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Effects of Genotype, Plant Growth Regulators and Explant Source on Callus Induction in Cotton (*Gossypium hirsutum* L.)

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ABSTRACT

In this study callus induction of 5 elite upland cotton genotypes ('PIL-8', 'F 1378', 'F 1861', 'LH 1995' and 'LH 2076') were tested on five different callus induction (MS + 1.0 mg/l Kin + 1.5 mg/l IAA; MS + 2.0 mg/l NAA + 0.1 mg/l Kin; MSB (MS + Vit. B5) + 1.0 mg/l ZT + 1.0 mg/l Kin + 560 mg/l proline; MSB +2.5 mg/l ZT + 1.0 mg/l 2,4-D; MS + 1.0 mg/l 2,4-D + 1.0 mg/l Kin + 560 mg/l proline) and somatic embryo initiation-maturation media that were previously found to be capable of inducing somatic embryogenesis in cotton. All genotypes tested produced callus on different media within 4 to 6 weeks. Analysis of variance revealed significant differences in callus induction, days taken to callus induction and callus growth index in different explants, genotypes and media composition. Of the two explants compared, hypocotyls and cotyledons, the former were most responsive to callus induction and proliferation than the latter. Hypocotyls showed a significantly higher percentage of callusing ranging from 35.05 to 78.44% with an average of 59.45% while the corresponding values for cotyledons were 27.75 to 59.92% and 45%, respectively. PIL-8 recorded the highest percentage of callus induction (78.44%) and was the only genotype that induced callus within 30 days. Different types of media tested revealed that plant growth regulator type had a significant effect on callus induction and physical appearance. The callus induced on MS medium containing 2,4-D was brown and of low quality compared to that produced on MS media containing NAA, Kin and ZT combinations. However, medium containing Kin resulted in the formation of compact callus with a number of roots emerging from it. The results of this study will pave the way for establishing a future *in vitro* regeneration system and transformation methods for elite Indian cultivars.

Keywords: callus induction, cotyledon, *Gossypium hirsutum*, hypocotyls, plant growth regulator

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important commercial crops of the world valued for its fiber, oil and other by-products. It is grown in more than 70 countries and over 180 million people around the globe are involved with the fiber industry which produces US$ 20-30 billion of raw cotton (John 1997). Although significant progress has been made in cotton breeding programs in past, the genetic improvement of this crop through conventional breeding techniques still have several limitations, such as access to a limited gene pool, crossing barriers, inefficient selection and being time consuming for improved variety development (Munro 1987; Abdellatef and Khalafalla 2008). To overcome such problems of conventional breeding, advanced biotechnological methods which have emerged as most important tools in agricultural research can be applied as alternative approaches for genetic improvement of this crop. Plant biotechnology seems to be an attractive way to improve cotton plants, but its use requires an effective *in vitro* culture system using somatic tissues of plant. *In vitro* culture allows circumventing aforementioned difficulties: e.g. callus obtained from any explant is an ideal material for genetic transformations (Finer and McMullen 1990). Comprehensive studies have been conducted on callogenesis in cotton from several cultivars using various explants and growth regulator combinations for the initiation and maintenance of callus in different *Gossypium* species by number of laboratories. The main factors that influence the efficiency of callogenesis and determine the tissue culture response in cotton and other recalcitrant crops include genotype (Seabrook and Douglas 2001), donor plant and type of plant growth regulators, or PGRs (Trolinder and Goodin 1988; Sun *et al*. 2006) and culture medium (Popelka and Altpeter 2001). The current standard strategy for cotton transformation involves gene transfer and regeneration via somatic embryogenesis, but it is successful only for a handful of cultivars. The objective of the present study was to investigate the effect of genotype, PGR in culture medium and explant type on cotton callus induction and proliferation, which is a major prerequisite for an *in vitro* plant regeneration system involving elite Indian upland cotton genotypes.

