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Effect of growth hormones, genotypes and explants on callus induction in cotton (*Gossypium hirsutum* L.)

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ABSTRACT : Seven days old cotyledon and hypocotyl explants of Coker genotypes (Coker 310 and Coker 312) and Indian genotypes (MCU 5 and SVPR 2) were evaluated with different levels of plant growth hormones in MS medium for callus induction. In the present study, the growth hormones *viz.*, IBA, 2,4-D with KT, 2,4-D with TDZ, pIC with KT, pIC with TDZ, NAA with BA, NAA with TDZ and ZT was supplemented to evaluate the callus induction frequency. The best callus induction (85.10 %) was identified from MS medium containing pIC and TDZ followed by (83.09 %) from the MS medium supplemented with pIC and KT. Among the genotypes studied, the highest callus induction frequency was exhibited by Coker 310 (79.59 %) and the lowest callus induction was recorded by MCU 5 (61.65 %). Hypocotyl recorded the mean value of 72.34 per cent for the callus induction in different hormonal combinations studied. The mean value of callus induction was 66.56 per cent in cotyledon and it took more time for the callus induction. Hypocotyl was identified as the best explant for the callus induction.

Key words: Callus, cotton, explants, genotypes

Cotton (*Gossypium hirsutum* L.) is one of the most important commercial crops grown throughout India. Cotton contributes 29.9 per cent of the Indian agricultural gross domestic product and provides livelihood to nearly 6 crore people with half of this population employed directly by the textile industry.

Genetic improvement of cotton through conventional breeding has limitation due to several factors like lack of useful variation and prolonged periods that are required to complete one breeding cycle. To overcome the problems of conventional breeding, advanced biotechnological method emerged as most important tool in agricultural research which can be applied as alternative approach for genetic improvement. Establishment of efficient callus induction protocol is pre requisite to cell and tissue culture techniques in crop breeding. Many factors influences the efficiency of callogenesis procedure. The main factors determining the tissue culture response in cotton and other recalcitrant crops include growth regulators and genotypes. Hence the present investigation was undertaken to develop an efficient callus induction protocol, which is a major pre requisite for *in vitro* regeneration system.

MATERIALS AND METHODS

Laboratory experiments were carried out at the Genetic Transformation Laboratory, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during 2007-2010.

Seed germination and explant preparation: Coker genotypes (Coker 310 and 312) and Indian genotypes (MCU 5 and SVPR 2) were acid delinted and seeds were surface sterilized with 70 per cent ethanol for 1 min and then washed 3 times with sterile distilled water. These were again surface sterilized with 0.1 per cent mercuric chloride for 10 min followed by 3 washes with sterile distilled water. The surface sterilized seeds were germinated on ½ strength MS medium supplemented with one per cent (w/v) sucrose (30 g/l) and agar (8 g/l). The inoculated seeds were incubated at 25 ± 2° C in 16 / 8 h light/dark for seed germination. Explants of hypocotyl (4-6 mm^2) and cotyledonary leaf (16 mm^2) sections from 7 days old seedlings of Coker

310, Coker 312, MCU 5 and SVPR 2, were used for callus induction.

Growth harmones: Seven days old explants were placed on MS medium with different concentrations of IBA (Indole Butryic Acid) alone, 2,4-D (2, 4-Dichlorophenoxyacetic acid) with KT (Kinetin), 2,4-D with TDZ (Thiadiazuron), pIC (Picloram) with TDZ, pIC with KT, NAA (Naphthaleneacetic acid) with BA (Benzyl adenine purine), NAA with TDZ and ZT (Zeatin) alone (Table 1). The experiment was conducted in 16h photoperiod (1000 lux) followed by 8h dark condition for callus induction. MS salts were supplemented with different growth hormones, maltose (30 g/l) and solidified with agar (8 g/l).

RESULTS AND DISCUSSION

The analysis of variance showed that highly significant difference for callus induction was due to phytohormones, genotypes and explant types. The interaction effects between hormones and genotypes, hormones and explant types, genotype and explant types were highly significant indicating that the differences due to hormones for callus induction were influenced by both genotypes and explants.

