International Journal of Current Science Research
www.drbgrpublications.in

IP Indexing - Impact factor – 3.5

Volume: 4; Issue: 1; January-2018; pp 1497-1505. ISSN: 2454-5422

Optimization of hormones for the induction of callus, shoot and root buds of cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae)

C.Edward[1], A.Ganesh Kumar[*2] and T.Selvarathinam[2]

[1]Department of Microbiology, K.R. College of arts and science , Kovilpatti, India
[2]Department of Zoology, ANJA College (Autonomous), Sivakasi, India
[*]Corresponding author email: maga1zoo@gmail.com

Abstract

The present work is based on developing a protocol for the callus induction, shoot and root bud multiplication in cotton, *Gossypium hirsutum* L. from the shoot explant. The sterilized explant was inoculated in MS-medium containing various combination of auxin such as naphthalene acetic acid (NAA) and cytokinins such as kinetin and 6 penzyl amino purine (BAP). High frequency of callus induction was found in MS media with the combination BAP (0.5 mg/l) and NAA (1.5 mg/l) which was followed by BAP (0.5 mg/l) and IAA (1.5 mg/l). The highest frequency of shoot bud regeneration (57.2%) was observed on MS basal medium containing KIN (0.5 mg/l) and IAA (2.0 mg/l). The highest mean number of shoot bud multiplication was observed on MS medium with KIN (3.0 mg/l) and IAA (0.5 mg/l). The highest mean number of shoot bud multiplication was observed on MS medium with KIN (3.0 mg/l) and IAA (0.5 mg/l). The highest frequency of rooting was observed on MS medium containing 0.3 mg/l of IBA and 0.05 mg/l KIN. The protocol developed here can be used for genetic transformation experiments of Indian Cotton cultivars.

Keywords: Hormones, callus, shoot, root, *Gossypium herbaceum* and *Gossypium hirsutum*

Introduction

Cotton is an important economic and fiber crop, grown in 70 countries in the world. Over 180 million people are associated with the fiber industry that produces 20 to 30 billion dollars worth of raw cotton. Both diploid (*Gossypium herbaceum*) and tetraploid

(*Gossypium hirsutum*) cultivars are cultivated in different regions of Iran and are considered as important crop plants of the country. Over 100 years ago, Haberlandt envisioned the concept of plant tissue culture for the cultivation of plant tissues and organs. Plant tissue culture is now a well established technology. Around the mid of twentieth century, there is a notion that plants could be regenerated or multiplied from either callus or organ culture through either micropropagation or clonal propagation. Cotton regeneration was first observed in *Gossypium hirsutum* cv. Coker (Davidonis and Hamilton 1983). Regenerated plants have been obtained from explants such as hypocotyl, cotyledon, root, and anther and from various cotton species (Ozyigit *et al.,* 2007). *In vitro* plant regeneration from cells, tissues and organ cultures is a prerequisite for the application of plant biotechnology to plant propagation (Kehie *et al.*, 2011). The present study was undertaken to standardize the hormones for the induction of callus, shoot and root of cotton, *Gossypium hirsutum* by *in vitro* culture.

Materials and Methods

The experimental material *Gossypium hirsutum* obtained from Transgene Institute for Biotechnology and Bioinformatics, Trichy, Tamil Nadu, South India. The seeds were sterilized with 0.1 (w/v) aqueous Mercuric chloride ($HgCl_2$) solution for 5 minutes followed by washing with distilled water and then transferred to hormone and vitamin B5 added MS medium in test tubes to germinate. The seedlings were grown at 24±2 ºC with 55% relative humidity under 16h photoperiod provided by white fluorescent tubes. Nine day old seedlings were selected as source of explants. Shoot tips and node from *in vitro* seedlings or regenerated shoots were excised and cultured on a nutrient medium for callus induction.

Callus induction

Different seedling explants were cultured on Ms medium containing, 3% (w/v) sucrose and 0.8% (w/v) agar-agar at various concentrations of NAA (0.5, 1.0 and 1.5 mg/l) and IBA (0.5, 1.0 and 1.5 mg/l) in combination with BAP (0.5 mg/l) for callus induction. The effect of hormones on callus induction was studied.

Shoot bud regeneration and shoot multiplication

Calli were transferred to regeneration medium containing MS-basal medium, 3% (w/v) sucrose, 0.8% (w/v) agar-agar and 0.1 mg activated charcoal at different concentrations

of NAA (0.1, 0.5, 1.0 and 2.0 mg/l), IBA (0.1, 0.5, 1.0 and 2.0 mg/l) and IAA (0.1, 0.5, 1.0 and 2.0 mg/l) in combinations with KIN (0.05 mg/l) for shoot initiation. Shoot bud regeneration and differentiation of shoot bud could be observed for 20-26 days. The shoot buds were sub-cultured on shoot bud multiplication medium containing various concentration of BAP and KIN (1, 2 and 3 mg/l) in combination with IAA (0.5 mg/l) for further growth and multiple shoot bud formation.