MATERIALS AND METHODS

Seed germination and cultivation of sterile seedlings

Seeds of five cotton cultivars viz. 'PIL-8', 'F 1378', 'F 1861', 'LH 1995' and 'LH 2076' used as source material were obtained from the Cotton Section, Department of Plant Breeding and Genetics, and were delinted by using concentrated commercial H_2SO_4. The seeds were continuously stirred in H_2SO_4 by a wooden rod for 5~10 min until the surface of seeds appeared shiny. Some water was then added and stirred for a few seconds. The seeds were washed thoroughly five times with tap water to remove the acid completely, left in a beaker of water for few minutes, after which those floating on the water surface were discarded. Plump, mature seeds were chosen and washed in a solution containing a few drops of Tween 20 to which water was added, vigorously shaken and then thoroughly washed thrice by autoclaved water. Surface sterilization of seeds was done by using $HgCl_2$ (0.1%) + Bavistin (1.0%) solution for 6 min followed by 4-5 washings with autoclaved distilled water. The seeds were soaked in autoclaved distilled water for 6 hours and then sown in jam jars containing MS

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Table 1 List of media used for callus induction in present study (all values in mg/l).

Media code	Composition
M1	MS + 1.0 Kin + 1.5 IAA
M2	MS + 2.0 NAA + 0.1 Kin
M3	MSB (MS + Vit. B5) + 1.0 ZT + 1.0 Kin + 560 Pro
M4	MSB (MS+ Vit. B5) + 2.5 ZT + 1.0 2,4-D
M5	MS + 1.0 2,4-D + 1.0 Kin + 560 Pro
List of media used for callus proliferation (CP) in present study	
CP1	MS + 1.0 Kin + 1.0 IAA
CP2	MS + 1.0 Kin + 0.5 2,4-D
CP3	MS + 1.0 Kin + 1.0 NAA
CP4	MS (Vit. B5) + 1.0 ZT + 1.0 NAA
CP5	MS (Vit. B5) + 1.0 ZT + 1.0 2,4 -D
CP6	MS + 1.0 ZT + 1.0 IAA
CP7	MS + 1.0 BAP + 0.5 2,4 –D
CP8	MS + 1.0 BAP + 1.0 IAA

Abbreviations: BAP, 6-benzylaminopurine; 2,4-D, 2,4-dichlorophenoxy acetic acid; IAA, indole-3-acetic acid; Kin, kinetin; MS, Murashige and Skoog; MSB, MS+ Vit. B5; NAA, α-naphthalene acetic acid; Pro, proline; ZT, zeatin

(Murashige and Skoog 1962) medium supplemented with 8 g/l agar for germination at 28 ± 2°C in the dark. All sterilization work was performed in a laminar airflow cabinet.

Initiation and proliferation of callus

After five days of radical emergence, hypocotyl sections (3-5 mm in length), cotyledonary leaf pieces (10~16 mm^2 surface area), of sterile seedlings were placed on different media (**Table 1**). The pH of the media was adjusted to 5.8 and PGRs were added to the medium which was then autoclaved for 20 min at 121°C at 12 psi. Then, 40-50 explants (both hypocotyls and cotyledons separately) per treatment per genotype were placed on the media in jam jars (3-4 explants per jar) and test tubes (one explant per test tube) and incubated in the dark for 4 weeks at 28 ± 2°C for the induction of callus.

