al. 1987; Trolinder and Goodin 1987; Zhang and Wang 1989; Voo et al. 1991;

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Kolganova et al. 1992; Peeters et al. 1994; Zhang et al. 1996, 1999). The regeneration protocols have been used to obtain genetically modified plants (Rajasekraran et al. 1996). Although efficiency of cotton regeneration has been significantly improved but some difficulties still remain like low efficiency of somatic embryogenesis have been reported (Zhang et al. 1993; Zhang and Zhao 1997). Regeneration in cotton is limited to a few cotton cultivars (Trolinder and Zhixian 1989). Further, long culture time and complicated protocols confine the application of biotechnology in cotton. 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 Kn on multiple shoot induction in a newly developed indigenous mutant NIAB-999 cultivar of cotton. Results of the present study will facilitate the application of plant tissue culture and plant biotechnology for the improvement of cotton.

Materials and Methods

The present research was conducted in Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Seeds of cotton cultivar NIAB-999, a mutant developed through physical mutagens, were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Seeds were delinted using H_2SO_4 @ 15 ml per 100 g of seeds. Seeds were disinfected with 0.1% $HgCl_2$ for 20 min followed by 70% ethanol for ten minutes. Sterilized seeds were rinsed with double distilled water for two - three times and cultured on MS to raise seedlings in aseptic condition at 25 ± 2°C for 72 hrs.

Shoot apex with cotyledonary nodes; 0.5 cm hypocotyl without cotyledons, with both cotyledons and single cotyledon attached were used. Seedlings were grown on MS basal salts. Explants were cultured on MS macro and micro salts, vitamins of B5 medium (Gamborg et al. 1968), 30% glucose, solidified on 1.4 g/l. pytagel. The pH of the medium 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 supplemented with following doses of Kn: K1. MS (control), K2. MS + 0.10 mg/l Kn, K3. MS + 0.25 mg/l Kn, K4. MS + 0.50 mg/l Kn and K5. MS + 1.0 mg/l Kn.

Elongated shoots (4 - 5 cm) were excised and cultured on MS basal media with following concentrations of growth hormones: T1. MS (control), T2. MS + 0.5 mg/l NAA, T3. MS + 0.1 mg/l Kn and T4. MS + 0.5 mg/l NAA + 0.1 mg/l Kn.

Rooted shoots were taken out, washed with tap water, planted in pots having sterilized sand. Half strength of MS was applied to moisten the sand, which was then covered with polythene bags. Pots were placed under 2500 lux

light at 16 hrs photoperiod for one week. After three - four days holes were made in the polythene bags to gradually expose them to the external environment. After ten days they were transferred to larger pots containing 50% sand and 50% peat moss and shifted to greenhouse.

Results and Discussion

Seeds were disinfected through delinting with concentrated H_2SO_4 followed by 0.1 $HgCl_2$ for 20 minutes and with ethanol for ten minutes. Germination was observed after 48 hrs. Within three days well developed root system with expanded cotyledon was produced. The germination of cotton seeds on agar has also been observed by Shoemaker et al. (1986) and Zhang (1994).

Explants containing shoot apices attached to both cotyledons and 0.5 cm hypocotyls produced multiple shoots more efficiently (Fig. 1A). Explants containing shoot apex with a single cotyledon were also able to produce multiple shoots but with less efficiency (Fig. 1B). Non-regenerable green callus was observed in explant without cotyledon (Fig. 1C). Variable regeneration efficiency of explants has been reported by other scientists as well (Zhang et al. 2001; Sakhanokho et al. 2001) suggesting that type of explants has influence on their ability to regenerate. Induction of multiple shoots was 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 of the embryonic apical meristem axes of cotton.

Highest average multiple shoots developed on 0.25 mg/l Kn (Table 1). Further increase in shoot number was not observed with increasing concentrations of cytokinin. Jorge et al. (1998) found that higher concentration of growth hormone yields fewer shoots in cotton (*Gossypium hirsutum* L. cv. Guazuncho II). The effect of single growth hormone was studied by Hemphil et al. (1998) in which they observed best development of shoots on MS containing 0.3 mg/l BA in cotton (*Gossypium hirsutum* L. cv. Stoneville 7A). Multiple shoots elongated within same media. Agarwal et al. (1997) obtained highest number of shoots in cotton (*Gossypium hirsutum* L. Anjali-LRK 516) by culturing cotyledonary nodes devoid of apical meristem in MS basal medium supplemented with BA and Kn 2.5 mg/l each. However, shoot could not elongate in same media. Elongation of shoots was observed in liquid media or in MS solidified with agar. This could be attributed to the difference in the concentration of growth hormone and the cultivar.