Root induction

Larger shoot buds were excised and transformed to MS basal medium containing 3% (w/v) agar at different concentration of IBA (0.1, 0.2 and 0.3 mg/l) and IAA (0.1, 0.2 and 0.3 mg/l) in combination with KIN (0.05 mg/l) for root initiation. Rooting could be observed after 15-18 days.

Results and Discussion

From the above mentioned methods, the highest frequency of callus induction was observed on MS medium containing 1.5 mg/l NAA and 0.5mg/l BAP combination which was followed by BAP (0.5 mg/l) and IAA (1.5 mg/l). Callus initiation from hypocotyls explants varied from 45.8-72.3% in cotton (Table 1). Highest percentage of callus induction from shoot tips of *Cassia obtusifolia* L. was reported by using a combination of 2, 4 D and kinetin (Hasan *et al.*,2008). Earlier workers reported the use of very high concentration of BAP for maximal shoot proliferation from shoot tip explants of capsicum (Christopher and Rajam 1994; Sanatombi and Sharma 2008). The highest frequency of shoot bud regeneration (57.2%) was observed on MS basal medium containing KIN (0.5 mg/l) and IAA (2.0 mg/l) which was followed by KIN (0.5 mg/l) and IAA (1.0 mg/l) (Table 2). The highest mean number of shoot bud multiplication (18.7) was observed on MS medium with KIN (3.0 mg/l) and IAA (0.5 mg/l) (Table 3). IAA was successfully employed for rooting in *C. chinense* (Sanatombi and Sharma 2008) and it was more effective root induction as compared to NAA (Kehie *et al.*,2011).

In cotton tissue cultures, rooting problems are also one of the major problems and many scientists applied different methods to solve this problem (Ouma *et al.*,2004; Ozyigit *et al.*,2007). However, results of rooting success in many literatures are unclear and there are less data on rooting. In this study, the highest frequency of rooting was observed on

MS medium containing 0.3 mg/l of IBA and 0.05 mg/l KIN (Table 4). The percentage of rooting increased with increase in the concentration of auxin. IBA induced maximum frequency of rooting. The effect of single growth hormone was in which the best development of shoots was observed on MS containing 0.3 mg/l BA in cotton (*Gossypium hirsutum* L. cv. Stoneville 7A) (Hemphil *et al.,* 1998). The rooting by culturing isolated shoots on MS basal salts supplemented with NAA in cotton (*Gossypium hirsutum* L. cv. Khandwa-2) (Gupta *et al.*,1997).

Different hormonal combinations of auxin (2,4-D) and cytokinin (KT) at varying concentrations have been previously tested in basal MS medium (Kumria *et al.,* 2003; Leelavathi *et al.,* 2004; Rajasekaran *et al.,* 2000; Trolinder and Goodin 1987). Inclusion of an auxin and cytokinin will be necessary for callus growth (Wani *et al.,* 2010). The combination of auxins and cytokinins at definite proportions are very critical and found to be essential for the induction of shoot and root in many species (George *et al.*,1987; Lazzeri and Dunwell 1986; Ono *et al.,* 1994; Reddy and Bahadur 1989; Shankar and Ram 1990). The effects of auxins on root induction in *in vitro* regenerated chili plantlets have been reported (Agrawal *et al.*,1989; Christopher and Rajam 1996).

Effect of types of cytokinin used for *in vitro* shoot proliferation on the subsequent rooting of shoots was studied and 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 (Bennett *et al.*,1994). Effective *in vitro* callus mass production can be beneficial for high yield of secondary metabolites and optimization of alkaloid production. The methods used for *in vitro* regeneration and rooting can be applied to increase the efficiency of transformation protocols using shoot and nod as explants source.

Acknowledgements

The authors are grateful to the Management and the Principal of Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu, India for their encouragement and providing the facilities to conduct the experiments.

References

Agrawal S, Chandra N and Kothari SL, Plant regeneration in tissue cultures of pepper (*Capsicum annum* L cv. Mathania). *Plant Cell Tiss Org Cul* 16 (1989) 47-55.

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

Christopher T and Rajam MV, *In vitro* clonal propagation of Capsicum spp. *Plant Cell Tiss Org Cul* 38 (1994) 25-29.

Christopher T and Rajam MV, Effect of genotype, explant and medium on *in vitro* regeneration of red pepper. *Plant Cell Tiss Org Cul* 46 (1996) 245-250.

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

George L, Bapat VA and Rao PS, *In vitro* multiplication of sesame (*Sesamum indicum*) through tissue culture. *Annals of Botany*, 60 (1987) 17-21.

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

Hasan MF, Das R, Rahman MS, Rashid MH, Hossain MS and Rahman M, Callus induction and plant regeneration from shoot tips of chakunda (*Cassia obtusifolia* L.). *Int J Sustain Crop Prod*, 3 (2008) 6-10.

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

Kehie M, Kumaria S and Tandon P, *In vitro* plantlet regeneration from nodal segments and shoot tips of *Capsicum chinense* Jacq. cv. Naga King Chili. *Biotech 3* (2011) 31-35.

Kumria R, Sunnichan VG, Das DK, Gupta SK, Reddy VS, Bhatnagar RK and Leelavathi S, High-frequency somatic embryo production and maturation into normal

plants in cotton (*Gossypium hirsutum* L.) through metabolic stress. *Plant Cell Rep* 21(2003) 635-639.

