Research Article

Optimization of shoot apex based cotton regeneration system

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Abstract

Cotton (*Gossypium* spp.) belonging to the genus *Gossypium* in the family Malvaceae. Cotton is an important fibre crop of global importance. It is grown in tropical and subtropical regions of more than 80 countries with an annual production of 20 million tonnes. It is an important source of oil and high quality protein meal and plays a significant role in the national economy. Among the cotton producing countries, India has the distinction of having the largest cotton growing area in the world with all four cultivated species under cultivation. The recalcitrance of cotton to regeneration through somatic embryogenesis has slowed down the development of transgenic cottons. Hence alternate strategies to obtain transgenic cotton *via* organogenesis in some varieties have been taken up. A direct shoot regeneration protocol was optimized for three cultivars of cotton Coker310, MCU-5, SVPR-2. Shoot apex from vigorous seedlings were placed in six different shoot apex media composition for shoot development. The efficiency of shoot development was higher when the shoot apices were cultured on SAM1 (MS+ 0.1 mg/l kinetin) irrespective of the genotypes. The nine day-old explants had a significant effect on shoot development. Among the different Regeneration media tried, the best rooting response was observed on RM2 (MS+ 0.1 mg/l GA_3 + 1.0 mg/l IAA).

Key Words-Cotton, Direct organogenesis, Shoot apex

Introduction

Cotton is the most important source of natural fiber. Many factors *viz*, biotic, abiotic stress attribute for the low yield of cotton which needs genetic enhancement. Among the biotic stresses, bollworms are very important pests which cause severe damage and crop losses. Conventional breeding techniques to infuse genetic resistance from cultivated and wild relatives have not resulted in successful genotypes. Hence, the alternative procedures like molecular breeding techniques are attempted in this crop worldwide. Success has also been achieved in these programmes and hybrids impregnated with genetic resistance have been developed and released for general cultivation. Genetic engineering offers a direct method of plant breeding that selectively targets one or few traits for introduction into the crop plant. The development and commercial release of transgenic cotton plants relies exclusively on two basic requirements. The first one is a method that can transfer a gene into the cotton genome and govern its expression in the progeny. The other requirement is the ability to regenerate fertile plants from transformed cells. This is achieved by regenerating plants *via* somatic embryogenesis or from direct organogenesis.

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Materials and method

Surface sterilization and preparation of culture media

Acid-delinted seeds of MCU 5, SVPR 2 and Coker 310 were surface sterilized with 70 per cent ethanol for 2 min and then washed three times with sterile distilled water. They were again surface sterilized with 0.1 per cent mercuric chloride for 10 min followed by three washes with sterile distilled water. The surface sterilized seeds were germinated on half strength MS medium supplemented with one per cent (w/v) sucrose and 10 g/l agar. The pH of the medium was adjusted to 5.7-5.8 (by using 0.1N KOH or 0.1N HCl) prior to autoclaving.

Shoot apex isolation

Shoot apices were isolated from 9-11 day-old seedlings. The shoot apex was exposed by pushing down one cotyledon until it broke away. The apex was removed just below the attachment of the unexpanded leaf. Additional tissue was removed to expose the base of the shoot apex. The isolated shoot apex was then placed on shoot apex medium for shoot development.

Shoot development

The isolated shoot apices from the three cotton varieties MCU 5, SVPR 2 and Coker 310 were placed on shoot apex medium with different concentration and combination of plant growth regulators (Table 1) for two weeks to induce shoot development. Shoot development was recorded for each variety.

Effect of shoot apex age

To test the effect of age of the shoot apex on shoot development, shoot apex were excised from 5, 7, 9, 11 day-old seedlings. The shoot development frequency of apex was assessed on SAM1.

Rooting of shoots and Hardening

The well developed shoots were then transferred from shoot apex medium to rooting medium. Different media compositions tried for rooting are listed in Table 2. After three weeks, the number of rooted shoots was recorded. The rooted shoots were subsequently transplanted in pots containing sterilized soil. The plants were covered with polyethylene bags and kept in the culture room. After 15 days, the polyethylene bags were removed and well established plants were transferred to greenhouse.

