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In vitro Shoot Development from Three Different Nodes of Cotton (*Gossypium hirsutum* L.)

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Abstract

In plant tissue culture studies, obtaining new plantlets from many different parts of a plant is a very important feature with direct or indirect ways and all nodes are considerable sources, although they show different regeneration capacities from one species to another. In this research, *in vitro* direct shoot developments from different nodes of cotton (*Gossypium hirsutum* L. *var*. Nazilli 84S) were comparatively studied. Cotyledonary nodes, first and second leaf nodes together with hypocotyl, shoot and epicotyl pieces from 35-day-old *in vitro* grown plants were cultured on MS media supplemented with 0.1 mg/l KIN (kinetin). Cultured seeds and explants were kept at growth chamber with a photoperiod for 16 hours light (7500 lx) and 8 hours dark, at 25 °C and 70 % humidity. A rapid *in vitro* shoot development was obtained from all three types of explants and no significant differences among young plants were observed. Shoot development was maximum 74.2 % in cotyledonary nodes, followed by 60.7 % and 41.6 %, respectively for first and second leaf nodes. All these shoots were capable of rooting in Woody Plant Medium (WPM), supplemented with 1 mg/l IBA (indole-3-butyric acid), and establishing in soil after 15-30 days.

Keywords: cotton, tissue culture, cotyledonary node, leaf node

Introduction

Cotton (*Gossypium hirsutum* L.) is the single most important textile fiber in the world, accounting for about 40 % of all fibers produced. Besides, its edible oil contributes 65-70 % to the local oil industry as well as of other industrial products (Méndez-Natera *et al*., 2007; Khan *et al*., 2009). China is the largest producer and consumer of cotton in the world (24 %) and its cotton production is characterized by intensive cultivation due to limited arable land per capita and availability of a large pool of agricultural labor (Hsu & Gale, 2001; Dong *et al*., 2004; Dong *et al*., 2005; Meyer *et al*., 2007). On average, the United States provides 20 % of the global cotton production, and is also the leading supplier in the international market. Other major cotton producer countries are Australia, India, Pakistan, Uzbekistan, Brazil, and Turkey (Meyer *et al*., 2007; Ozyigit & Gozukirmizi, 2009).

Advanced biotechnology provides both an innovation method for cotton breeding and germplasm multiply and accelerates the process of cotton breeding. The plant breeding methods can be combined with tissue culture methods in order to form genetic variability for desired traits (Naz *et al*., 2007; Ozyigit & Gozukirmizi, 2008). The key to successful application of biotechnology in plant breeding is the establishment of an efficient regeneration system, which can be used in interspecific hybridization, mutation, combination, hybrid variety breeding, rapid propagation and transformation (Rauf *et al*, 2004; Efe, 2005).

Cotton plants are severely limited in their regeneration *in vitro* from callus, protoplast or leaf tissues (Gould *et al*., 1991; Rashid *et al*., 2004). This problem presently restricts improvement of the few potential commercial genotypes through genetic engineering (Rashid *et al*., 2004; Ozyigit *et al*., 2007a). In addition, tissue culture and gene transfer systems are genotype dependent and also browning and rooting problems have not been fully solved yet (Ozyigit *et al*., 2007a). Even though problematic establishment of cotton tissue culture and gene transfer systems, at the beginning of the 1990s, genetically modified cottons, which carry insect- and herbicide-resistant genes were obtained successfully and then transgenic cotton cultivars became commercially available in 1995 (Collins, 1996; Song *et al*., 2000). Recently, transgenic cotton varieties are available in over 70 % of cotton planted acres, in the USA (Meyer *et al*., 2007).

