Journal

J. Biol. Chem. Environ. Sci., 2009, Vol. 4(4): 151-165
www.acepsag.org

DIRECT REGENERATION OF EGYPTIAN COTTONS (*GOSSYPIUM BARBADENSE* L) FROM COTYLEDONARY NODES AND SHOOT APEX

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ABSTRACT

Plant transgenic technology is an attractive means for introduction of foreign genes into cotton, but it requires an effective plant regeneration system. This study was aimed to develop a high frequency shoot regeneration technique from cotyledonary nodes and shoot apex of Egyptian Cottons (*Gossypium barbadense* L.). Shoot proliferation from different explants of seven Egyptian cotton varieties namely: Giza 45, Giza 70, Giza 83, Giza 86, Giza 88, Giza 89 and Giza 90 was studied in culture. The proliferation was carried out on B_5 Gamberg medium supplemented with different cytokinins (6 combinations). The results indicated that the addition of cytokinins in the medium induced the number of produced shoots compared to the control, however differences based on the cytokinin type and concentration and nature of the explants were observed. Cotyledonary nodes produced higher number of shoots compared to shoot apex of the same vareity. The cotyledon nodes and shoot apex explants of the Giza 70 produced higher numbers of shoots compared to the other varieties. The best medium for the proliferation of cotton from cotyledonary nodes of all the tested varieties was the medium supplemented with 3 mgl^{-1} Kin (M1), while the combination of 1 mgl^{-1} Kin and 1 mgl^{-1} 2ip (M6) gave the highest number of shoots per shoot apex compared to the other hormonal combinations. The isolated shoots were rooted on basal medium supplemented with α-naphthaleneacetic acid and were transferred to acclimatization on mixture of soil and peat moss (1:1) under nonsterile conditions.

Key words: cotton, cotyledonary nodes, *Gossypium barbadense*, proliferation, propagation, regeneration, shoot apex.

Abbreviation: Kin - Kinetin; BA - 6-benzyladenine; 2ip - (τ-τ dimethylallayl-amino)-purine MS - Murashige and Skoog medium (1962); B_5 - Gamborg (1968); NAA - α-naphthalene acetic acid.

INTRODUCTION

Cotton is an economic plant of world importance. It is the world's leading textile fiber crop and it is also a source of other products such as oil, live-stock feed (cotton seed cake) and cellulose (Anderson, 1999; Frelichowski *et al*., 2006)). In Egypt its importance is derived from being one of the main sources of foreign currency as well as the principle raw material for the national textile industry and one of the important sources of edible oil (Adawy *et al*., 2006). Although conventional breeding programs have made steady improvements in agronomic traits in cotton, genetic improvement is limited by several factors such as lack of useful variation and long time periods required (Wang *et al*., 2006).

Somatic embryogenesis is an important tool in breeding cotton species because of its great potential in germplasm enhancement (Zhang *et al*., 2004). Since Davidonis and Hamilton (1983) reported plant regeneration via somatic embryogenesis in *G. hirsutum* cv. Coker 310, a number of protocols have been exploited for somatic embryogenesis and plant regeneration from various explants of cotton (Finer, 1988; Zhang *et al*., 2000; Sakhanokho *et al*., 2004; Jin *et al*., 2005; Sun *et al*., 2006). To take the full advantage of somatic embryogenesis, a high-frequency, synchronous embryogenic system is needed. Recently, a simple and efficient method was developed to improve somatic embryogenesis frequency and synchronous development of mass somatic embryos from cultured cells of the cotton cultivar Coker 201 (Jing-Lin *et al*., 2008).

Plant transgenic technology is an attractive means for introduction of foreign genes into cotton, but it requires an effective plant regeneration technique that results in production of large number of shoots and allows the faithful multiplication of transferred gene without causing variations. On the basis of the previous studies, the present study was conducted to develop an efficient and reproducible

regeneration protocols for clonal multiplication of Egyptian cottons using cotyledonary nodes and shoot apex. The results from this study will be useful in genetic improvement of cotton through biotechnology by providing an efficient regeneration system.