Effect of plant growth regulators: Plant growth regulators are widely known as one of the most important factors affecting callus induction and formation. Different species and varieties have specific requirements for nutrients and hormones. The best callus induction (85.10 %) was identified from MS medium containing pIC and TDZ followed by (83.09 %) from the MS medium supplemented with pIC and KT (Table 2). TDZ induced a 30 fold increase in the growth of callus cultures over other plant growth regulators. In addition, the callus absorbed less TDZ than other plant growth regulators, thereby indicating a relatively high intrinsic activity of TDZ. Meanwhile the best callusing response obtained in the present investigation was MS medium containing 2,4-D with KT produced 76.13 per cent of callus with well proliferation. The most effective auxin for callus induction and proliferation was 2,4-D, which is powerful suppressant of organogenesis and often induced callus even in the absence of endogenous cytokinin. Most of the published works also indicated that the MS based medium containing 2,4-D (auxin) and kinetin (cytokinin) is suitable for callus induction (Cao *et al.,* 2008).

Effect of genotypes: The highest callus induction frequency was exhibited by Coker 310 (79.59%) and the lowest by MCU 5 (61.65%). Zouzou *et al.*, (2008) also observed significant genotypic differences in callus initiation response. This positive response of Coker on callogenesis was already reported by Rajeswari *et al.*, (2010). The order of callus induction in the

Table 1. MS medium supplemented with different growth hormones with different concentrations used for callus induction

Experiment	Harmones	Treatments (mg/l)					
1	IBA alone	0.1	0.5	1.0	1.5	-	-
2	2,4-D	0.1	0.1	0.5	0.5	-	-
	KT	0.1	0.5	0.1	0.5	-	-
3	2,4-D	0.1	0.1	0.5	0.5	-	-
	TDZ	0.01	0.05	0.01	0.05	-	-
4	pIC	0.5	0.5	1.0	1.0	-	-
	KT	0.1	0.5	0.1	0.5	-	-
5	pIC	0.5	0.5	1.0	1.0	1.0	1.0
	TDZ	0.01	0.05	0.01	0.05	0.01	0.05
6	NAA	0.5	1.0	0.5	1.0	-	-
	BA	1.0	1.0	-	-	-	-
	TDZ	-	-	0.05	0.05	-	-
7	ZT	0.1	0.5	1.0	1.5	-	-

Table 2. Effect of plant growth hormones in genotypic differences for callus induction (%) in cotyledon and hypocotyl

S. No.	Plant growth hormones	Cotyledon				**Mean**	Hypocotyl				**Mean**	**Overall mean**
		Coker 310	Coker 312	MCU 5	SVPR 2		Coker 310	Coker 312	MCU 5	SVPR 2		
1	IBA alone	66.04	58.54	43.95	48.33	**54.22**	66.54	61.88	48.33	48.75	**56.87**	**55.55**
		(54.93)	(49.99)	(37.71)	(40.14)	**(45.65)**	(56.73)	(52.17)	(40.14)	(40.38)	**(47.36)**	**(46.5)**
2	2,4-D, KT	82.29	77.92	67.21	70.63	**74.51**	86.04	80.83	70.62	73.54	**78.07**	**76.13**
		(65.99)	(62.05)	(55.1)	(57.2)	**(60.08)**	(70.22)	(64.14)	(57.2)	(59.06)	**(62.66)**	**(61.37)**
3	2,4-D,TDZ	52.71	56.88	48.54	55.96	**54.27**	59.38	64.79	55.00	59.37	**59.63**	**56.95**
		(46.42)	(48.97)	(43.86)	(50.27)	**(47.38)**	(50.51)	(54.07)	(47.65)	(50.84)	**(50.69)**	**(49.03)**
4	pIC, KT	91.04	80.00	73.33	76.46	**80.20**	93.54	88.13	76.46	85.83	**85.99**	**83.09**
		(72.8)	(63.25)	(58.95)	(60.99)	**(64.07)**	(76.31)	(69.96)	(60.99)	(67.91)	**(68.79)**	**(66.43)**
5	pIC, TDZ	94.58	79.17	74.44	80.97	**82.29**	96.94	87.92	81.11	85.94	**87.92**	**85.10**
		(78.42)	(62.98)	(59.72)	(64.34)	**(66.37)**	(82.92)	(69.92)	(64.43)	(67.94)	**(71.3)**	**(68.83)**
6	NAA, BA, TDZ	65.00	50.42	39.17	53.96	**52.14**	70.21	56.46	51.46	53.96	**58.02**	**55.08**
		(55.62)	(45.07)	(38.32)	(47.49)	**(46.63)**	(58.68)	(49.1)	(45.92)	(47.49)	**(50.3)**	**(48.46)**
7	ZT	75.63	55.83	51.46	60.00	**60.73**	96.58	67.71	58.75	60.00	**70.76**	**65.74**
		(60.71)	(48.40)	(45.98)	(50.91)	**(51.5)**	(80.83)	(55.56)	(50.17)	(50.91)	**(59.37)**	**(55.45)**
Overall mean		**76.61**	**66.44**	**58.04**	**65.14**	**66.56**	**82.57**	**73.56**	**65.25**	**68.00**	**72.34**	**69.45**
		(63.19)	**(55.00)**	**(49.27)**	**(53.74)**	**(55.3)**	**(68.93)**	**(59.98)**	**(53.82)**	**(55.78)**	**(59.63)**	**(57.46)**
		T	G		E	TG	GE		TE	TGE		
SED		0.239	0.169		0.119	0.478	0.239		0.338	0.677		
CD (p=0.05)		0.473	0.334		0.236	0.947	0.473		0.669	1.770		

