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High frequency induction of multiple shoots and plant regeneration from zygotic embryo axis explants of cotton cultivar L-604 (*Gossypium hirsutum* L.).

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Abstract: An efficient, robust regeneration protocol has been developed using zygotic embryo axis explants of an elite cultivar L-604 (*Gossypium hirsutum* L.). Explants regenerated on MSB5 medium supplemented with 0.5 mg^{-1} Thidiazuron, 0.1 mg^{-1} Naphthalene acetic acid, 5.0 mg^{-1} Silver nitrate and 1.0 g^{-1} activated charcoal, was identified as the best combination for producing the maximum number (12.67) of multiple shoots per explant. In addition, the highest shoot elongation (5.30 cm) and maximum regeneration frequency (89.0%) was also recorded in the same hormonal combinations. *In vitro* shoots were successfully rooted on ½ MSB5 medium containing 1.5 mg^{-1} IBA. Well rooted plants were subsequently transferred to polyhouse and greenhouse conditions for primary and secondary hardenings respectively and about 95% of the plants were established successfully. The conditions optimized in this study for inducing multiple shoots could be best suitable for development of transgenic L-604 variety for agronomically important traits.

Key words: *Gossypium hirsutum*; Organogenesis; Multiple shoots; Zygotic embryo axis

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the world's most important cash crops and it plays a major role in the global economy. It is the richest source of fiber and the backbone for the textile industry. Further, cotton seed is used for oil extraction and seed cake is also used as animal feed. It is commercially grown worldwide in almost 70 countries and the source of livelihood for around 180 million people (John, 1997; Rahman *et al.,* 2012). Among major cotton growing countries, India (11.6 Mha) stands first in the area of cultivation followed by China (6.3 Mha), USA (4.2 Mha), Pakistan (3 Mha), Uzbekistan (1.45 Mha), Brazil (1 Mha) and Turkey (0.5 Mha). However, China leads highest in productivity (1265 kg ha^{-1}) followed by USA (985 kg ha^{-1}), Uzbekistan (831 kg ha^{-1}), Pakistan (599 kg ha^{-1}) and India (560 kg ha^{-1}) (Juturu *et al.,* 2015). Low productivity in spite of high acerage in India is due to its susceptibility to various biotic and abiotic stresses and lack of resistant lines with good agronomic traits. Genetic engineering has become a viable alternative to overcome these problems associated with narrow genetic base and sexual incompatibility of classical breeding to develop the resistant lines.

Genetically engineered cotton against lepidopteran pest is highly successful throughout the world, more than 24 Mha of cotton grown throughout the world is genetically modified cotton hybrids for insect resistance, which is equivalent to ~81% of total cotton growing area (James Clive, 2012). Despite of this handsome achievement, cotton transformation in important and high yielding varieties is facing serious limitations; one among them is lack of robust, reproducible tissue culture protocols. A reliable, reproducible and efficient regeneration system is prerequisite for the development of a robust genetic transformation system. Like other crops, genetic transformation of cotton is difficult and challenging because of its recalcitrant nature to *in vitro* regeneration. Somatic embryogenesis was predominantly reported in cotton compared to organogenesis since after the report of Davidonis and Hamilton (1983). However, many of the reports were on coker background cultivars because of its good regenerating ability although they were agronomically poor. Moreover, Somatic Embryogenesis approach has many limitations such as, prolonged culture periods, low fertility of regenerated plants, genotype dependency and somaclonal variations[3]. To circumvent these problems, organogenesis was considered as one of the alternative potential methods in several species including cotton. Several reports in cotton through organogenesis have been published especially with cotyledonary nodal explants (Rauf *et al.,* 2005;

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Abdellatef and Khalafalla, 2007; Yang *et al.,* 2010; Obembe *et al.,* 2011; Mushke *et al.,* 2012; Chakravarthy, 2013) or shoot tip (Hemphill *et al.,* 1998; Satyavathi *et al.,* 2002; Tripathy *et al.,* 2002) and hypocotyl explants (Divya *et al.,* 2008). However, the lower regeneration frequency of these reports ultimately affects the transformation frequency. Thus, there is an urgency to establish robust and efficient regeneration protocols by reconsidering the explants like zygotic embryo axes which are more feasible for transformation. In this report, we featured an efficient method for *in vitro* multiple shoot induction using zygotic embryonic axes explants of Indian high yielding, drought and aphid resistant cultivar L-604. As per our knowledge there are no protocols are available for multiple shoot induction of L-604 variety.

