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Optimization of Shoot Tip-Based *in Vitro* Plant Regeneration in Cotton (*Gossypium* spp.)

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ABSTRACT

An *in vitro* regeneration system in cotton has been optimized from explants comprising pre-existing shoot apices of aseptically grown seedlings. This system has been found to be useful with a variety of genotypes (*arborium* and *hirsutum*). Shoot regeneration among genotypes on different media ranged from 69 to 80%. The age of explants showed a significant effect on shoot tip regeneration. On an average, 5 day-old shoot tips recorded highest regeneration of 76% as compared to 3 or 9 day old seedlings on ½ MS medium + 6% sucrose. The genotypic differences for regeneration rate were significant, indicating that the regeneration of shoot tips was genotype-dependent. Rooting efficiency between genotypes ranged from 60 to 67% was not significantly different indicated that rooting efficiency is genotype independent under *in vitro* conditions. However, root induction was greatly influenced by media composition as it varied from 55 to 72%. Half MS + 0.05 mg/l α-naphthalene acetic acid medium recorded the highest percentage of root induction (72%) closely followed by half MS (68%). Root and shoot formation was observed in all genotypes. After plantlet formation, *in vitro*-raised plantlets were shifted to test tubes containing a little cotton soaked with a small amount of water for two weeks and the water from these tubes was changed daily. The hardened plantlets were then transferred to small polythene bags filled with sand: soil mixture in the greenhouse and finally transferred to earthen pots in glasshouse. These plants grew into healthy green plants and reached maturity.

Keywords: age of explant, *Gossypium*, *in vitro*, shoot regeneration

INTRODUCTION

Cotton (*Gossypium* spp.) is an excellent source of textile fiber and cultivated in many countries (Meyer *et al*. 2007; Ozyigit and Gozukirmizi 2009). Besides, its edible oil contributes 65-70% to the oil industry as well as of other industrial products (Méndez-Natera *et al*. 2007; Khan *et al*. 2009). 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 lack of necessary variation, especially resistance against pests and diseases. Advanced biotechnology provides both an innovation method for cotton breeding, germplasm multiplication and accelerates the process of cotton breeding. Plant tissue culture techniques provide an alternative means of crop improvement (Zhang and Zhao 1997). The plant breeding methods can be combined with tissue culture methods in order to form genetic variability for desired traits (Naz *et al.* 2007; Ozyigit and Gozukirmizi 2008). 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 and highly genotype dependent (Trolinder and Chen 1989; Sanghera *et al*. 2009). Aside from the genotype limitation, many of the plants regenerated from callus as somatic embryos were abnormal (Cousins *et al*. 1991; Trolinder and Goodin 1987; Rajasekaran *et al*. 1996) and the transformation efficiencies are generally low due to the low frequency of embryogenesis and the difficulty in germination of transformed embryos (Wilkins *et al.* 2000; Zhao *et al*. 2006). Since genetic transformation has played an important role in modern cotton breeding and had a significant impact on its production. To take advantage of this promising technology, a reliable and genotype-independent regeneration system is essential. One of the technologies for delivery of genes into plant tissues is biolistic gun which requires regenerable tissues may be callus, suspension cells, leaves, meristem tips or any other explant. Compared with somatic embryogenesis, the shoot tip mediated regeneration techniques are easy and less time-consuming process (Ganesan and Jayabalan 2006). Cotton plants have proved to be difficult to manipulate in tissue culture (McCabe and Martinell 1993). In recent years, there has been increasing focus on the use of meristems and shoot axes as the source of tissue explants for transgenic cotton production (Satyavathi *et al*. 2002; Ganesan *et al*. 2009). Lack of standard protocol for regeneration of diploid (*Gossypium arboreum*) and or tetraploid genotypes of cotton (*G. hirsutum*) prevents genetic enhancement in these species by genetic engineering and tissue culture. Shoot-tip meristem and apex culture offers unique advantages to regenerate plants directly from inoculated shoots on simple Murashige and Skoog (MS) medium (Nandeshwar *et al*. 2009).

The objective of this research is to improve shoot regeneration and rooting efficiency in shoot tip based cotton regeneration system. Three factors *viz*. effect of seed sterilization method, shoot tip age and media composition that could affect the *in vitro* regeneration and rooting efficiency of shoot apices were investigated. The protocol developed for shoot and root development from shoot tips is quite simple and has been found to be useful with a variety of genotypes (both *arborium* and *hirsutum*).