Observations on the number of days taken for callus induction were recorded as and when callus initiation was noticed. The percentage of callusing and callus growth score were recorded 35 days after inoculation. Growth score was recorded by visual observations adopting the following scoring system: no callusing (1), poor callusing (2), moderate callusing (3), good callusing (4) and callus index was calculated using the formula: Callus index = per cent callusing × growth score (Jeevajothi *et al*. 2005). Potential calli were identified and sub-cultured on various media containing different concentrations of IAA (indole-3-acetic acid) + Kinetin (Kin), Kin + 2,4-D (2,4-dichlorophenoxy acetic acid), NAA (α-naphthalene acetic acid) + Kin, IAA + Zeatin (ZT), ZT + Kin, ZT + 2,4-D, NAA + BAP (6-benzylaminopurine), 2,4-D + BAP and BAP + IAA (**Table 1**). All chemicals and PGRs used in the study were procured from HiMedia Pvt. Ltd., Mumbai, India. The percentage of callus induction, days taken for callus induction, the callus index and morphogenetic response were recorded. The effects of hormonal regimes, genotype and explants on aforementioned observations were analyzed.

Experimental design and statistical analysis

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

RESULTS AND DISCUSSION

The current standard strategy for cotton transformation involves gene transfer and regeneration via somatic embryogenesis, but it is successful only for a handful of cultivars. Five upland Indian cotton lines were tested on different callus induction and embryo initiation–maturation media that were previously found (Trolinder and Goodin 1988; Sakhanokho *et al*. 2001; Zhang *et al.* 2001; Haq 2005; Laleefe 2005; Rao *et al.* 2006) to be capable of inducing somatic embryogenesis in diverse cotton species by using hypocotyl and cotyledon explants. The results showing the effects of genotype, PGRs and explants are reported next.

Effect of genotype on callus induction

Five cotton cultivars viz. 'PIL-8', 'F 1378', 'F 1861', 'LH 1995' and 'LH 2076' were evaluated *in vitro* for their potential to induce callus, its proliferation and somatic embryogenic potential or embryogenicity. All genotypes tested produced callus on different media within 4 to 6 weeks. Callus formation was observed in all genotypes although different genotypes showed variable callus formation response on different media. Callus was initiated at cut ends of explants first when placed in culture media; thereafter, cells of explants proliferated actively, forming callus (**Fig. 1A, 1B**). It was inferred that endogenous hormones were released from cut sides that helped to produce more callus. Statistical analysis of arc sine-transformed data revealed significant differences among genotypes with respect to percentage induction of callus. The average percentage of callus induction

Table 2 Effect of media and genotype on callus induction in hypocotyls explants.

Genotypes/Media code	Callus induction (%)					Mean
	PIL 8	F 1378	F 1861	LH 1995	LH 2076	
M1	72.58 (58.42)	52.86 (46.63)	58.47 (49.87)	52.09 (46.19)	68.63 (55.71)	60.52
M2	57.36 (49.23	67.36 (55.13)	53.42 (46.96)	57.61 (49.37)	58.14 (49.68)	58.77
M3	78.44 (62.33	59.67 (50.57)	74.91 (59.94)	61.20 (51.47)	66.39 (54.56)	68.12
M4	60.73 (51.19)	53.69 (47.11)	35.05 (36.30)	53.42 (46.96)	58.09 (49.65)	52.19
M5	55.33 (48.05)	52.34 (46.34)	60.92 (51.30)	50.16 (45.09)	57.36 (49.23)	57.62
Mean	64.88	57.18	58.95	54.89	61.32	
CD at (0.05)	Genotypes: 5.35; Media: 8.30; Genotypes × Media: 10.85					

Values in the parentheses are arc sine transformed

Table 3 Effect of media and genotype on callus induction in cotyledonary explants.

Genotypes/Media code	Callus induction (%)					Mean
	PIL 8	F 1378	F 1861	LH 1995	LH 2076	
M1	59.92 (50.72)	43.35 (41.17)	44.81 (42.02)	48.02 (43.86)	53.33 (46.33)	49.68
M2	47.28 (43.44)	40.50 (39.52)	39.98 (39.21)	42.20 (40.50)	50.16 (45.09)	44.02
M3	55.22 (47.99)	44.31 (41.73)	52.50 (46.26)	42.33 (40.58)	54.82 (47.46)	49.83
M4	43.41 (41.21)	35.57 (36.61)	27.75 (31.78)	40.87 (39.73)	52.16 (46.23)	39.95
M5	44.84 (42.03)	40.55 (39.55)	45.11 (42.19)	36.28 (37.03)	40.87 (39.43)	41.53
Mean	50.13	40.85	42.30	41.94	50.06	
CD at (0.05)	Genotypes: 4.15; Media: 6.48; Genotypes x Media: 7.65					