Materials and Methods

Cotton seeds (*cv* L-604) were collected from Acharya N G Ranga Agricultural University, Lam farm, Guntur, India. The seeds were delinted by treating with concentrated sulphuric acid (H_2SO_4) for 2-3 min followed by sodium bicarbonate wash to nullify the acid effect. Then the seeds were thoroughly rinsed with sterile distilled water 3-4 times and dried for 2 days at 40 ± 2 ºC. Healthy and matured seeds were selected and disinfected with 1% (w/v) bavistin solution for 10 min followed by 70% (v/v) ethanol for 5 min. Then, the seeds were surface sterilized with 0.1 % (w/v) mercuric chloride ($HgCl_2$) solution for 20 min and thoroughly washed with sterile distilled water for 5-6 times to remove the traces of $HgCl_2$. The seeds were then soaked for overnight in sterile distilled water under dark.

Mature zygotic embryos were separated from overnight soaked seeds by removing the seed coat and zygotic embryo axis were carefully prepared by dissecting the primary cotyledonary leaves and radical and inoculated vertically upright position with the hypocotyl cut end slightly embedded in shoot induction media (SIM) MSB5 (Murashige Skoog salts and B5 vitamins) supplemented with different concentrations of Kn (0.1, 0.5, 1.0, 2.0 mg^{-l}), BAP and TDZ (0.1, 0.5, 1.0, 2.0 and 3.0 mg^{-l}) in combination with NAA (0.1 and 0.5 mg^{-l}). Overall, 23 different combinations of media were used and designated as SIM1-SIM4 containing Kn, SIM5-SIM14 containing BAP and SIM15-SIM22 containing TDZ. In all the media combinations (SIM1-SIM22) 0.1, 0.5 mg^{-l} NAA and 5.0 mg^{-l} silver nitrate was included. The control experiment without any plant growth regulators (SIM0) was also conducted simultaneously. After 15 days of initial culture, the emerging shoot buds were transferred to fresh SIM medium supplemented with half amount of initial concentration of the growth regulators and then incubated for another four weeks. A total of 35 explants were inoculated in each treatment in five replicates as 7 explants per each culture and repeated three times.

Shoots induced in different growth media were shifted basal MSB5 medium without supplementing growth regulators and cultured for two weeks. The elongated shoots which were more than 3 cm in length were kept for rooting on root induction medium containing half-strength MSB5 medium supplemented with different concentrations of IBA and IAA (0.5, 1.0 and 1.5 mg^{-l}) with 1.5% maltose and cultured for another three weeks. Frequency of rooting was recorded. In all the media, 3% (w/v) maltose as a carbon source and 0.8% (w/v) phyto agar (Hi-Media) as gelling agent was used. The pH of the media was set to ~5.8 with 0.1N NaOH or 0.1N HCl before autoclaving at 121 ºC at 15 lbs pressure for 15 min. All the stages of cultures were maintained at 16/8 h light/dark photo period under cool white fluorescent lamps (50 $\mu Em^{-2}\ s^{-1}$) at 25 ± 2 ºC. All the experiments were executed as triplicates and data was statistically analyzed using statistical package SPSS 22.0 version.