MATERIALS AND METHODS

This study was conducted at School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab. Delinted seeds of four cotton genotypes *viz.* 'LD 210' and 'LD 694' (*G. arborium* L.) and 'LH 1995' and 'LH 2076' (*G. hirsutum* L.) obtained from

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Table 1 List of media used for *in vitro* shoot regeneration in present study.

Media code	Composition
SR1	Half MS
SR2	Half MS + 6% sucrose
SR3	Half MS + 2 mg/l Kin + 0.5 mg/I IAA
SR4	Half MS + 2 mg/l Kin + 1.0 mg/I IAA
SR5	Half MS + 2 mg/l BAP + 0.5 mg/I Kin
SR6	Half MS + 2 mg/l BAP+ 1.0 mg/I Kin
SR7	DKW
SR8	DKW + 1.0 mg/l BAP
List of media used for *in vitro* rooting in present study	
R1	Half MS
R2	Half MS + 0.1 mg/l NAA
R3	Half MS + 0.05 mg/l NAA
R4	Half MS + 0.2% activated charcoal

Abbreviations: BAP, 6-benayleaminopurine; DKW, Driver and Kuniyuki; IAA, indole-3 acetic acid; Kin, kinetin; MS, Murashige and Skoog; NAA, α-naphthalene acetic acid

the Cotton Section, Department of Plant Breeding and Genetics, PAU, Ludhiana, used as source material were disinfected via three methods: (i) treatment with $HgCl_2$ (0.1%) solution (ii) treatment with Bavistin (1.0%) solution and (iii) treatment with $HgCl_2$ (0.1%) + Bavistin (1%) solution for 4, 6, 8 and 10 min duration. After treatment, seeds were rinsed at least three times with sterile double-distilled water and then soaked for 6 hours in autoclaved distilled water at room temperature and placed on seed germination medium. Eight to 10 seeds were placed in each jar/Petri dishes containing MS (Murashige and Skoog 1962) medium supplemented with 8 g/l agar plus 3% sucrose. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. The seed jars were kept in the dark for germination at 28 ± 2°C for 7 days. The number of elongated shoots was counted. Contamination was determined by visual inspection for fungal and/or bacterial growth.

Shoot tips were excised from 3 to 9-days old seedlings. The seedling apex was removed just below the attachment of the largest unexpanded leaf. Additional tissue was removed to expose the base of the shoot apex. The isolated shoot apices were then placed on agar solidified (8.0 g/l) shoot elongation media dispensed (20-25 ml) in test tubes (**Table 1**). The well developed shoots were transferred to four different media composition for rooting (**Table 1**). The rooted shoots were then transferred to test tubes containing a little cotton soaked with small amount of water and incubated in a culture chamber (27°C) for 2 weeks and the water from these tubes was changed daily during hardening. The hardened plantlets were then transferred to small polythene bags filled with sand soil mixture in the greenhouse and finally transferred to earthen pots in glass house and grown to maturity. 36 to 48 shoot tips were used for each treatment and each genotype. All cultures were maintained at 27 ± 2°C at a constant photosynthetic photon flux density (PPFD) of 113.20 µmol m^{-2} sec^{-1} under a 16-h photoperiod in the culture chamber.

The data recorded were analyzed according to completely randomized design (simple and factorial) analysis (Snedecor and Cochran 1967) using statistical software CPCS-1 package developed by Cheema and Singh (1990). The data of percent were converted to an arc sine value for the analysis of variance (ANOVA). The significance of variation among the treatments were observed by applying the 'F' test and critical differences (CD) at the 5% level of significance and used to compare the means of treatments; interpretations were made accordingly.

RESULTS AND DISCUSSION

Cotton seeds from the field are highly contaminated as they contain large numbers of small hairs that can hold spores of fungi and bacteria. To obtain the best explants for isolating the shoot tips, three seed sterilization methods were compared in this study. The number of visually contaminated seeds and the number of germinated seeds (shoot elongation) were recorded after 5 days. Of the three sterilization methods used in this study method 3 i.e. (1.0% Bavistin + 0.1% $HgCl_2$) gave the best surface disinfection with minimum contamination of seed (0 to 2%) and germination as good as (69-80%) (**Table 2**) when treatment duration was 6 min in different cotton genotypes (**Fig. 1A-C**).