MATERIALS AND METHODS

Plant material

This study included six Egyptian cotton varieties belonging to *Gossypium barbadense* L. namely: Giza 45, Giza70, Giza 83, Giza 86, Giza 88, Giza 89 and Giza 90 (Table1). Pure seeds of the genotypes were obtained from the Cotton Research Institute (CRI), Agriculture Research Center (ARC), Giza, Egypt.

This work was carried out at the Plant Biotechnology Laboratory (PBL), Faculty of Agriculture, Cairo University, Giza, Egypt, during two successive seasons from 2006 to 2008.

Table 1. List of cotton varieties used in the study, their pedigree, staple length and the location in which they are cultivated

Varieties	Parents	Staple length	Climatic region
Giza 45	G 28 X G 7	Extra-long	Delta
Giza 70	G 59A X G 51B	Extra-long	Delta
Giza 83	G 67 X G 72	long	Upper Egypt
Giza 86	G 75 X G 81	long	Delta
Giza 88	G 77 X G 45	Extra-long	Delta
Giza 89	G 75 X Russia 6022	long	Delta
Giza 90	G 83 X Dandra	long	Upper-Egypt

Seeds sterilization and germination

Cotton seeds were surface-sterilized by immerse in 70% ethanol for 1min (Ouma, *et al*., 2004a), followed by 40% Clorox for 20min, then 0.1% (w/v) mercuric chloride ($HgCL_2$) solution for 15min followed by three washes with sterile distilled water and then stored overnight in sterile distilled water for germination (Wang *et al*., 2006).

The seeds were surface-sterilized by immerse in 10% Clorox for 1min at the next day followed by three washes with sterile distilled water. The seed coats were removed and placed on half strength MS (Murashig and Skoog, 1962) medium supplemented with 1.5% (w/v) sucrose, solidified with 0.7% agar and adjusted to pH 5.8 before autoclaving. The medium was distributed into the culture jar 800ml where each jar contained 100ml of the media. The seeds without coats were germinated under dark conditions for 2days and then transferred to a 16h light, 8h dark light condition at 28±2°C. Light was provided by white fluorescent lamps giving intensity of 1000 lux (Suresh Kumar *et al*., 2003).

Shoot induction and multiplication

Shoot apex and cotyledon nodes were excised from 21 days old sterile seedlings that were used as explants for shoot induction by culturing on B_5 (Gamborg *et al*., 1968) medium supplemented with 30g l^{-1} sucrose, 100mg l^{-1} myo-inositol, 10mg l^{-1} thiamine, 7g l^{-1}agare and various concentrations of different cytokinin hormones (Table 2) according to Gupta *et al*. (1997). Plant growth regulators were added before autoclaving at 121ºC for 20min and pH of the media was adjusted to 5.8. The medium was distributed into the culture jar 250ml where each jar contained 35ml of the media. The explants after culturing transferred to the same of conditions were used of the germination for 4 weeks.

Table 2. The hormone combination in the induction media of shoots and multiplication

Treatments	Hormones combination (mg l^{-1})		
	Kin	2ip	BA
M0 (Control)	0.0	0.0	0.0
M1	3.0	0.0	0.0
M2	0.0	3.0	0.0
M3	0.0	0.0	3.0
M4	1.0	0.0	1.0
M5	0.0	1.0	1.0
M6	1.0	1.0	0.0

Rooting of shoots

Elongated shoots (1.5 to 2.5cm long) were isolated and transferred to half strength MS media and vitamins supplemented with 15g l^{-1} sucrose, 0.5mg l^{-1} NAA and 7g l^{-1} agar for rooting (Rauf *et al*., 2004a).

Acclimatization

Plantlets were transplanted after rooting into green house and washed with tap water for three times to remove all traces of agar then soaked in vita fax and cultured in black plastic pots containing a mixture of pet moss and sand (1:1) under nonsterile conditions. After 15 days, the plantlets maintained under partial shade and irrigated daily.

Statistical analysis

Each experiment was performed three times and an analysis of variance was performed for each experiment. The least significance difference test (LSD at p= 0.05) was used for multiple mean comparisons.