Result and Discussion

Effect of genotype on shoot apex medium

The efficiency of shoot development was higher when the shoot apices were cultured on SAM1 (MS+ 0.1 mg/l kinetin) irrespective of the genotypes tested (Table 3). The highest frequency was observed in Coker 310 (56%) followed by MCU 5 (53.3%) and SVPR 2 (44.7%) (Plate 1a-b). Regeneration on Full and Half strength MS medium has been earlier observed in *G.hirsutum* shoot apices by Gould *et al*. (1991) and Zapata *et* al (1999).Vigrous shoot growth was observed when kinetin is supplemented to the media

The age of explants had a significant effect on shoot development induction (Table 4). On an average, the frequency of shoot development was 42.6 per cent from 5 day-old explant; 48.4 per cent from 7 day-old explants; 51.5 per cent from 9 day-old explant and 54.6 per cent from 11 day-old explant. Shoot apex from 9 day-old seedlings showed best response for shoot and root formation.

Thirty well developed shoot tips of each variety were transferred to different media compositions (RM1–RM6) to induce rooting for 3 weeks. The rooting response of the elongated shoots cultured on different media is presented in Table 5 and Plate 1c-d. Among the different media tried, the best rooting response was observed on RM2 (MS+ 0.1 mg/l GA_3 + 1.0 mg/l IAA) (Table 6). After two weeks culture, the plantlets were transferred in to pots containing autoclaved soil and cultured in the chamber under high humid for one week. The elongation of shoot and root development took place simultaneously resulting in the normal plantlet regeneration (Plate 1e-f). Shoot apex from 9 day-old seedlings showed best response for shoot and root formation. Similar result was observed by Bajaj and Gill (1986); Gould *et al*. (1991); Gupta *et al*. (1997) and Saeed *et al. (*1997). Protocol involving proliferation of cotton shoots (Agrawal *et al*.,1997, Gupta *et al*., 1997) or cotton shoot regeneration (Hemphill *et al*., 1998) have been published. The regeneration was carried out without a callus regeneration phase. Rooted cotton plants were obtained between 6weeks to 3 month depending on the root induction method used (Hemphill *et al*., 1998). In the present study, a complete protocol for successful *in vitro* regeneration of cotton genotypes through shoot apices was standardized (Appendix 1). With this protocol, the period from shoot development to establishment of plants can be achieved in a period of 5-6 weeks, and they could be transferred directly to soil. Two weeks later they could be transferred to the greenhouse.

Conclusion

In conclusion, the development of transgenic cotton requires an efficient means for the transformation and regeneration of fertile plants.. A direct and simple procedure of regeneration from shoot was developed and rooting was also achieved. This regeneration procedure could be coupled with *Agrobacterium*-mediated transformation for rapid introduction of value-added traits directly in to high-fiber yielding cotton genotypes.

Reference

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Table 1. Different media composition used for Shoot apex cultures

Medium	Media composition*
SAM 1	MS+ 0.1mg/l kinetin
SAM 2	MS+ 0.3mg/l kinetin
SAM 3	MS+ 0.3mg/l GA_3
SAM 4	MS+ 0.1mg/l BAP
SAM 5	MS+ 0.2mg/l BAP
SAM 6	MS +1.5mg/lBAP + 0.5mg/l kinetin

* SAM: Shoot Apex Medium; All media were supplemented with MS salts and 30 g/l maltose and solidified with 10 g/l agar.

Table 2. Different media compositions used for rooting of regenerated shoots

Medium	Media composition*
RM 1	MS basal
RM 2	0.1mg/l GA_3 + 1.0 mg/l IAA
RM 3	0.1mg/l GA_3 +1.0 mg/l NAA
RM 4	0.1mg/l NAA
RM 5	Half strength MS + 0.1mg/l IAA

* RM: Regeneration Medium; All media were supplemented with MS salts, and 30 g/l sucrose and solidified with 0.4 per cent phytagel (Sigma)