Differential response to plant regeneration from apical shoot tips owing to the media composition, genotypic differences, explant age and root induction have been reported by earlier groups (Bajaj and Gill 1986; Gould *et al.* 1991; Gupta *et al.* 1997). Therefore, to develop efficient plant regeneration system, four promising cotton genotypes viz., LD 210, 'LD 694' (diploid) and 'LH 1995' and 'LH 2076' (tetraploid) were screened for regeneration response from apical shoot tips by studying the effect of different media compositions, age of explants and standardize other factors like root induction, *in vitro* hardening and establishment in soil. The results obtained from different experiments are described below.

Effect of genotype and media on shoot tip culture

In first experiment, 5-6 days old *in vitro* raised seedlings were used for excision of shoot apical tips (0.5-0.7 cm) which were cultured on different media compositions (**Fig. 1D**) based on ½ MS and DKW salts. About 60-80 shoot tips of each genotype were cultured per treatment. All ½ MS based media were supplemented with 3% sucrose except one that was fortified with 6% sucrose only without any plant growth regulators (PGRs).

It was observed that the cultured shoot tips, irrespective of media composition started to grow after 3-4 days of incubation on regeneration medium and one shoot was regenerated after 2 weeks from each shoot tip cultured (**Fig. 1E**). Percentage shoot regeneration efficiency was recorded and

Table 2 Effect of different seed surface sterilants and treatment duration on percent seed germination and contamination in cotton after 1 week.

Varieties	LD210		LD694		LH1995		LH2076	
Treatment				**Mercuric chloride (0.10%)**				
Duration (Min)	**Germ (%)**	**Cont (%)**	**Germ (%)**	**Cont (%)**	**Germ (%)**	**Cont (%)**	**Germ (%)**	**Cont (%)**
4	66.75 (54.76)	6.37	71.75 (57.89)	8.29	73.45 (58.98)	9.62	67.75 (55.39)	7.48
6	67.77 (55.39)	4.47	75.37 (60.24)	5.17	77.70 (61.80)	6.66	75.55 (60.36)	5.28
8	53.99 (47.27)	5.62	63.22 (52.66)	4.37	61.58 (51.69)	5.51	59.37 (50.40)	3.16
10	34.87 (36.19)	2.65	39.65 (39.02)	2.01	43.50 (41.26)	3.59	37.57 (37.80)	2.12
Bavistin (1.00%)								
4	73.25 (58.85)	12.79	71.52 (57.74)	16.02	71.00 (57.41)	12.37	77.32 (61.56)	10.01
6	69.65 (56.55)	9.98	77.56 (61.72)	12.07	75.65 (60.43)	12.07	78.42 (62.31)	7.66
8	65.82 (54.22)	5.20	62.63 (52.31)	7.97	62.25 (52.09)	7.50	66.64 (54.71)	5.08
10	60.65 (51.13)	3.07	61.37 (51.57)	3.95	59.70 (50.59)	5.01	64.85 (53.63)	4.36
Bavistin (1.00%) + mercuric chloride (0.10%)								
4	71.25 (57.55)	4.25	70.52 (57.11)	3.87	68.62 (55.96)	4.54	71.42 (57.68)	6.12
6	71.37 (57.63)	1.20	69.52 (56.47)	2.01	78.42 (62.31)	1.00	80.00 (63.43)	1.90
8	59.10 (50.22)	0.00	63.64 (52.91)	1.25	51.47 (45.84)	0.00	61.62 (51.71)	1.25
10	27.65 (31.70)	0.00	31.50 (34.14)	0.00	31.50 (34.14)	0.00	33.45 (35.33)	0.00

CD at 0.05 for germination: Varieties = 0.91; Sterilants = 0.78; V x S = 1.57; Durations = 0.90; V x D = 1.81; S x D = 1.57; V x S x D = 3.15
CD at 0.05 for contamination: Varieties = 0.13; Sterilants = 0.11; V x S = 0.23; Durations = 0.13; V x D = 0.27 ; S x D = 0.23; V x S x D = 0.47