Values in the parentheses are arc sine transformed

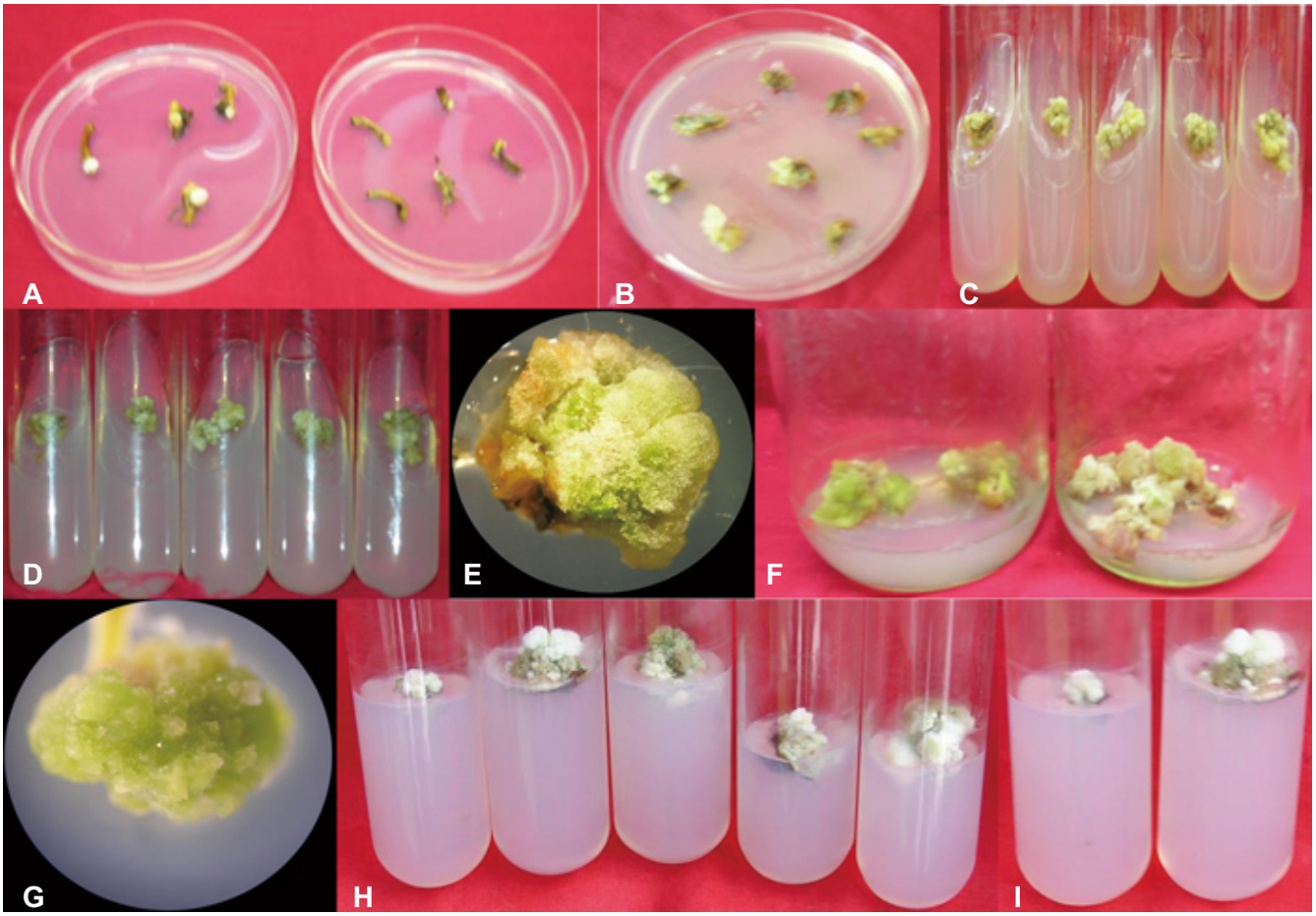

Fig. 1 ***In vitro*** **callus induction in cotton.** (**A, B**) Callus initiation at cut ends of hypocotyl and cotyledon explants on M3 medium; (**C**) Rapid callus growth from hypocotyls explants; (**D**) Callus induced on MS media supplemented with Kin (1.0 mg/l); (**E**) Stereoscopic view; (**F**) Callus on MSB media supplemented with ZT (1.0 mg/l); (**G**) Stereoscopic view; (**H**) Callus grown on MS medium supplemented with 2,4-D (1.0 mg/l); (**I**) Callus growth on MSB medium supplemented with NAA (1.0 mg/l) + ZT (1.0 mg/l).

Table 4 Effect of media and genotype on the days taken for callus induction in cotton (explant: hypocotyl).

Genotypes/Media code	Days taken for callus induction					Mean
	PIL 8	F 1378	F 1861	LH 995	LH 2076	
M1	29.58	42.16	40.58	32.38	32.38	36.19
M2	27.69	36.70	38.83	34.33	35.33	34.57
M3	24.92	37.53	39.66	38.11	38.41	35.72
M4	26.55	39.56	40.16	40.08	37.95	36.86
M5	25.35	37.48	38.41	37.00	36.41	34.93
Mean	26.81	38.68	39.52	37.15	36.09	
CD at (0.05)	Genotypes: 2.35; Media: 1.48; Genotypes x Media: 2.65					

Table 5 Effect of media and genotype on the days taken for callus induction in cotton (explant: cotyledon).

Genotypes/Media code	Days taken for callus induction					Mean
	PIL 8	F 1378	F 1861	LH 995	LH 2076	
M1	33.62	42.66	47.55	40.38	42.16	41.27
M2	36.53	40.93	45.05	42.11	45.50	42.48
M3	34.38	40.93	45.65	44.83	45.16	42.19
M4	37.38	44.91	46.00	43.88	46.41	43.71
M5	34.49	44.16	45.11	44.38	45.96	42.82
Mean	35.28	43.18	45.87	43.11	45.03	
CD at (0.05)	Genotypes: 3.85; Media: 1.65; Genotypes x Media: 2.40					