The well rooted plants were washed carefully under running tap water to remove any traces of agar sticking to the roots and transferred to poly bags containing 1:1 (W/W) of autoclaved vermiculite: sand for primary hardening and they were maintained under poly house to minimize transpiration losses. These plantlets were slowly acclimatized in 10-15 days by gradually exposing them to the open environment. Acclimatized plants were subsequently transferred to bigger poly bags containing soil and farm yard manure in 3:1 (W/W) and grown till their maturity under controlled conditions. The survival percentage of plants during acclimatization was recorded.

Results and Discussion

The cultivar L-604 used in the present study is a high yielding with drought tolerance and aphid resistance variety. Zygotic embryo axis explant is the best choice for regeneration since it is having high number of meristamatic cells, can be readily reprogrammed compared to any other explants. On SIM, explants turned green and underwent considerable enlargement within first week of culture initiation. During second week, multiple shoot buds formed apical meristmatic regions of the zygotic embryo axis explants (Fig. 1b, c). In all 23 different hormonal combinations, SIM1 and SIM16 medium gave lowest (0.17 ± 0.03) and highest (12.67 ± 0.84) shoot number respectively (Table 1). Explants kept on hormone free medium were not showed any effect of regeneration, which implies that the phytohormones played a key role in reprogramming for multiple shoot induction.

Table 1: The effect of various combinations of plant growth regulators on regeneration of cotton with zygotic embryo axis explants. The data represent means from three replicates ± standard error. Means denoted by different letters differ significantly at $P = 0.05$.

Treatments	Plant growth regulators (mg^{-1})				Mean no of shoots	Shoot length (cm)	Regeneration frequency (%)
	KN	BAP	TDZ	NAA			
SIM1	0.1			0.1	0.17 ± 0.03g	1.93 ± 0.97f	54.0%
SIM2	0.5			0.1	0.30 ± 0.06g	3.73 ± 0.07d	58.0%
SIM3	1.0			0.1	0.13 ± 0.09f	2.73 ± 0.03f	62.0%
SIM4	2.0			0.1	0.47 ± 0.20ef	3.23 ± 0.09e	66.0%
SIM5		0.1		0.1	1.40 ± 0.15ef	4.80 ± 0.10b	68.0%
SIM6		0.5		0.1	2.27 ± 0.19de	4.13 ± 0.09c	72.4%
SIM7		1.0		0.1	2.50 ± 0.25e	3.73 ± 0.27d	63.2%
SIM8		2.0		0.1	4.90 ± 1.01b	2.73 ± 0.03f	68.2%
SIM9		3.0		0.1	2.70 ± 0.19de	2.30 ± 0.17g	60.1%
SIM10		0.1		0.5	2.67 ± 0.34de	3.83 ± 0.07cd	66.2%
SIM11		0.5		0.5	2.47 ± 0.12de	3.83 ± 0.19cd	58.2%
SIM12		1.0		0.5	2.87 ± 0.03d	3.43 ± 0.24de	60.0%
SIM13		2.0		0.5	4.87 ± 0.50b	2.30 ± 0.25g	72.5%
SIM14		3.0		0.5	3.10 ± 0.25cd	2.93 ± 0.13ef	68.5%
SIM15			0.1	0.1	5.30 ± 0.26b	4.63 ± 0.18b	84.0%
SIM16			0.5	0.1	12.67 ± 0.84a	5.30 ± 0.23a	89.0%
SIM17			1.0	0.1	3.33 ± 0.38cd	3.60 ± 0.12d	79.2%
SIM18			2.0	0.1	0.60 ± 0.10fg	1.10 ± 1.12i	58.0%
SIM19			0.1	0.5	5.10 ± 0.35b	3.93 ± 0.09cd	58.0%
SIM20			0.5	0.5	3.77 ± 0.35c	4.13 ± 0.15c	78.0%
SIM21			1.0	0.5	2.93 ± 0.27d	4.83 ± 0.09b	66.0%
SIM22			2.0	0.5	0.67 ± 0.15fg	1.17 ± 0.09h	52.0%

