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 & Gozukirmiz, 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 & Gozukirmiz, 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.,...
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, cotyledonal 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 cotyledonal 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 cotyledonal 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 cotyledonal 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, Stonewell 7A and Stonewell 474). They observed that cotyledonal, 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 cotyledonal 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 cotyledonal 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 cotyledonal 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) cotyledonal 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 cotyledonal nodes are more capable and preferable sources than other leaf nodes. Although less than cotyledonal nodes, in this research, obtained higher shooting capacity of first and second leaf nodes of Nazilli...
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