(T - Treatment, G - Genotype, E - Explant; The values in paranthesis are sine transformed values)

different genotypes was Coker 310 > Coker 312 > SVPR 2 > MCU 5.

Effect of explants: The requirement of different growth regulators for the initiation of callus from explants in different genotypes may be due to endogenous levels of hormones in the explants. Of the two explants used for callus induction, hypocotyl (72.34 %) recorded higher callusing percentage than cotyledons (66.56 %) irrespective of the genotypes. Such high callusing response in hypocotyl was also reported by Ghasemi *et al.*, (2011) and Han *et al.*, (2009) (Table 3). Rapid callus development in hypocotyl tissue may shorten the culture duration, thus reducing the occurrence of somoclonal variation, a major problem in cotton tissue culture.

Callus morphology: A callus characteristic such as colour, texture and friability plays a major role in the successful regeneration of cotton *via* somatic embryogenesis. In the present study, callus induced from different growth regulators differed in their colour, texture and friability indicated that it was highly dependent on the combination of auxins and cytokinins (Table 3). Calli produced in the media having a low level of 2,4-D with KT was smooth, highly friable and appeared creamy white to yellow or brown in colour. Increased level of 2,4-D produced brown coloured watery callus with low friability. Among the hormonal combinations, 2,4-D combined with kinetin produced faster growth with highly embryogenic calli.

CONCLUSIONS

Development of an efficient tissue culture protocol for cotton varieties is the first step towards the application of transgenic technology to improve cotton breeding. We have established callus induction protocol for Coker genotypes and Indian genotypes. This protocol will pave the way for the development of *in vitro* regeneration system for this elite cultivars and

Table 3. Characteristics of calli produced on various hormonal combinations

Growth regulators	Days taken for callus induction	Colour	Texture	Friability	Response
IBA	9-12	Brown, Yellowish green	Compact, hard	Medium	Non embryogenic calli
2,4-D, kinetin	5-7	Yellowish green, light yellow, creamy white, brown	Smooth, homogenous	High	Embryogenic
2,4-D, TDZ	9-12	Creamy white turning brown, brown	Smooth	Medium	Embryogenic
Picloram, kinetin	10-12	Greenish yellow and transparent	Smooth, homogenous	Medium	Embryogenic
Picloram, TDZ	12-15	Light yellow, creamy white and brown	Smooth	Low	Embryogenic
NAA, BA	10-12	Light green	Compact	Low	Calli with rooting
NAA, TDZ	10-15	Greenish yellow	Compact, hard	Low	Calli with rooting
Zeatin	12-15	Grayish yellow	Friable, heterogenous	Low	Embryogenic

Observations were recorded on two months old culture

consequently will promote the application of plant tissue culture technology in the area of selecting resistance, production of artificial seeds, and genetic transformation.

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