among the genotypes ranged from 57.18 to 64.88% when hypocotyls were used as explants (**Table 2**), while in cotyledon explants the average response was low and varied from 40.85 to 50.13% (**Table 3**). The genotype 'PIL-8' recorded the highest callus induction (78.44%) in M3 medium using hypocotyl explant followed by 'F1861' (74.91%) on the same medium. Similarly, varietal differences were observed for per cent callus induction when cotyledons were used as explants. Again, genotype 'PIL-8' exhibited the maximum callus induction (59.92%) in M1 medium closely followed by 55.22% in M3 medium while 'LH 2076' ranked second with respect to callus induction, recording 54.82 and 53.33% in M3 and M1 media, respectively (**Table 3**). The genotype 'LH 1995' scored the lowest level of callus induction (54.89%). The genotypic influence on callusing in cotton has been studied by earlier workers (Trolinder and Goodin 1987; Laleefe 2005; Haq 2005) who reported that differential response of genotypes for callus induction and proliferation depended on apparently minor differences in the genotype of mother plants.

Data regarding the effect of genotype on days taken for callus induction revealed that there was a significant dif-

ference among genotypes for days taken to callus induction. Mean number of days for callus induction among different genotypes using hypocotyl explants varied from 26.81 to 39.52 days (**Table 4**) while in cotyledon explants the corresponding values ranged from 35.28 to 45.87 days (**Table 5**). In both the cases, 'PIL-8' was only the genotype that induced callus within 25 to 35 days, while other genotypes took more than 40 days for callus induction.