Literature indicates that direct regeneration and somatic embryogenesis are main regeneration methods for cotton tissue cultures (Gupta *et al*., 2000; Kumria *et al*.,

2003). While hypocotyls are the main explant sources for callus induction and indirect somatic embryogenesis, cotyledonary nodes and meristematic shoot tips are the primary sources for direct regeneration (Gould *et al*., 1991; Hemphill *et al*., 1998; Zapata *et al*., 1999; Gupta *et al*., 2000; Kumria *et al*., 2003; Ozyigit & Gozukirmizi, 2009). However, the results obtained are, correlated with plant material such as explant age or genotype, culture conditions such as hormones, medium composition or other physical culture conditions (Ozyigit *et al*., 2007a). Furthermore, in plant tissue culture studies, one part of a genotype could be more adaptive to regeneration than another part of other genotype (Ozyigit *et al*., 2007b). This condition shows that, explant source is also an important factor, like genotype for many species, which are studied.

In this research, *in vitro* direct shoot developments of three different nodes of 35-day-old cotton were studied. Same medium, hormone and culture conditions were used and it was observed that different nodes were more adaptive to *in vitro* shoot development and that shooting capacities were showing different properties.

Materials and methods

Seeds of cotton v*ar*. Nazilli 84S, were obtained from Nazilli Cotton Research Institute, Aydın-TURKEY. Before surface sterilization, cottonseeds were kept under flowing tap water for 1 hour and they were surface sterilized by immersion in 70 % ethanol for 3 minutes, followed by stirring in 20 % commercial bleach for 20 minutes. The surface sterilized seeds were rinsed 3 times with sterile distilled water for 5 minutes and they were dried onto filter papers. Seed coats were removed with sterile scalpel prior to germination. The seeds were germinated on hormone free MS (Murashige & Skoog, 1962) medium, which contained 1 mL MS vitamin solution, 30 g sucrose and 2.2 g phytagel. The pH of the media was adjusted to 5.7 with 1M NaOH before autoclaving. 20 mL MS media were poured into Magenta vessels and 5 seeds were germinated in each vessel. Seeds were kept at growth chamber with a photoperiod of 16 hours light (7500 lx) and 8 hours dark at 25 °C and 70 % humidity. After germination, 35-day-old hypocotyl, epicotyl and shoot pieces which carrying two cotyledonary nodes, first and second leaf nodes were dissected out from seedlings with sterile scalpel and then cultured on MS media supplemented with 0.1 mg/l KIN. Five explants were put in each Petri plate and each cultured plant pieces (explant) had two lateral nodes. Explants were cultured under the same culture conditions of germination. *In vitro* direct shoot development started in one week and shoots were removed from explants and then subcultured in one-week intervals. For rooting, obtained shoots were transferred into culture tubes, which included Woody Plant Medium (WPM) and 1 mg/l IBA. In general, WPM has the same ingredients with MS but amounts of some supplements are different from it (McCown & Lloyd, 1981).

Results and discussion

The germination frequency of cotton *var*. Nazilli 84S was around 95-100 %. After 35 days from germination, cotyledonary nodes, first and second leaf nodes together with hypocotyl, epicotyl and shoot pieces were dissected out from seedlings and then cultured on MS media supplemented with 0.1 mg/l KIN. Different shoot development ratios were obtained from each explant sources. The best shooting efficiency was seen with cotyledonary nodes (74.2 %) while first leaf nodes and second leaf nodes showed 60.7 % and 41.6 % respectively. In addition, there were two nodes on each explant and in general, only one node showed development. In explants containing cotyledonary nodes, 40.4 % of both nodes showed direct shooting efficiency together at the same time, while the first leaf node carrying explants showed 11.7 % and the second leaf node carrying explants showed 6.6 % (Picture).