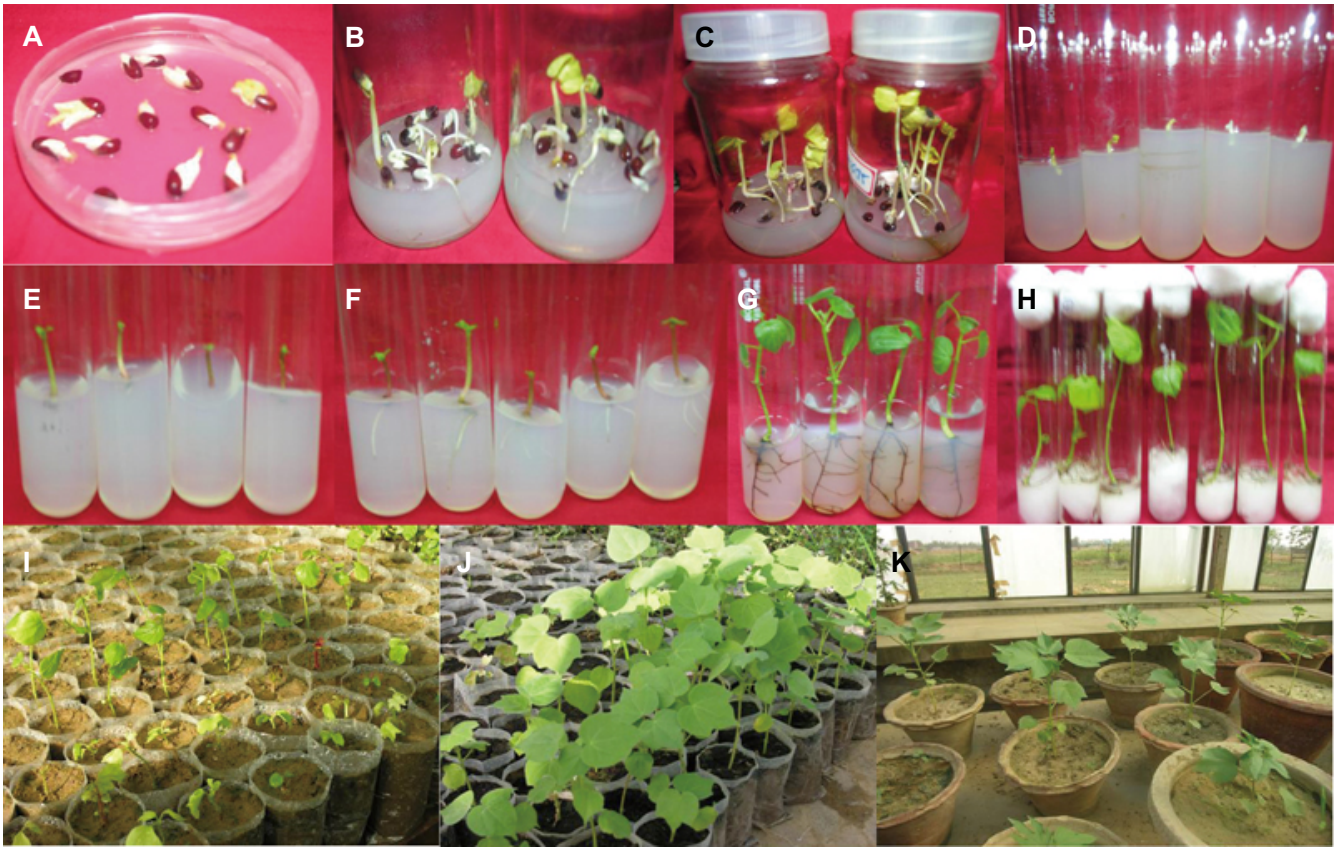

Fig. 1 ***In vitro*** **shoot tip regeneration in cotton.** (**A**) Seed germination in cotton variety LH2076; (**B-C**) Axenic seedlings produced with bavistin 1.0% +$HgCl_2$ 0.1% treatment; (**D**) Shoot tips put on ½ MS + 6% sucrose medium; (**E**) Elongation of shoot tips after 2 weeks; (**F**) Shoots showing root induction; (**G**) Plantlets showing well developed shoot and root; (**H**) Plantlets hardened on water for 2 weeks; (**I-J**) *In vitro* regenerated plantlets established in soil and (**K**) Plantlets showing normal growth in earthen pots in glasshouse.

compared with respect to genotypes and different media used following statistical analysis conducted after arc sine transformation of data using factorial complete randomized design. The results showed significant differences among the genotypes for shoot regeneration on all the media tested (**Table 3**).