RESULTS AND DISCUSSION

Seeds sterilization and germination

Poor and nonuniform germination was a problem at the beginning of the experiment because of the variability in water absorption and, possibly, seed age and vigor. This problem has been overcame by soaking the sterilized seeds in distilled water overnight and aseptically removing the seed coats before transferring them to the germination medium (Fig. 1A). This technique is relatively easy, and it proved to be useful in improving the germination rate (Fig. 1B). This improved germination rate may be particularly useful when there is a need to submit a uniform set of seedlings to a treatment. In addition, this technique reduced the contamination problem arising from the seed coats (Sakhanokho *et al*., 2001).

Shoot induction and multiplication

In this study, regeneration with 21-day-old explants of all several varieties was successful. Our results are in a disagreement with those found by Luo and Gould (2000) who cultured 7-, 14-, 21-, 28-, 34-, and 45-day old cotyledonary nodes containing hypocotyl pieces and

obtained the best regeneration response was for 14 and 35-day old explants.

Different types and concentrations of cytokinins were studied for proliferation of several Egyptian cotton varieties. Shoot cultures were induced by placing cotyledon nodes and shoot apex explants in B_5 medium supplemented with 30g l^{-1} sucrose, 100mg l^{-1} myo-inositol, 10mg l^{-1}thiamine, 7g l^{-1}agare and combination of different cytokinins (Table 2).

The results showe that cotyledonary nodes of all the tested varieties produced higher number of shoots (Table 3, Fig. 1C) compared to shoot apex of the same variety (Table 3). The cotyledon nodes (Fig. 1C) and shoot apex explants of the variety Giza 70 produced higher numbers of shoots compared to the other varieties (Table 3, Fig. 2). The shoot apex of the variety Giza 88 and the cotyledon nodes of the variety Giza 83 gave the lower number of shoots per explant (Table 3).

Table 3. Effect of varieties on the number of shoots per explants

Varieties	Explants	
	Cotyledonary nodes	Shoot apex
Giza 45	1.95 a	1.60 b
Giza 70	2.03 a	1.82 a
Giza 83	1.61 c	1.61 b
Giza 86	1.73 bc	1.09 c
Giza 88	1.72 bc	1.08 c
Giza 89	1.81 abc	1.71 ab
Giza 90	1.72 bc	1.14 c
LSD at 0.05	0.23	0.17