This research showed that cotyledonary nodes are more capable compared to other leaf nodes for *in vitro* tissue culture systems of cotton *var*. Nazilli 84S. Similar to this study, Hemphill *et al*. (1998) reported the use of BA for induction of multiple shoots from pre-existing meristems of nodal explants (14-day-old) of four cotton genotypes (Paymaster HS2A, CA-3076, Stoneville 7A and Stoneville 474). They observed that cotyledonary, primary and secondary leaf nodes were more responsive than apices when cultured on MS medium plus 0.3 or 0.5 mM BA. Agrawal *et al*. 1997, cultured cotyledonary nodes of 35-day-old cotton (cv. Anjali-LRK 516) seedlings and they demonstrated multiple shoots on MS medium plus BA and KIN (2.5 mg/l each). In another study, Gupta *et al*. 1997, obtained the best regeneration response from 6-day-old explants using 5-10-day-old cotyledonary nodes of 10 different genotypes. The regeneration responses were between 40-91.7 % and showed genotype dependency. In a similar study, 7, 14, 21, 28, 35 and 45-day-old cotyledonary nodes containing hypocotyl pieces were cultured and the best regeneration response was obtained from 14 and 35-day-old explants (Luo & Gould, 2000). Lately, Ozyigit & Gozukirmizi 2008, studied with the same genotype's (Nazilli 84S) cotyledonary nodes (one-week old) and they cultured explants on MS + 0.1 mg/l KIN + 1 g/L PVP (polyvinylpyrrolidone) performing the same culture conditions; they obtained 80 % shoot induction. The results above claimed that in cotton tissue culture studies, firstly genotype, and then explant source, age and culture conditions are important factors affecting success of *in vitro* regeneration, and cotyledonary nodes are more capable and preferable sources than other leaf nodes. Although less than cotyledonary nodes, in this research, obtained higher shooting capacity of first and second leaf nodes of Nazilli