Three major cytokinins or cytokinin like substances (Kn or BAP or TDZ) along with mild concentrations of NAA were tried for *denovo* regeneration. Varied response was observed with different plant growth regulator combinations as expected. Kinetin along with NAA promoted shoot tip elongation instead of multiple shoot induction as a result most of the explants did not induced multiple shoots at all. Kinetin promotes mostly single shoot proliferation[10]. Therefore, this combination of plant growth regulators has very minimal effect on multiple shoot induction. BAP induced multiple shoots in all the concentrations and highest number (~ 4.9) was observed in SIM8 (2.0 mg^{-1} BAP + 0.1 mg^{-1} NAA) and SIM13 (2.0 mg^{-1} BAP + 0.5 mg^{-1} NAA) with no significant difference (Table 1). However, increased concentrations of BAP from 2 mg^{-1} to 3 mg^{-1}, not only resulted in significant reduction in number of shoots, but also increased the abnormal shoot formation. Therefore, higher concentrations of BAP inhibit shoot regeneration and similar results were also observed by Morre *et al.,* (1998) and Banerjee *et al.,* (2003) with the zygotic embryo axis explant. Chakravarthy (2013) also noted decrease in multiple shoot number besides abnormal morphology from cotyledonary node explants of cotton, when BAP concentration increased to 3 mg^{-1}.

In the present study TDZ showed superior effect over BAP and KN on multiple shoot induction as well as high frequency regeneration. Lower concentrations of TDZ along with NAA predominantly induced multiple shoots. TDZ at 0.5 mg^{-1} concentration in combination with 0.1 mg^{-1} NAA induced maximum number of multiple shoots/explants (12.67) with highest regeneration frequency (89%) besides highest shoot elongation (5.30 cm) (Table 1). However, increased concentrations of TDZ and NAA resulted in the formation of callus which was leading to significant reduction of shoot number. Similar results were also reported by Sathyavathi *et al.,* (2002) and Divya *et al.,* (2008) with shoot tip and hypocotyl explants respectively in the Indian elite cultivars with higher concentrations of TDZ. Similarly, Chakravarthy (2013) observed decrease in mean shoot number and percent response due to callus formation at the base of the cotyledonary node explants at higher concentration of TDZ. TDZ a phenylurea compound originally developed as herbicide, but exploited as a growth regulator in tissue culture especially in woody plants for the first time. Since then many researchers used TDZ in *in vitro* regeneration studies and in fact, Singh *et al.,* (2003) proved it is more potent than BAP in *in vitro* regeneration of pigeon pea cultivars. Our results were also convincible with the results of Singh *et al.,* (2003). Therefore, TDZ can also be used in the tissue culture experiments of wide range of plant species. Silver nitrate (5 g^{-l}) an anti ethylene factor has been used in the shoot induction medium to reduce hyper hydrosity nature of explant which has been well reported for the same in various species *viz. Brassica* sp (Palmer, 1992), Peanut (Pestana *et al.,* 1995; Ozudogru *et al.,* 2005) and Pomegranate (Naik and Chand, 2003) including Cotton (Tripathy *et al.,* 2002; Abdellatef and Khalafalla, 2007; Divya *et al.,* 2008). Further, woody plants especially cotton produces enormous amount of phenolics from the cut ends of the tissues, which causes deleterious effects on regenerating tissues, to minimize this effect plant tissue culture grade activated charcoal (1.0 g^{-l}) was used in this study in all the media excepting in

elongation medium, and thereby achieved less browning or necrosis. Subculture of explants to SIM with reduced concentrations of hormones and final culture on MSB5 basal medium without any hormones resulted in simultaneous elongation of multiple shoots while proliferation (Fig. 1f).