Effect of media on callus induction

Different hormonal combinations [auxins (IAA, NAA; 2,4-D) and cytokinins (Kin; ZT)] were used in MS and MSB basal media that permitted induction of callus in *G. hirsutum* L. genotypes. Explants, when placed on media containing sucrose, nutrients and phytohormones, swelled, and callus was initiated at cut ends first. The contact area of cut sides produce more callus. All genotypes tested produced callus on different media within 4 to 6 weeks. Significant differences in callus induction and days taken to callus induction existed for different media. The mean percentage of callus induction among various media ranged from 52.19 to 68.12% in hypocotyl explants and 39.95 to 49.83% in cotyledon explants. Medium M3 recorded the highest (68.12%) average callus induction followed by M1 (60.52%) and M2 when explants were hypocotyls (**Table 2**). In case of cotyledon explants the performances of media were in similar order but showed lower percentage of callus induction (**Table 3**). While, medium M4 recorded the lowest percentage of callus induction (52.19%, 39.95%) in both the explants, indicating the insufficient auxin: cytokinin concentration for induction of callus. Both low (1.0 mg/L) and high (2.5 mg/L) concentrations of ZT could induce hypocotyl and cotyledon explants to produce callus. A wide range of callus induction on hypocotyl and cotyledon in cotton using different concentration of ZT (0.1-7.0 mg/l) has been documented by Zhang *et al.* (2001). Addition of 2,4-D promoted the formation and growth of cotton callus. This might be due to the direct interference of 2,4-D with IAA synthesis in the tissues or hastening of IAA degradation as reported by Elliot *et al.* (1978). Different plant growth regulators (PGRs) at different concentrations and various combinations have been tried for their effects on cotton callus culture by other workers (Shoemaker *et al.* 1986; Trolinder and Goodin 1987; Zhang *et al.* 2001; Rao *et al.* 2006; Abdellatef and Khalafallah 2008) who observed differences based on PGR type and concentration. These results are similar to those in Zhang *et al.* (1996) which showed that suitable medium for callus induction was MS + Vit B5 + 0.1 mg/L 2, 4-D + 0.5 mg/L Kin. It is clear from this study that suitable concentration of kin/ZT plays some role in callogenesis.

Further, data recorded regarding the effect of media on days taken for callus induction revealed a non-significant difference among various media for days taken to callus induction. Mean number of days for callus induction among different media using hypocotyl explants varied from 34.57 to 36.86 days (**Table 4**) while in cotyledon explants the corresponding values ranged from 41.27 to 43.71 days (**Table 5**).

Effect of explant source on callus induction

Two explants (hypocotyls and cotyledon) excised from *in vitro* grown 5-6 days old seedlings of five cotton cultivars were placed on different media to investigate their effects on callus formation and its subsequent regeneration. When the two explants were compared for callus induction, hypocotyls recorded significantly higher percentage of callusing ranged from 52.19 to 68.12% with an average of (59.44%) than that of cotyledon for which the corresponding figures were 39.95 to 49.83% and (45.00%), respectively (**Table 6**). Hypocotyl explants were most responsive to callus induction and proliferation. The maximum callus percentage (68.12%) was recorded in M3 medium followed by M1 (60.52%) and M2 (58.77%) with hypocotyls as explants while corresponding figures for these media in cotyledons as explants were 49.83, 49.68 and 44.02%, respectively. The medium M4 exhibited the minimum (52.19, 39.95) percentage callus induction in both explants (**Table 6**). A high percentage of callus induction for hypocotyls has been reported in different studies (Shoemaker *et al.* 1986; Zhang *et al.* 2001; Lashari *et al.* 2008). Statistical analysis of data revealed significant differences for callus induction, days taken to callus induction in different explants and media composition. Of the two explants compared, callus induction was more rapid (35.65 days) in hypocotyls than in cotyledons (42.49 days) (**Table 7**). This might be due to the presence of juvenile tissues where rate of cell division would be higher than in other parts. Hypocotyl explants formed callus more readily than explants from cotyledons, a result that agrees with earlier findings (Sakhanokho *et al.* 2001; Zhang *et al.* 2001; Rao *et al.* 2006; Abdellatef and Khalafallah 2008). Furthermore, callus initiation and production was maximized by increasing more contact between the cut surface of explants and media by longitudinally dissecting explants (hypocotyls) placed on media.