The average shoot regeneration among different 1/2 MS based and DKW media over varieties ranged from 68.74 to 79.87%. The maximum shoot regeneration (79.87%) was recorded in 1/2 MS + 6% sucrose followed by DKW + 1.0 mg/l BAP (76.05%), 1/2 MS + 2.0 mg/l BAP+ 1.0 mg/I Kin (75.85%) and 1/2 MS (74.96%) while other were statistically at par among themselves. Similarly, shoot regeneration among genotypes over media was maximum for 'LD 694' (80.72%) which was at par with 'LH 2076' (79.76%) followed by 'LH 1995' (73.50%). Different genotype × media combinations revealed significantly high shoot regeneration (86.97, 82.81%), (84.37, 85.41%) and (84.23, 82.73%) in two varieties ('LD 694' and 'LH 2076') on ½ MS + 6 % sucrose, ½ MS and DKW + 1.0 mg/l BAP media, respectively (**Table 3**). The genotypic differences in *G. arboreum* L. as well as *G. hirsutum* genotypes for shoot regeneration from cultivated shoot apices on modified MS medium has been reported by Kaur (2002), wherein variation in response ranged from 65.91% ('LD 327') to 85.37% ('RG 8') for *G. arboreum* cotton varieties and it ranged from 75.47% ('PIL 43') to 100% ('LHH 144') in *G. hirsutum*. Bajaj and Gill (1986) also reported variation in shoot regeneration from shoot tip culture in various diploid cotton genotypes ranged from 41.6% (*G. arboreum* x *G. stocksii*) to 72.7% (*G. arboreum* x *G. anomalum*). Similarly, shoot regeneration ranging from 40% (*G. arboreum* cv. 'Lohit') to 91.7% ('Pusa 37') have been documented in the study conducted by Gupta *et al.* (1997). Sharma *et al.* (2007) reported high frequency plant regeneration and multiple shoot induction from apical tips in two *G. arboreum* genotypes. Maximum plant regeneration (87% in 'LD 694' and 83% in 'DS-5') was obtained on ½ MS medium containing 0.2% (w/v) activated charcoal (AC). It can be inferred that screening of genotypes for regeneration response should be carried out; this helps to choose the best one for genetic transformation. Importance should be given to regeneration response owing to its effect on successful transformation, which would lead to higher success during successive steps. From the above media, ½ MS + 6% sucrose and DKW + 1.0 mg/l BAP were further used to study the effect of explant age on shoot rege-

Table 3 Effect of different genotypes and media composition on shoot regeneration in cotton.

Genotype/	Shoot Regeneration (%)				
Media code	LD210	LD694	LH1995	LH2076	Mean
SR1	56.25	84.37	73.81	85.41	74.96
	(48.57)	(66.70)	(59.20)	(67.52)	(59.97)
SR2	72.67	86.97	77.04	82.81	79.87
	(58.46)	(68.85)	(61.35)	(65.49)	(63.34)
SR3	60.52	71.40	71.16	71.87	68.74
	(51.05)	(57.66)	(57.51)	(57.96)	(56.00)
SR4	69.15	72.45	71.76	72.07	71.36
	(56.24)	(58.33)	(57.91)	(58.08)	(57.64)
SR5	65.08	73.07	71.16	76.70	71.50
	(53.77)	(58.72)	(57.55)	(61.13)	(57.73)
SR6	68.54	77.89	76.62	80.36	75.85
	(55.87)	(61.95)	(61.06)	(63.69)	(60.56)
SR7	63.38	74.94	71.47	71.16	70.24
	(52.74)	(59.98)	(57.74)	(57.50)	(56.93)
SR8	63.21	84.23	74.04	82.73	76.05
	(52.64)	(66.61)	(59.35)	(65.45)	(60.69)
Mean	64.85	80.72	73.50	79.76	
	(53.20)	(63.95)	(59.01)	(63.26)	

CD at (0.05) Genotypes 1.90; Media 2.13; Genotypes x Media 4.26; Values in parentheses have been arc-sine transformed

neration. The results of the present study thus are in agreement with those of Sharma *et al*. (2007) where the superiority of MS salts and DKW over other media compositions was observed. In the present study, shoot tips were established successfully on both PGR-supplemented and PGR-free growth medium. Similar PGR-autonomous growth of shoot tips in cotton has also been reported by Gould *et al.* (1991) in *G. hirsutum* and Chinchane *et al.* (2005) in *G. arboreum*. Likewise, Zapata *et al*. (1999) reported a high percentage of regenerated shoots on PGR-free MS media (full and half) devoid of AC.