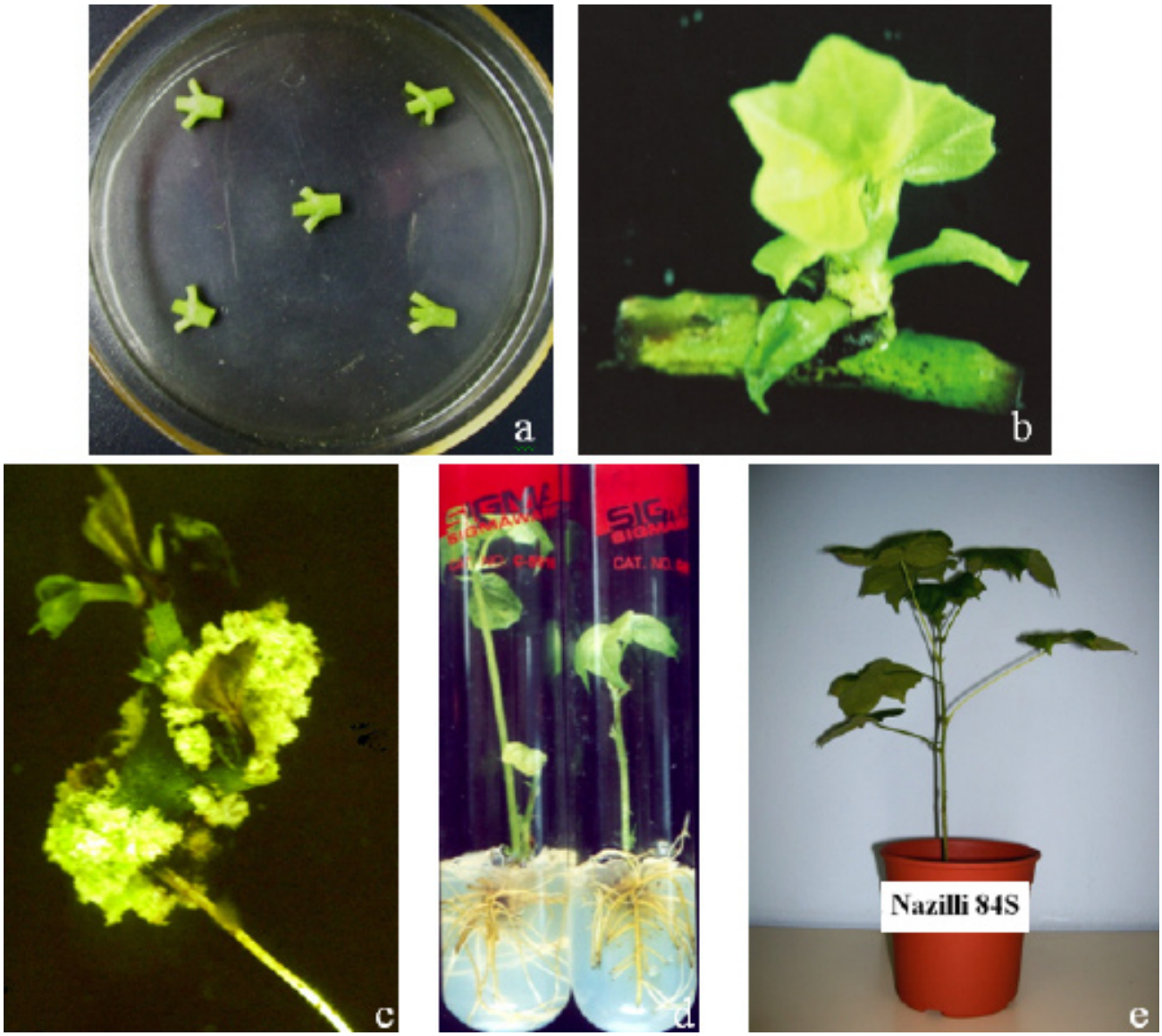

Fig. 1. 35-day-old explants of cotton (*Gossypium hirsutum* L.) var. Nazilli 84S cultured on MS medium supplemented with 0.1 mg/l KIN (a). Direct shoot development from one cotyledonary node (b) and both leaf nodes (c). Rooting cotyledonary node (left) and leaf node (right) in WPM + IBA (d). Adaptation of plant to the soil in pot (f).

84S is remarkable when compared to the results of previous researches with other cotton genotypes.

In this study, two nodes were present in one explant and shooting capacities of both nodes together at the same time were also investigated. It was observed that cotyledonary nodes on hypocotyl pieces were more successful than other leaf nodes on the stem (40.4 %). Moreover, in this study, only 0.1 mg/l KIN was used and multiple shoots were not mostly obtained while Rauf *et al*., 2004 resulted multiple shoots by using four different concentrations (0.1, 0.25, 0.5 and 1.0 mg/l) of KIN in cotton cv. NIAB-999. According to the literature, BA is one of the most effective hormones for obtaining multiple shoots from cotyledonary nodes (Hemphill *et al*., 1998; Gupta *et al*., 2000). Thidiazuron is also known as a promoter of multiple shoots in cotton explants, especially for cotyledonary nodes (Caramori *et al*., 2001).

Obtained and subcultured shoots were transferred into culture tubes, which included Woody Plant Medium (WPM) and 1 mg/l IBA. After 15-30 days, rooting was observed. The rooted plantlets were potted in a mixture of soil and perlite (1:1, v/v), and grown in a greenhouse conditions for adaptation to natural environments.

Cotton tissue culture systems are genotype dependent and different explant sources show different regeneration affinities. Although worldwide, most of the planted cottons are transgenic, only a few varieties could be genetically modified. Different local varieties were adapted to their own area's climate, soil and altitude properties. Thus, all varieties and possible explant sources are valuable and

establishment of rapid *in vitro* regeneration systems from different varieties is very important. Success with local varieties will become alternative solutions to some problems for genetically modified plants in the future.

References

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

Caramori, L. P. C., S. Fávaro and L. G. E. Vieira (2001). Thidiazuron as a promoter of multiple shoots in cotton explants (*Gossypium hirsutum* L.). Maringá. 23(5):1195-1197.

Collins, J. R (1996). BXN Cotton: Marketing plans and wed control programs utilizing buctril. p. 201. In Proc. Beltwide Cotton Conf., Nashville, TN. 9-12 Jan.1996. National Cotton Council, Am., Memphis, TN.

Dong, H. Z., W. J.Li , W . Tang and D. M Zhang (2004). Development of hybrid Bt cotton in China-A successful integration of transgenic technology and conventional techniques. Current Science. 86:778-782.

Dong H, Li W, Li Z, Tang W, Zhang D, 2005. Evaluation of a production system in china that uses reduced plant densities and retention of vegetation branches. The Journal of Cotton Science, 9: 1-9.