Table 3. Effect of media composition on shoot development

Medium	Shoot tip induction (%)		
	Cocker 310	MCU 5	SVPR 2
SAM 1	56.0 ±0.8^{a}	53.3±0.5^{a}	44.7±0.5^{a}
SAM 2	40.0±0.8^{b}	36.0±0.8^{bc}	32.7±0.5^{b}
SAM 3	36.7±0.5^{c}	33.3±0.5^{cd}	29.3±0.5^{c}
SAM 4	41.3±0.5^{b}	30.0±0.8^{e}	24.7±0.5^{d}
SAM 5	34.7±0.5^{c}	32.0±0.8^{de}	26.0±0.8^{d}
SAM 6	35.3±0.5^{c}	38.7± 0.5^{b}	30.7±0.5^{bc}

Values represent the mean ± standard error of three replications.
In a column, means followed by same letters are not significant at 5% level by LSD.\

Table 4. Effect of genotype and age of the shoot apex on shoot development media

Cotton Variety	Age of Explants				
	5 days	7 days	9 days	11 days	Mean
Coker310	46.6±0.6^{a}	50.6±1.1^{a}	57.3±1.1^{a}	58.6±1.1^{a}	53.2
MCU-5	42.6±0.3^{ab}	49.3±1.2^{ab}	50.6±1.3^{b}	54.6±0.6^{ab}	49.2
SVPR-2	38.6±1.3^{b}	45.3±1.3^{b}	46.6±1.3^{b}	50.6±1.3^{b}	45.2
Mean	42.6	48.4	51.5	54.6	

Values represent the mean ± standard error of three replications.
In a column, means followed by same letters are not significant at 5% level by LSD

Table 5. Frequency of rooting on rooting media

Varieties	Number of explants cultured	Number of shoots rooted	Rooting efficiency (%)
Coker 310	30	23	76.6
MCU 5	30	18	60.0
SVPR 2	30	12	40.0

Table 6. Effect of different media on rooting

Medium*	Resultant growth of somatic embryos
RM1	Reduce rooting and shooting
RM2	Normal rooting; shooting
RM3	Root elongation ; delayed shoot elongation
RM4	Root elongation, with no shooting
RM5	Very slow rooting; no shoot elongation

***RM 1 to RM 5 as in Table 2.**

Appendix 1. Regeneration of cotton genotype via shoot apex

TIME	STAGE	MEDIA
9-11 days	Seed germination	SGM
↓	↓	↓
2 weeks	Shoot apex development	SAM1
↓	↓	↓
3 weeks	Root development	RM2
↓	↓	↓
	Greenhouse	Soil

SGM- Seed germination medium

Plate1. Shoot apex based cotton regeneration system

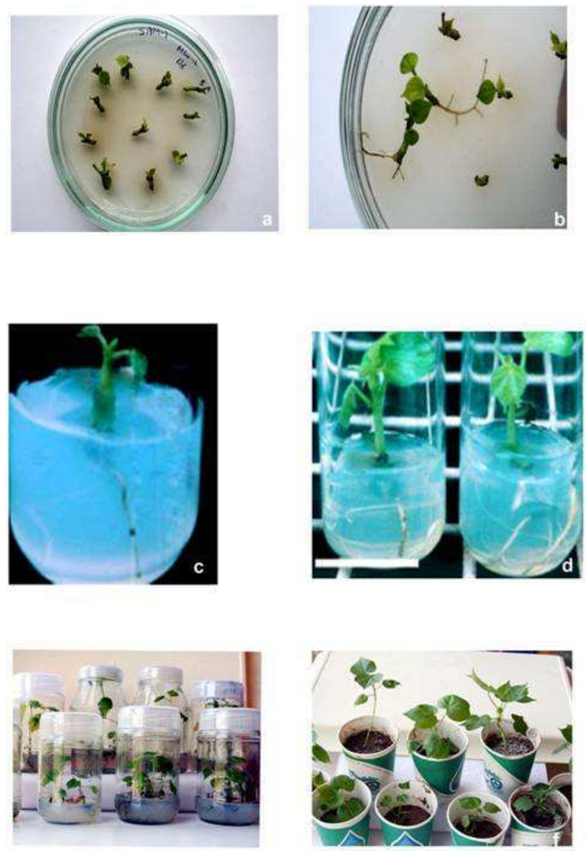

Plate 1. Shoot apex based cotton regeneration system

a & b. Shoot development on SAM
c & d. Rooting on regeneration medium
e & f. Regenerated plantlets