Effect of age of explant on shoot regeneration

The age of explants used for isolating shoot tips was examined in this experiment. 24-30 of 3, 5 and 9 day-old seedlings of each of the four genotypes were used to excise shoot apices. The excised tips were placed on two media viz. ½ MS medium + 6% sucrose and DKW + 1.0 mg/l BAP (based on an earlier experiment) to induce shoot elongation for 2 weeks. The age of explants has a significant effect on shoot tip elongation (**Table 4**). On an average, 54.29% of shoot tips from 3 day-old explants had elongated, 76.29% of shoot tips from 5 day-old had elongated and 68.93% of shoot tips from 9 day-old explants had elongated on ½ MS medium + 6% sucrose medium (**Table 5**) while the corresponding figures recorded on DKW + 1.0 mg/l BAP medium were 50.73, 72.33 and 68.93%, respectively (**Table 6**). Importance of age of seedlings was also reported by Kaur (2002). A relatively good survival (430-571 shoots/1000 excised) of shoot apices excised after 5 days of germination was observed by Gould *et al.* (1991). The elongation rates between media were not significantly different. The elongation rates of the 4 genotypes were significantly different from each other, which indicates that the elongation of shoot tips on elongation medium was genotype-dependent it could be due to the fact that genotypes included in experiment belong to *G. arboreum* and *G. hirsutum*. Among genotypes, 'LH 2076' recorded highest shoot elongation (70.84, 67.03%) percentage followed by 'LD 694' (68.39, 65.53%), while 'LD 210' exhibited lowest elongation (60.55, 58.75%) in 1/2 MS + 0. 6% sucrose and DKW + 1.0 mg/l BAP media, respectively. These results are in support of earlier findings (Mittal 2005) who observed differential response among 4 genotypes at all the ages of seedling and reported that 5-day old seedlings exhibited maximum shoot regeneration in 'LD 784' (58.8%) followed by 'LD 210' (55.5%), whereas least response was recorded in 'RG 8' (44.4%). In general ½ MS + 6% sucrose medium supported higher shoot regeneration than DKW + 1.0 mg/l BAP medium. Theoretically, each excised apex should develop into a rooted plant; however, the yield of shoots *in vitro* from isolated apices depends on the incidence of contamination and rooting efficiency (Gould *et al*. 1991). The isolated shoot tips began to grow in one week. In earlier studies, the age of seedlings less than 10 days has been reported for isolation and regeneration of shoot apices (Bajaj and Gill 1986; Gould *et al*. 1991; Gupta *et al*. 1997; Nasir *et al*. 1997). It was also observed that some tips grew into callus; this may be because the BAP was used in the medium to promote cell division and aid in growth. No multiple shoot formation was observed in this experiment. It may be because of apical dominance.

Table 4 Analysis of variance table for investigation of effect of age of explants on shoot regeneration.

Source	DF	Mean square	F value
Variety	3	4.94	1.85
Age	2	128.36*	48.25
Variety x Age	6	2.38*	0.89
Error	22	2.66	

* Significant at 5%

Table 5 Effect of age explants on shoot regeneration in cotton on 1/2 MS + 6% sucrose medium.

Genotype/	Shoot regeneration (%)			Mean
Age of explant	3 days	5 days	9 days	
LD 210	51.15	69.50	61.01	60.55
LD 694	56.04	78.19	70.95	68.39
LH1995	50.80	76.06	71.83	66.22
LH2076	59.17	81.43	71.93	70.84
Mean	54.29	76.29	68.93	

CD at (0.05) Genotypes = 1.65; Age of explants = 4.68; Genotype x Age =7.77

Table 6 Effect of age explants on shoot regeneration in cotton on DKW + 1.0 mg/l BAP medium.