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 higher amount of medium available in the culture vessel

(50 mg/l bottle). Positive influence of larger culture vessel in vitro has also been documented by McClellend and Smith (1990) in woody species. Highest root length 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 mg/l NAA and 0.46 mg/l Kn in ten different cotton cultivars. Number of roots and root length were highest with 0.5 mg/l

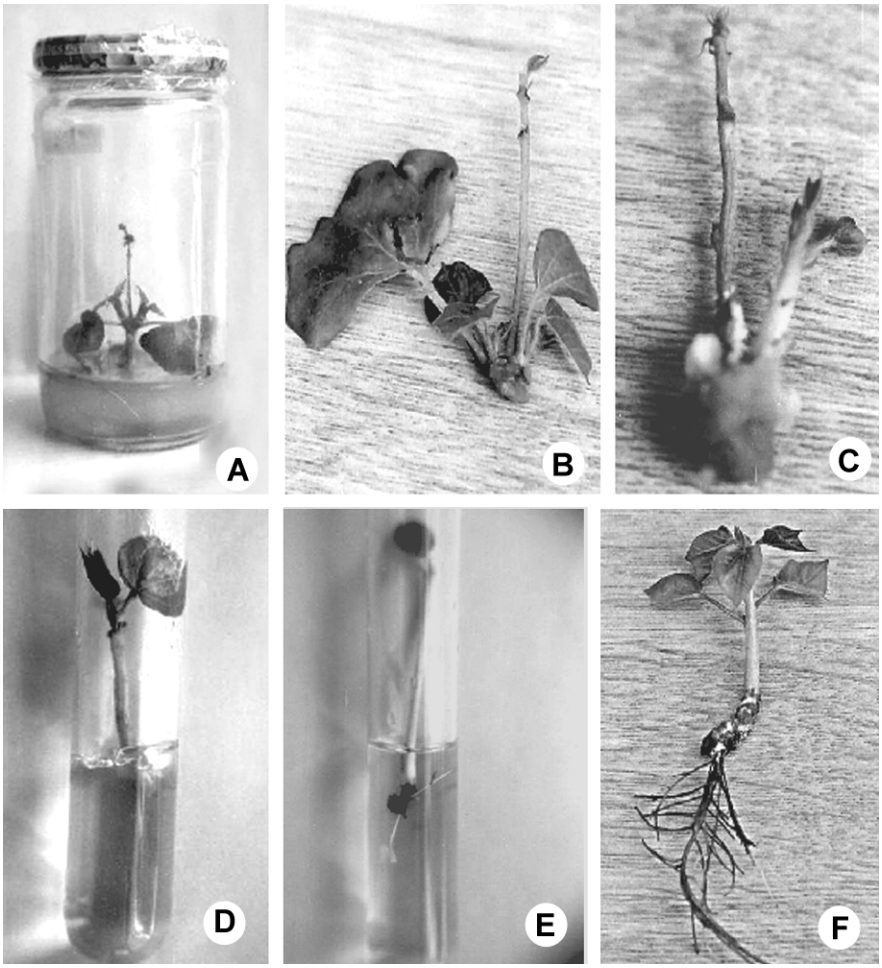

Fig. 1. A. Induction of multiple shoots from cotyledonary node with both cotyledons. B. Induction of multiple shoots from cotyledonary node with single cotyledon. C. Multiple shoots from cotyledonary node without cotyledon with green callus at the base. D. Excised shoot inoculated for rooting. E. Rooted shoot. F. Complete plantlet of cotton cultivar NIAB-999.

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

Treatments + media	Kinetin (mg/l)	Cotyledonary node					
		Single cotyledon (%)	Shoots/ explant (mean ± SD)	Both cotyledons (%)	Shoots/ explant (mean ± SD)	Without cotyledon (%)	Shoots/ explant (mean ± SD)
K1 + MSB	0.0	80.0	1.00 ± 0.40	91.70	1.00 ± 0.40	61.00	0.90 ± 0.30
K2 + MSB	0.10	100.0	1.33 ± 0.40	100.00	1.60 ± 0.40	100.00	1.90 ± 0.40
K3 + MSB	0.25	100.0	2.61 ± 0.70	100.00	3.43 ± 0.32	91.17	2.21 ± 0.83
K4 + MSB	0.50	85.0	2.07 ± 0.44	82.00	2.62 ± 0.66	71.23	1.94 ± 0.41
K5 + MSB	1.00	60.0	1.60 ± 0.90	67.00	2.08 ± 0.49	43.00	1.53 ± 0.54

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

Treatments + medium	NAA (mg/l)	Kn (mg/l)	No. of shoots evaluated for rooting	No. of shoots rooted	Rooting (%)	Root length (cm ± SD)
T1 + MSB	0.0	0.0	30	21	70.00	3.85 ± 0.50
T2 + MSB	0.50	0.0	30	25	83.33	4.60 ± 0.54
T3 + MSB	0.0	0.10	30	23	76.66	4.02 ± 0.52
T4 + MSB	0.50	0.10	30	28	93.33	5.85 ± 0.93

NAA and 0.1 mg/l Kn (Table 2). Presence of 0.5 mg/l NAA improved root length of the plantlet as compared to control and T3 (Table 2). Gupta et al. (1997) also observed rooting by culturing isolated shoots on MS basal salts supplemented with NAA in cotton (*Gossypium hirsutum* L. cv. Khandwa-2). Root number and length was higher than control in the presence of 0.1 mg/l Kn, which showed that presence of low cytokinin enhances root formation (Table 2). Rooting of all shoots was high. Efffect of types of cytokinin used for *in vitro* shoot proliferation on the subsequent rooting of shoots was studied by Bennett et al. (1994) found that shoots of *Eucalyptus* from the multiplication medium containing Kn produced more roots and remained healthy for a longer period on the rooting medium as compared to shoots taken from multiplication medium containing BAP.