Lazzeri PA and Dunwell JM, *In vitro* Regeneration from Seedling Organs of *Brassica oleracea* var. *italica* Plenck cv. Green Comet I. Effect of Plant Growth Regulators, *Ann Bot* 58 (1986) 689-697.

Leelavathi S, Sunnichan SG, Kumria R, Vijaykanth GP, Bhatnagar RK and Reddy VS, A simple and rapid Agrobacterium-mediated transformation protocol for cotton (*Gossypium hirsutum* L.): Embryogenic calli as a source to generate large numbers of transgenic plants. *Plant Cell Rep* 22 (2004) 465-470.

Ono Y, Takahata Y and Kaizuma N, Effect of genotype on shoot regeneration from cotyledonary explants of rapeseed (*Brassica napus* L.). *Plant Cell Rep* 14 (1994) 13-17.

Ouma JP, Young MM and Reichert NA, Rooting of *in vitro* regenerated cotton (*Gossypium hirsutum* L.) is influenced by genotype, medium composition, explant type and age. *Afr J Biotech* 3 (2004) 313-318.

Ozyigit II, Kahraman MV and Ercan O, Relation between explant age, total phenols and regeneration response in tissue cultured cotton (*Gossypium hirsutum* L.), *Afr J Biotech* 6 (2007) 3-8.

Rajasekaran K, Hudspeth RL, Cary JW, Anderson DM and Cleveland TE, High frequency stable transformation of cotton (Gossypium hirsutum L.) by Particle bombordment of embryogenic cell suspension cultures. *Plant Cell Rep* 19 (2000) 539-545.

Reddy KRK and Bahadur B, Adventitious bud formation from leaf cultures of castor (*Ricinus communis* L.). *Cur Sci* 58 (1989) 152-154.

Sanatombi K and Sharma GJ, *In vitro* propagation of Capsicum chinense Jacq. *Biologia Plantarium* 52 (2008) 517-520.

Shankar S and Ram HYM, Plantlet regeneration from tissue cultures of Sesbania grandiflora *Cur Sci* 59 (1990) 39-43.

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

Wani M, Pande S and More N, Callus induction studies in *Tridax procumbens L*. *Int J Biotech Appl* 2 (2010) 11-14.

Table 1. Effect of different concentrations of NAA, IAA, IBA in combination with 0.5 mg/l BAP on callus induction frequency in *Gossypium hirsutum* ($\overline{X} \pm SD$)

MS medium + Hormone mg/l		**Callus induction frequency**
NAA	BAP	Callus
0.1	0.5	45.8±1.8
0.5	0.5	52.2±0.7
1.0	0.5	63.1±1.2
1.5	0.5	72.3±0.8
IAA	BAP	
0.1	0.5	35.6±1.2
0.5	0.5	42.8±0.9
1.0	0.5	58.9±1.2
1.5	0.5	67.1±1.2
IBA	BAP	
0.1	0.5	42.2±0.8
0.5	0.5	51.3±1.3
1.0	0.5	59.2±0.9
1.5	0.5	62.1±0.2

Table 2. Effect of different concentrations of NAA, IBA and IAA in combination with 0.5 mg/l KIN on shoot bud differentiation in *Gossypium hirsutum* ($\overline{X} \pm SD$)

MS medium + Hormone mg/l		**Shoot bud differentiation frequency**
NAA	KIN	Immature leaf
0.1	0.5	32.7±1.7
0.5	0.5	38.2±1.3
1.0	0.5	41.3±0.9
2.0	0.5	48.2±1.3
IBA	KIN	
0.1	0.5	33.5±1.4
0.5	0.5	36.4±1.5
1.0	0.5	42.3±0.8
2.0	0.5	48.4±1.3
IAA	KIN	
0.1	0.5	33.3±1.3
0.5	0.5	41.4±1.9
1.0	0.5	52.3±2.1
2.0	0.5	57.2±1.8

Table 3. Frequency of shoot bud induction on MS basal medium supplemented with difference of BAP and Kin in combination with 0.5 mg of IAA in *Gossypium hirsutum* ($\overline{X} \pm SD$)

MS medium + hormones mg/l	**Shoot induction frequency**	
BAP +	IAA	Immature leaf
1.0	0.5	3.8±0.1
2.0	0.5	6.9±0.2
3.0	0.5	10.8±1.2
KIN	IAA	
1.0	0.5	5.9±0.1
2.0	0.5	11.1±1.2
3.0	0.5	18.7±0.6

Table 4. Effect of different concentration of IAA, IBA along with 0.05 mg/l kin on the root induction from regenerated shoots in *Gossypium hirsutum* ($\overline{X} \pm SD$)

MS medium + hormones mg/l	**Root induction frequency**	
BAP +	KIN	Immature root
0.1	0.05	1.8±0.9
0.2	0.05	9.3±1.3
0.3	0.05	10.2±1.2
IBA	KIN	
0.1	0.05	3.8±1.2
0.2	0.05	7.3±1.7
0.3	0.05	8.2 ±1.3