Genotype/	Shoot regeneration (%)			Mean
Age of explant	3 days	5 days	9 days	
LD 210	46.07	69.70	60.50	58.75
LD 694	52.06	73.97	70.57	65.53
LH1995	49.42	72.13	71.34	64.29
LH2076	55.37	73.53	72.21	67.03
Mean	50.73	72.33	68.65	

CD at (0.05) Genotypes = 1.85 ; Age of explants = 3.38; Genotype x Age =6.57

In vitro rooting

30-40 regenerated shoots of each genotype from above experiment were subjected to root induction on four ½ MS-based media fortified with NAA and AC to induce rooting for 3 weeks. The experiment was repeated three times. Roots initiated after 15 days of shoot regeneration in all the four genotypes. **Fig. 1F-G** shows root induction and well developed plantlets in 'LH 2076'.

The statistical analysis of data showed that the genotypic differences with respect to percentage root induction were non-significant in all the media tested. However, significant differences existed among different rooting media tested (**Table 7**). ½ MS + 0.05 mg/l NAA recorded the highest percentage of root induction (71.63%) followed by ½ MS (67.56%), ½ MS + 0.2% AC (58.42%), while ½ MS + 0.1 mg/l NAA exhibited the minimum root induction of 55.05%. Similarly, Banerjee (2001) observed high percentage of rooting (85%) in cultivar cv. 'LRK-516' followed by 'NHH-44' (82.50%) and H-8 in ½ MS + vitamins medium supplemented with 0.05 mg/l NAA. After three weeks culture, the plantlets were then transferred to test tubes containing a little cotton soaked with small amount of water (**Fig. 1H**) and incubated in a culture chamber (27°C) for 2 and 3 weeks and the water from these tubes was changed daily during hardening. Hardening of *in vitro* regenerated plantlets of cotton in water revealed that 2 weeks duration resulted in better survival (60.91%) than 3 weeks (42.15%) and resulted in higher (50.50%) establishment in soil. The hardened plantlets were then transferred to small polythene bags filled with sand soil mixture in the greenhouse (**Fig.**

Table 7 Effect of different media composition on root induction in cotton.

Genotype/ Media code	Root induction (%)				
	LD210	LD694	LH1995	LH2076	Mean
R1	65.05 (53.78)	72.06 (58.09)	73.15 (58.79)	72.00 (58.05)	67.56 (55.28)
R2	54.04 (47.31)	55.72 (48.28)	56.00 (48.44)	54.44 (47.54)	55.05 (47.89)
R3	68.12 (55.62)	74.49 (59.66)	68.64 (55.94)	75.28 (60.18)	71.63 (57.81)
R4	54.01 (47.30)	66.25 (54.48)	56.18 (48.54)	57.15 (49.11)	58.42 (49.84)
Mean	60.30 (50.94)	67.13 (55.01)	63.48 (52.82)	64.71 (53.55)	

CD at (0.05) Genotypes NS; Media 7.30; Genotype x Media 9.85; Values in parentheses have been arc-sine transformed.

1I-J) and finally transferred to earthen pots in a glasshouse and grown to maturity. The plants appeared normal (**Fig. 1K**). In this study the shoot tip culture procedure was used for the regeneration of cotton plants, because cotton is recalcitrant and has proved difficult to manipulate in tissue culture (McCabe and Martinell 1993). For transformation a rapid, reliable regeneration system is required so this procedure can be used for gene delivery either by *Agrobacterium* transformation or particle bombardment. Regeneration through shoot apex has been used successfully in *Agrobacterium*-mediated transformation of cotton (Gould *et al*. 1997, 2002). Trolinder *et al*. (2006) used chilled apical shoot tips to develop transgenic plants. Kategari *et al*. (2007) reported *Agrobacterium*-mediated genetic transformation of elite Indian genotype Bikaneri Narma (*G. hirsutum*) using shoot apical meristems. Recently, Nandeshwar *et al*. (2009) developed an *Agrobacterium*-mediated gene transfer protocol for diploid cotton (*G. arboreum*) cv 'RG8' using meristematic shoot tips, followed by direct shoot organogenesis or multiple shoot induction of putative transformants. Efforts have been made to couple this regeneration procedure with particle gun/*Agrobacterium*-mediated transformation for rapid introduction of value-added traits directly into high-yielding cotton genotypes.

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