Efe, L. (2005). Callus formation and plant regeneration from two cotton species (*Gossypium hirsutum* L. and G. barbadense L.). Pakistan Journal of Botany. 37(2):227-236.

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

Gupta, S. K., A. K. Srivastava, P. K. Singh and R. Tuli (1997). In vitro proliferation of shoots and regeneration of cotton. Plant Cell Tissue and Organ Culture. 51(2):149-152.

Gupta, S. K., P. K. Singh, S. V. Sawant, R. Chaturvedi and R Tuli (2000). Effect of light intensity on in vitro multiple shoot induction and regeneration of cotton (*Gossypium hirsutum* L. cv Khandawa-2). Indian Journal of Experimental Biology. 38:399-401.

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

Hsu, H. H. and F. Gale (2001). Regional shifts in China's cotton production and use. Cotton Wool Situation Outlook. 11:19-25.

Kumria, R., V. G. Sunnichan, D. K. Das, S. K. Gupta, V. S. Reddy, R. K. Bhatnagar and S. Leekavathi (2003). High-frequency somatic embryo production and maturation into normal plants in cotton (*Gossypium hirsutum* L.) through metabolic stress. Plant Cell Reports. 21:635-639.

Khan, N. U., G. Hassan, K. B. Marwat, M. B. Kumbhar, I. Khan, Z. A. Soomro, M. J. Baloch and M. Z. Khan (2009). Legacy study of cotton seed traits in upland cotton using Griffing's combining ability model. Pakistan Journal of Botany. 41(1):131-142.

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

McCown, B. H. and G. Lloyd (1981). Woody Plant Medium (WPM) - a mineral nutrient formulation for microculture for woody plant species. Horticultural Science. 16:453.

Méndez-Natera, J. R., A. Rondón, J. Hernández and J. F. Merazo-Pinto (2007). Genetic studies in upland cotton (*Gossypium hirsutum* L.) I. heterotic effects. Pakistan Journal of Botany. 39(2):385-395.

Meyer, L., S. MacDonald and L. Foreman (2007). Cotton Backgrounder: Outlook Report from the Economic Research Service. USDA (United States Department of Agriculture). CWS-07B-01. 1-33. www.ers.usda.gov.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15:473-497.

Naz, S., A. Ali, F. A. Siddique, J. Iqbal (2007). Multiple shoot formation from different explants of chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany. 39(6):2067-2073.

Ozyigit, I. I., M. V. Kahraman and O. Ercan (2007a). Relation between explant age. total phenols and regeneration response of tissue cultured cotton (*Gossypium hirsutum* L.). African Journal of Biotechnology. 6(1):3-8.

Ozyigit, I. I., N. Gozukirmizi and B. D. Semiz (2007b). Genotype dependent callus induction and shoot regeneration in sunflower (*Helianthus annuus* L.). African Journal of Biotechnology. 6(13):1498-1502.

Ozyigit, I. I. and N. Gozukirmizi (2008). High efficiency shoot and root formation from cotyledonary nodes of cotton (*Gossypium hirsutum* L.). Pakistan Journal of Botany. 40(4):1665-1672.

Ozyigit, I. I. and N. Gozukirmizi (2009). Efficient shoot and root formation from shoot apices of cotton (*Gossypium hirsutum* L.). Russian Journal of Plant Physiology. 56(4):527-531.

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

Rashid, B., T. Husnain and S. Riazuddin (2004). In vitro shoot tip culture of cotton (*Gossypium hirsutum*). Pakistan Journal of Botany. 36(4):817-823.

Song, P., J. L. Heinen, T. H. Burns and R. D. Allen (2000).

Expression of two tissue-specific promoters in transgenic cotton plants. The Journal of Cotton Science. 4:217-223.

Zapata, C., M. Srivatanakul, S. H. Park, B. M. Lee, M. G. Salas and R. G. Smith (1999). Improvements in shoot apex regeneration of 2 fiber crops: cotton and kenaf. Plant Cell Tissue Organ Culture. 56:185-191.