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References

Agarwal DC, Banerjee RR, Kolala AB, Dhage AV, Kulkarni AV, Nalawade SH and **Krishnamurthy KV** (1997) *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Reports **16**: 647-652.

Bennett IJ, McComb JA, Tonkin CM and **McDavid DAJ** (1994) Alternating cytokinins in multiplication media stimulates *in vitro* shoot growth and rooting of Eucalyptus globulus Labill. Annuals of Botany **74** : 53-58.

Chen ZX, Li SJ and **Trolinder NL** (1987) Some characteristics of somatic embryogenesis and plant regeneration in cotton cell suspension culture. Sci. Agric. Sin. **20** : 6-11.

Davidonis GH and **Hamilton RH** (1983) Plant regeneration from callus tissue of *Gossypium hirsutum* L. Plant Sci. Lett. **32** : 89-93.

Gamborg OL, Milelr RA and **Ojima K** (1968) Nutrient requirement of suspension culture of soybean root cells. Exp. Cell Res. **50** : 151-158.

Gupta SK, Srivastava AK, Singh PK and **Tuli R** (1997) *In vitro* proliferation of shoots and regeneration of cotton. Plant Cell, Tissue & Org. Cult. **51** : 149-152.

Hemphil JK, Maier CGA and **Chapman KD** (1998) Rapid *in vitro* regeneration of cotton (*Gossypium hirsutum* L.) Plant cell Report **17** : 273-278.

Jorge LM, Permingeat HR, Romagnoli MV, Heisterborg CM and **Ruben HV** (1998) Multiple shoot induction and plant regeneration from embryogenic axes of cotton. Plant Cell, Tissue & Org. Cult. **54** : 131-136.

Kolganova TV, Srivastava DK and **Mett VL** (1992) Callogenesis and regeneration of cotton (*Gossypium hirsutum* L. cv. 108-F). Sov. Plant Physiol. **39** : 232-236.

McClellend MT and **Smith MAL** (1990) Vessel type, closure and explant orientation influence in vitro performance of five woody species. HortSci. **25** : 797-800.

Peeters M, Willems K and **Swennen R** (1994) Protoplasm to plant regeneration in cotton (*Gossypium hirsutum* L. cv. Coker 312) using feeder layers. Plant Cell Rep. **13** : 208-211.

Rajasekaran K, Grula JW, Hudspeth RL, Pofelis S and **Anderson DM** (1996) Herbicide resistant Acala and Coker cotton transformed with a native gene encoding mutant forms of acetohydroxy acid synthesis. Mol. Breeding **2**: 307-317.

Saeed NA, Zafar Y and **Malik KA** (1997) A simple procedure of *Gossypium* meristem shoot tip culture. Plant Cell, Tisseu & Org. Cult. **51** : 201-207.

Sakhanokho HM, Allan ZF, Rajasekaran K, Saha S and **Sharma GC** (2001) Induction of highly embryogenic calli and plant regeneration in Upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) Cottons,. Crop. Sci. 41 : 1235-1240.

Shoemaker RC, Couche LJ and **Gilbrait DW** (1986) Characterization of somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep. **23** : 178-181.

Trolinder NL and **Goudin JR** (1987) Somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep. **6** : 231-234.

Trolinder NL and **Xhixian C** (1989) Genotype specificity of he somatic embryogenesis response in cotton. Plant Cell Rep. **8** : 133-136.

Voo SK, Rugh CL and **Kamalay JC** (1991) Indirect somatic embryogenesis and plant recovery from cotton (*Gossypium hirsutu* L.). In vitro Cell Dev. Biol. **27** : 117-124.

Zhang BH (1994) A rapid induction method for cotton somatic embryos. Chinene Sci. Bull. **39** : 1340-1342.

Zhang DL and **Wang ZZ** (1989) Tissue culture and embryogenesis of *Gossypium hirsutum* L. Acta Bot. Sin. **31** : 161-163.

Zhang BH, Li XL, Li L and **Li GD** (1996) Plant recovery from cotton somatic embryos. Acta Agric. Boreali-occidentalis Sinica **2** : 24-28.

Zhang BH, Li XL, Li L and **Li GD** (1996) Development of abnormal plantlets and their normalization during cotton tissue culture. Acta Botanica Sinica **38** : 845-852.

Zhang BH and **Zhao BS** (1997) Cotton biotechnology and its application. China Agricultural Press, Beijing.

Zhang BH, Feng R, Liu F and **Yao CB** (1999) Direct induction of cotton somatic embryogenesis. Chinese Sci. Bull. **44** : 766-767.

Zhang BH, Feng R, Fang L and **Qinglian W** (2001) High frequency somatic embryogenesis and plant regeneration of an elite Chinese cotton variety. Bot. Bull. Acad. Sin. **42** : 9-16.