Table 6 Effect of media and explants on callus induction in cotton.

Explants/	Callus induction (%)		Mean
Media code	Hypocotyls	Cotyledons	
M1	60.52	49.68	55.10
M2	58.77	44.02	51.39
M3	68.12	49.83	58.97
M4	52.19	39.95	46.07
M5	57.62	41.53	49.57
Mean	59.44	45.00	
CD at (0.05)	Explants: 7.85; Media: 3.65; Explants x Media: 10.40		

Table 7 Effect of media and explants on the days taken for callus induction in cotton.

Explants/	Days taken for callus induction		Mean
Media code	Hypocotyls	Cotyledons	
M1	36.19	41.27	38.73
M2	34.57	42.48	38.52
M3	35.72	42.19	38.95
M4	36.86	43.71	40.28
M5	34.93	42.82	38.87
Mean	35.65	42.49	
CD at (0.05)	Explants: 4.85; Media: 0.65; Explants x Media: 2.40		

Effect of hormonal regime and explants on callus proliferation and morphogenetic response

Different PGRs at different concentrations and combinations were tried for their effects on cotton callus culture. Calli were visually evaluated twice, first after one month of initiation and then after 45 days of culture. The results indicated that cotton callus was induced on MS basal medium supplemented with PGR. However, differences based on PGR type and concentration on the percentage of callus proliferation, callus growth score, callus index, morphogenetic response and embryogenicity were observed (**Table 8**).

When fast-growing calli were induced from hypocotyls (**Fig. 1C**) and cotyledon explants were sub-cultured on various media containing MS basal media and different concentrations of PGRs a wide range for percentage callus proliferation was observed that varied from 36.20% (CP8) to 68.12% (CP4) with an average of 55.53% for hypocotyls and 22.45 to 49.83% with a mean of 39.43% for cotyledon explants (**Table 8**). Hypocotyl explants also recorded higher mean callus growth index (126.99) than cotyledon (71.28) and callus easily proliferated. Among different media tested the callus growth index ranged from 72.40 in CP8 to 204.36 in CP4 for hypocotyls and 22.45 in CP8 to 119.85 in CP5 for cotyledon explants. Essentially, the effects of different PGRs on cotton callus induction and proliferation have

Table 8 Effect of hormonal regime and explant on callus proliferation and morphogenetic response in cotton.

Media code	Explant	Callus proliferation (%)	Callus growth score	Callus growth index	Callus colour	Compactness
CP1	H	60.52	+++	181.56	Greenish	Compact
	C	49.68	+	49.68		Compact
CP2	H	58.77	++	117.54	Green	Loose
	C	44.02	++	88.04		Loose
CP3	H	65.25	++	130.50	Green	Compact
	C	39.50	++	79.00		Compact
CP4	H	68.12	+++	204.36	Grayish yellow	Loose
	C	49.83	++	99.66		Compact
CP5	H	52.19	++	104.38	Greenish yellow	Friable
	C	39.95	+++	119.85		Loose
CP6	H	57.62	++	115.24	Light green	Compact
	C	41.53	++	83.06		Loose
CP7	H	45.50	++	90.00	Light yellow	Loose
	C	28.50	-	28.50		Friable
CP8	H	36.20	++	72.40	Greenish yellow	Loose
	C	22.45	-	22.45		Loose
Mean hypocotyl		55.53		126.99		
Mean cotyledon		39.43		71.28		