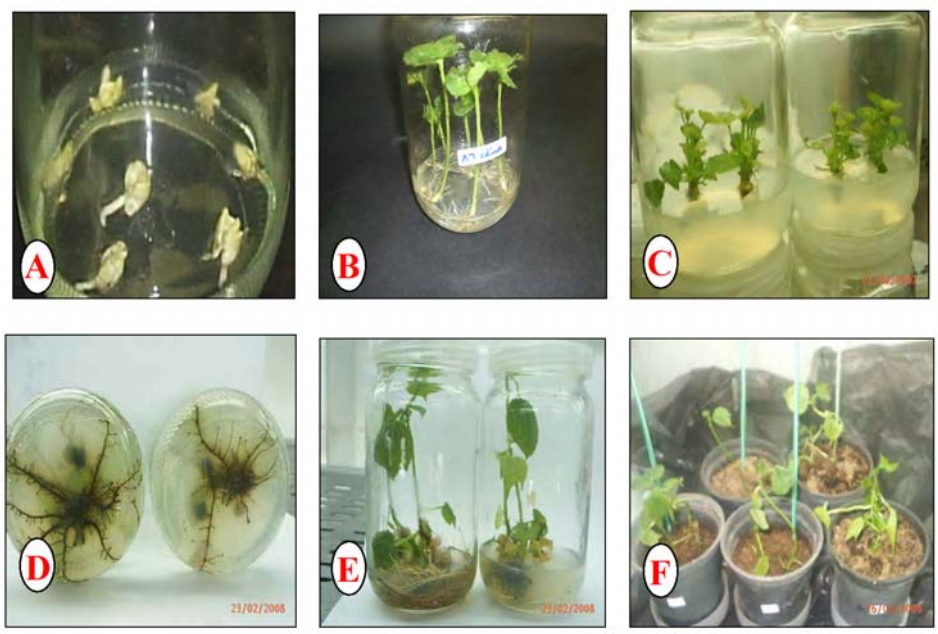

Fig.1. (A) Seeds without coats. (B) Germination from Giza 86. (C) Shoot induction from cotyledonary nodes of Giza 70. (D and E) Rooting of Giza 86 and Giza 88. (F) Acclimatized of all cultivars.

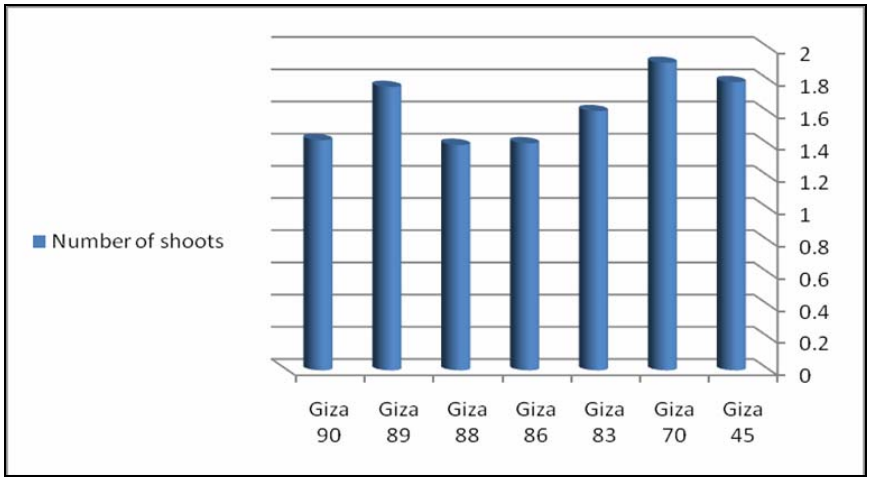

Fig.2. The effect of varieties on the number of shoots.

The results indicate that the addition of hormone in the medium induced the number of produced shoots compared to the control, however differences based on the cytokinin type and concentration and nature of the explants were observed (Tables 4, 5 and 6). The best medium for the proliferation of cotton from cotyledonary nodes of all the tested varieties was the medium supplemented with $3mgl^{-1}$ Kin (M1), while the medium containing $3mgl^{-1}$ BA (M3) gave the lowest number of shoots per node of most of the tested varieties (Tables 4 and 5). Abdellatef and Khalafalla (2007) induced shoot development was using kinetin (Kin) or benzyladenine (BA) alone. Both types and concentrations of growth regulators significantly influenced shoot proliferation. Kin proved to be more effective than BA. The best response, however was obtained when 2.0 mg L^{-1} Kin were used. This is mainly, because at higher cytokinin level cotyledonary node explants produced excessive callus and failed to improve the efficiency of shoot multiplication. Thiem (2003) reported that callus growth on explant usually interfere with the propagation process. The combination of 1 mgl^{-1} Kin and 1 mgl^{-1} 2ip (M6) gave the highest number of shoots per shoot apex compared to the other hormonal combinations, while the treatment containing 1 mgl^{-1} Kin and 1 mgl^{-1} BA (M4) produced the lowest number of shoots per shoot apex of most of the tested varieties (Tables 4 and 6).

Table 4. Effect of media on the number of shoots per explants

Media	Explants	
	Cotyledonary nodes	Shoot apex
M0 (Control)	0.76 e	0.72 e
M1	2.44 a	1.44 c
M2	2.04 bc	1.27 d
M3	1.48 d	1.75 b
M4	1.76 cd	1.17 d
M5	1.85 c	1.66 b
M6	2.23 ab	2.06 a
LSD at 0.05	0.30	0.15

M0: without growth regulators, M1: $3mgl^{-1}$ Kin, M2: $3mgl^{-1}$ 2ip, M3: $3mgl^{-1}$ BA, M4: $1mgl^{-1}$ Kin + $1mgl^{-1}$ BA, M5: $1mgl^{-1}$ 2ip + $1mgl^{-1}$ BA, M6: $1mgl^{-1}$ Kin + $1mgl^{-1}$ 2ip.

Cotton plants have proved to be difficult to manipulate in tissue culture. Shoot tip culture is a possible alternative to cope with the problem of recovering plants from callus tissue (Gould *et al*., 1991; Rauf *et al*., 2004a). Agarwal *et al*. (1997) obtained highest number of shoots by culturing cotyledonary nodes devoid of apical meristem in MS basal medium supplemented with 6-benzylaminopurine (BAP) and Kinetin 2.5 mgl^{-1} each. However, shoot could not elongate in same media. The advantages of shoot tip culture over other regeneration systems. Shoot regeneration from shoot tip is direct, relatively simple and needs less time to regenerate large number of plants (Saeed *et al*., 1997). Plants regenerated from shoot apices are true to phenotype with low incidence of somaclonal variation and chromosomal abnormalities (Bajaj and Gill, 1986). Shoot apex explant has few genotype limitations and considered as more appropriate because meristematic cells are programmed for direct shoot organogenesis without an intervening callus stage (Zapata *et al*., 1999).

Cytokinins are involved in shoot development of plants. Jorge *et al*. (1998) suggested that 6-benzylaminopurine (BA) is directly responsible for re-programming the embryonic apical meristem axes of cotton toward the production of multiple buds and subsequent shoot development. They reported that an average of 3.4 shoots per embryonary axis was obtained when explants were cultured on medium supplemented with 3 mg l^{-1} BA. Higher and lower concentrations of the growth regulator yielded fewer shoots per explants. Hemphill *et al*. (1998) described a plant regeneration procedure, which was applicable to diverse cotton germplasms and required specific concentrations of BA depending on the origin of the meristems. All shoots regenerated directly without a callus phase. Screening BA concentrations (0.0–10.0 μM) demonstrated that shoot meristems (apices), secondary leaf nodes, primary leaf nodes, and cotyledonary nodes derived from *in-vitro* grown 28-day-old seedlings (Paymaster HS26) varied in their ability to form elongated shoots depending on the level of BA.

Table 5. Effect of the media on cotyledonary nodes of all varieties

Media	Explants	Varieties						
		Giza 45	Giza 70	Giza 83	Giza 86	Giza 88	Giza 89	Giza 90
M0	Gotyledonary nodes	0.60 d	0.93 c	0.50 d	0.90 e	0.47 d	0.93 b	1.00 e
M1		2.70 a	2.75 a	2.22 a	2.50 a	2.50 a	2.33 a	2.28 a
M2		2.50 ab	2.25 ab	2.00 ab	1.84 bc	1.91 bc	2.00 ab	1.79 c
M3		1.50 c	1.50 bc	1.17 c	1.50 d	1.50 c	1.50 ab	1.70 cd
M4		1.83 c	2.06 ab	1.56 bc	1.58 d	1.83 bc	1.78 ab	1.72 cd
M5		2.00 bc	2.22 ab	1.67 abc	1.78 c	1.84 bc	1.89 ab	1.58 d
M6		2.50 ab	2.50 a	2.17 ab	2.00 b	2.00 b	2.22 a	2.00 b
LSD at 0.05		0.57	0.85	0.67	0.19	0.42	1.07	0.19

M0: without growth regulators, M1: 3mgl^{-1} Kin, M2: 3mgl^{-1} 2ip, M3: 3mgl^{-1} BA, M4: 1mgl^{-1} Kin + 1mgl^{-1} BA, M5: 1mgl^{-1} 2ip + 1mgl^{-1} BA, M6: 1mgl^{-1} Kin + 1mgl^{-1} 2ip.