*Cultures were evaluated at 45 days of culture. Number of + indicates the growth of induced callus; more +, more growth of induced callus; -, indicates trace growth of induced callus, H, hypocotyl; C, cotyledon

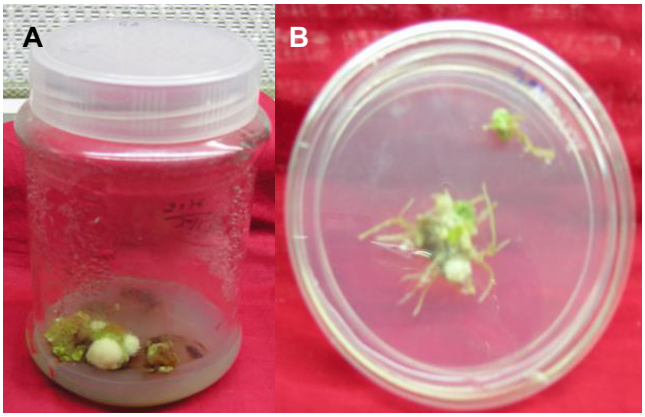

Fig. 2 ***In vitro*** **regeneration of callus culture.** (**A**) Callus resuming growth on regeneration medium and its browning. (**B**) Callus showing emergence of roots on regeneration medium supplemented with Kin (1.0 mg/l).

already been reported (Trolinder and Goodin 1987; Firoozabady *et al.* 1987; Sakhanokho *et al.* 1998).

Several types of calli were distinguishable based on their physical appearance. Callus induced on MS medium containing Kin was initially greener compared to other auxin-supplemented media (**Fig. 1D**). However, medium containing Kin resulted in the formation of compact callus. Callus on MS (VitB5) medium containing ZT (CP5) was initially grayish yellow and friable but grew into heterogeneous types (embryogenic and non embryogenic callus) after about 1 month (**Fig. 1E**). Callus on MS containing BAP (CP7) was homogeneous, loose and greenish. Of the three auxins used in media, callus on medium containing 2,4-D was homogeneous, loose and greenish (**Fig. 1H**). In contrast, callus on MSB medium containing 2,4-D and ZT and IAA was homogeneous and grayish yellow or gray and friable (**Fig. 1F**) while NAA with Kin (CP3) and ZT (CP4) promoted growth of cotton callus. Combinations of NAA with ZT (CP4) induced grainy, nodular texture of high quality callus (**Fig. 1I**). Non-embryogenic callus was slow-growing, compact, light brown or dark green. Some of these turned brown (**Fig. 2A**) and died. This types of variation with respect to color and texture of calli has been observed by several researchers (Shoemaker *et al.* 1986; Trolinder and Goodin 1987; Finer 1988; Zhang *et al.* 2001; Laleefe 2005). Among the different media tested three combinations showed embryogenic calli but when cultured on regeneration medium these turned brown and died. However, medium containing Kin (CP3) resulted in the formation of compact callus with large numbers of roots emerging from it (**Fig. 2B**).

Most of the successful strategies for cotton transformation involve the development of embryogenic callus and subsequent plant regeneration. Cotton is a recalcitrant crop and the success is limited to only those varieties which are regenerable, like Coker lines (Trolinder and Chen 1989). Although to date, no Indian genotype has been identified with such regeneration properties yet, however, attempts were undertaken to induce regeneration in different genotypes and to learn more about the regeneration process of Coker as standard using different explants and regeneration procedures. In the present investigation, conditions for initiation of callus in cotton were optimized in five local genotypes. Callogenesis showed a range of responses depending on medium formulation, PGR combinations and concentrations. The induction of callus from hypocotyls was better than that from cotyledons. Callogenesis was genotype-dependent.

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