Table 6. Effect of the media on shoot apex of all varieties

Media	Explants	Varieties						
		Giza 45	Giza 70	Giza 83	Giza 86	Giza 88	Giza 89	Giza 90
M0	Shoot apex	0.53 f	1.03 c	0.90 e	0.57 c	0.33 e	1.07 d	0.57 d
M1		1.50 d	1.89 ab	1.67 bc	1.17 ab	0.80 cd	1.78 abc	1.28 b
M2		1.24 e	1.83 ab	1.47 cd	1.00 bc	0.67 d	1.67 bc	1.00 c
M3		2.25 b	2.06 ab	2.00 ab	1.25 ab	1.50 b	2.00 ab	1.17 bc
M4		1.17 e	1.61 bc	1.17 de	1.00 bc	1.00 c	1.55 c	1.67 d
M5		2.00 c	2.00 ab	1.89 abc	1.17 ab	1.43 b	1.78 abc	1.33 b
M6		2.50 a	2.33 a	2.17 a	1.50 a	1.83 a	2.11 a	2.00 a
LSD at 0.05		0.21	0.71	0.74	0.45	0.30	0.41	0.22

M0: without growth regulators, M1: 3mgl^{-1} Kin, M2: 3mgl^{-1} 2ip, M3: 3mgl^{-1} BA, M4: 1mgl^{-1} Kin + 1mgl^{-1} BA, M5: 1mgl^{-1} 2ip + 1mgl^{-1} BA, M6: 1mgl^{-1} Kin + 1mgl^{-1} 2ip.

Rooting of shoots

The elongated shoots were rooted on half strength MS media supplemented with 15g l^{-1} sucrose, 0.5 mg l^{-1} NAA. This medium produced the highest percentage of root development and root length in all the tested varieties (Fig 1, E). The induction of *in vitro* roots is an important step in micro-propagation and genetic transformation protocols, but has often proved difficult in many recalcitrant species like cotton (Ouma *et al*., 2004b). Agrawal *et al*. (1997) used cotyledonary nodes and obtained rooting, but Saeed *et al*. (1997) used meristem shoot tips and found these to develop roots more readily than hypocotyls segments. Highest percentage (93.3%) of root development and root length (5.85) was obtained when shoots were cultured on MS media supplemented with 0.5 mgl^{-1} NAA and 0.1 mgl^{-1} Kinetin (Rauf *et al*., 2004b).

Acknowledgments

The authors are indebted to Dr. O. M. El-Shihy (the director of Plant Biotechnology Research Laboratory, Faculty of Agriculture, Cairo Univ.) for providing all the facilities during the practical part of this work and are grateful to Dr. S. A. Hamoda (doctor researcher in Agriculture Research Centre, Cotton Research Institute, Giza, Egypt) for his valuable assistance during the statistical analysis of this work.

REFERENCES

Abdellatef, E. and Khalafalla, M. (2007). Adventitious shoot and plantlet formation in medium staple cotton cultivar (*Gossypium hirsutum* L. cv Barace [67] B). international Journal of Agriculture & Biology. 6: 913-916.

Adawy, S.S., Hussein, E.H.A. and El-Itriby, H.A. (2006). Molecular characterization and genetic relationship among cotton genotypes 2-AFLP analysis. Arab J. Biotech. 9 (3): 478-492.

Agrawal, D.C., Banerjee, A.K., Kolala, R.R., Dhage, A.B., Kulkarni, A.V., Nalwade, S.M., Hazra, S. and Krishnamurthy, K.V. (1997). *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep. 16: 647-652.

Anderson, C.G. (1999). Cotton marketing. In: Smith, C.W, Cothren, J.T. (eds) Cotton: Origin, History, Technology and Production. Wiley, New York, pp: 659-679 (C.F. Frelichowski *et. al*., 2006).

Bajaj, Y.P.S. and Gill, M. (1986). Preservation of cotton (*Gossypium* spp.) through shoot tip and meristem culture. Indian. J. Exp. Biol. 24: 581-583.

Davidonis, G. H., and Hamilton, R. H. (1983). Plant regeneration from callus tissue of *Gossypium hirsutum* L. Plant Sci Lett. 32: 89–93.

Finer, J. J. (1988). Plant regeneration from somatic embryogenic suspension cultures of cotton, *Gossypium hirsutum* L. *Plant Cell Rep*. 7: 399–402.

Frelichowski, J.r.; Palmer, M.B.; Main, D.; Tomkins, J.p.; Cantrell, R.G.; Stelly, D.M.; Yu, J.; Kohel, R.J. and Uloa, M. (2006). Cotton genome mapping with new microsatellites from Acala "Maxxa" BAC-ends. Mol. Gen. Genomics, 275: 479-491.

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Gould, J., Banister, S., Hasegawa, O., Fahima, M. and Smith, R.H. (1991). Regeneration of *Gossypium hirsutum* and *G. barbadense* from shoot apex tissue for transformation. Plant Cell Rep. 10: 12-16.

Gupta, S.K.; Srivastava, A.K.; Singh, P.K. and Tuli, R. (1997). *In vitro* proliferation of shoots and regeneration of cotton. Plant Cell, Tissue and Organ Culture. 51: 149-152.

Hemphill, J.K., Maier, C.G. A. and Chapman, K.D. (1998). Rapid *in vitro* plant regeneration of cotton (*Gossypium hirsutum* L.). Plant Cell Rep. 17: 273-278.

Jin, S. X., Zhang, X. L., Liang, S. G., Nie, Y. C., Guo, X. P., and Huang, C. (2005). Factors affecting stable transformation and plant regeneration during transforming embryogenic callus of upland cotton (*Gossypium hirsutum* L.) with *Agrobacterium tumefaciens*. *Plant Cell Tiss Org Cult*. 81: 229–237.

Jing-Lin, C., Xian-Long, Z., Shuang-Xia, J., Xi-Yan, Y., Hua-Guo, Z., and Li-Li., F. (2008). An Efficient Culture System for Synchronization Control of Somatic Embryogenesis in Cotton (*Gossypium hirsutum* L.). Acta Agron Sin. 34(2): 224–231.

Jorge, L.M., Permingeat, H.R., Romagnoli, M.V.C., Heisterborg, M. and Ruben, H.V. (1998). Multiple shoot induction and plant

regeneration from embryonic axes of cotton. Plant Cell Tiss Org Cult. 54: 131-136.

Luo, J. and Gould, J.H. (2000). *In vitro* shoot tip grafting improves recovery of cotton plants from culture. *Plant Cell, Tissue Organ Cult.* 57: 211-213.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 80: 662-668.

Ouma, J.P.; Young, M.M. and Reichert, N.A. (2004a). Optimization of *in vitro* regeneration of multiple shoots from sections of cotton (*Gossypium hirsutum* L.). African Journal of Biotechnology. 3(3): 169-173.

Ouma, J.P.; Young, M.M. and Reichert, N.A. (2004b). Rooting of *in vitro* regenerated cotton (*Gossypium hirsutum* L.) is influenced by genotype, medium composition, explant type and age. African Journal of Biotechnology. 3(6): 313-318.

Rauf, S.; Rahma, H. and Khan, T.M. (2004a). Effect of kinetin on multiple shoots induction in cotton (*Gossypium hirsutum* L.) cv. NIAB-999. Iranian Journal of Biotechnology. 2(4): 279-282.

Rauf, S.; Usman, M.; Fatima, B.; Khan, T.M. and Khan, I.A. (2004B). *In vitro* regeneration and multiple shoot induction in Upland cotton (*Gossypium hirsutum* L.). International Journal of Agriculture & Biology. 6(4): 704-707.

Saeed, N.A., Zafar,Y., and Malik, K.A. (1997). A simple procedure of *Gossypium meristem* shoot tip culture, Plant Cell Tissue Org. Cult. 51 201–207.

Sakhanokho, H. F., Ozias-Akins, P., May, O. L., and Chee, P. W. (2004). Induction of somatic embryogenesis and plant regeneration in select georgia and Pee Dee cotton lines. *Crop Sci*. 44: 2199–2205.

Sakhanokho, H.F.; Zipf, A.; Rajasekaran, K.; Saha, S. and Sharma, G.C. (2001). Induction of highly embryogenic calli and plant regeneration in Upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) cotton. Crop Sci. 41: 1235-1240.

Sun, Y. Q., Zhang, X. L., Huang, C., Guo, X. P., and Nie, Y. C. (2006). Somatic embryogenesis and plant regeneration from

different wild diploid cotton (*Gossypium*) species. *Plant Cell Rep*. 25: 289–296.

Suresh Kumar, M.V.; Katageri, I.S.; Vamadevaiah, H.M.; Khadi, B.M. and Salimath, P.M. (2003). *In vitro* screening for regeneration in cotton (*Gossypiu* ssp.). *Indian J. Genet.* 63(1): 54-58.

Thiem, B. (2003). *In vitro* propagation of isoflavone-producing *Pueraria lobata* (Willd.) Ohwi. *Pl. Sci*. 165: 1123-1128.

Wang, Y., Wang, X., MA, Z., Zhang, G. and Hang, G. (2006). Somatic Embryogenesis and Plant Regeneration from Two Recalcitrant Genotypes of *Gossypium hurisutum* L. Agric Sci in China 5(5): 323-329.

Zapata, C., Srivatanakul, M., Park, S.H., Lee, B.M., Salas, M.G., and Smith, R.H. (1999). Improvements in shoot apex regeneration of two fiber crops: cotton and kenaf, Plant Cell Tissue Org. Cult. 12 43–50.

Zhang, B. H., Liu, F., Yao, C. B. (2000). Plant regeneration via somatic embryogenesis in cotton. *Plant Cell Tiss Org Cult.* 60: 89–94.

Zhang, X. L., Sun, Y. Q., Wu, J. H., Jin, S. X., Nie, Y. C. and Guo, X. P. (2004). Cell engineering and new germplasm development in Cotton. Cotton Sci. l6: 368–373 (in Chinese with English abstract).

الأكثار المباشر لنباتات القطن المصرى من القمم النامية والفلقات

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[3] معمل بيوتكنولوجيا النبات- كلية الزراعة – جامعة القاهرة – الجيزة – مصر.

تعنى تكنووجيا نقل الجينات النباتية أدخال جينات جديدة بداخل نبات القطن وبالطبع تحتاج هذه التكنولوجيا الى الوصول الى أفضل تقنية لأنتاج نباتات جديدة. ولذلك كان الهدف من هذه الدراسة هو انتاج أعلى عدد من النباتات الجديدة بأستخدام جزئين نباتين هما القمم النامية والفلقات للقطن المصرى.

أشتملت هذه التجربة على 7 أصناف نباتية هى جيزة 45 ، جيزة 70 ، جيزة 83 ، جيزة 86 ، جيزة 88 ، جيزة 89 و جيزة 90 . حيث تم زراعة الأجزاء النباتية المختلفة على B5 تشتمل على 6 توليفات من السيتوكينن المختلفة.

وقد أوضحت النتائج أن المعاملات االمختلفة أعطت عدد أعلى من النباتات الجديدة مقارنة بالبيئة التى لم يوضاف اليها اى منظمات نمو. وأوضحت أيضا أن الجزء الفلقات أعطت عدد أكبر من النباتات مقارنة القمم النامية لنفس النبات. كما أوضحت النتائج أيضا أن الفلقات والقمم النامية للصنف جيزة 70 أعطت أعلى عدد من النباتات الجديدة مقارنة بالأصناف الأخرى.

وكانت المعاملة التى تحتوى 3 ملجم كينيتين هى أفضل معاملة اعطت عدد أكبر من النباتات الجديدة مع الفلقات مقارنة بالمعاملة التى تحتوى على 1 ملجم كينيتين + 1 ملجم 2ip أعطت عدد أكبر من النباتات مع القمم النامية.

وبعد ذلك يتم نقل النباتات التى كونت جذور على بيئة تحتوى نفثالين اسيتك اسيد الى خليط من الرمل والبيت موس بنسبة 1:1 تحت ظروف غير معقمة.