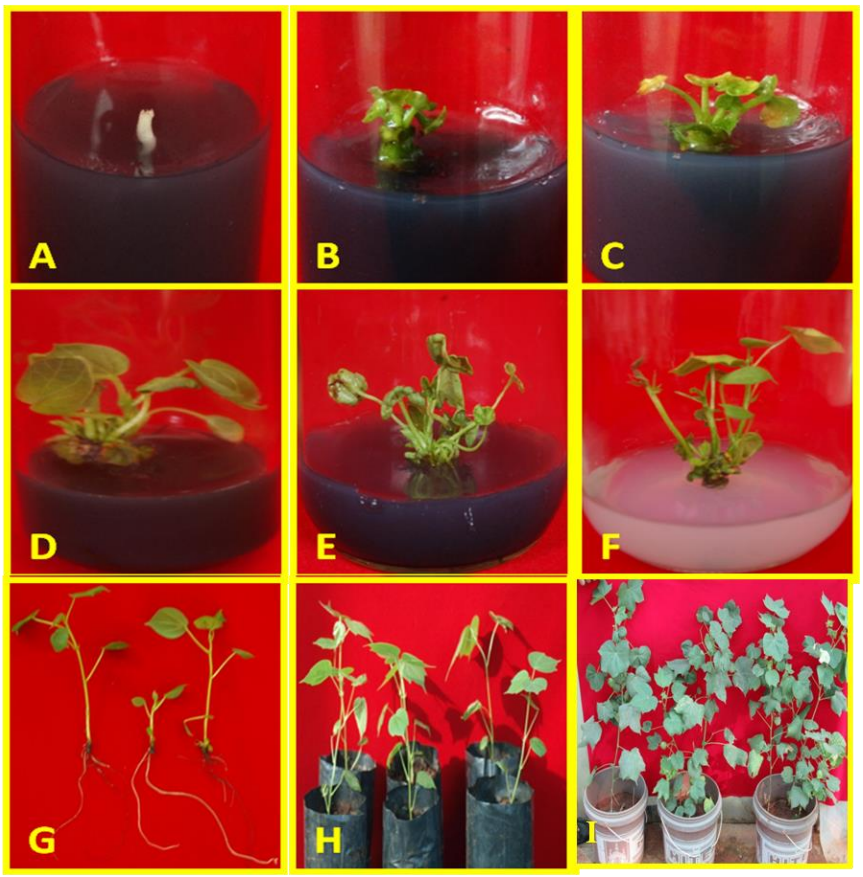

Figure 1: Regeneration of zygotic embryo axis explants of cotton (*Gossypium hirsutum* L. cv. L-604). (A) Overnight soaked zygotic embryo axis explants on shoot induction medium. (A-E) Different stages of multiple shoots arising from zygotic embryo axis explants. (F) Elongation of multiple shoots on shoot elongation medium. (G) Root formation of individual elongated shoots. (H) Well rooted plants in primary hardening stage. (I) Regenerated plant showing flowering.

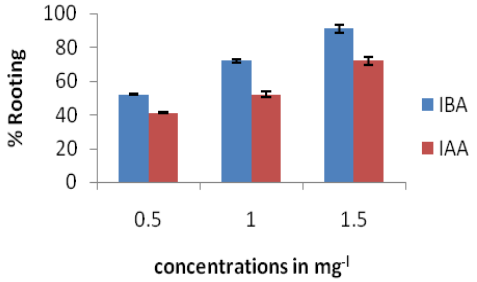

Figure 2: Effect of different growth regulators (IBA and IAA) on *in vitro* rooting of the cotton cultivar L-604.

The well elongated shoots were transferred to half strength MSB5 medium with various concentrations of IBA or IAA (0.5, 1.0 and 1.5 mg^{-l}) for *in vitro* rooting. Roots were induced from base of the shoots during first week of culture and well established healthy root system was developed within three weeks (Fig. 1g). Roots were induced on all hormone concentrations tested. However, the rooting frequency varied from 52-91 % in IBA and 41 – 72% in IAA containing medium. The varied response of different auxins under *in vitro* rooting may be due to their differential binding to auxin receptors involved in root formation of the shoots (Chakravarthy, 2013). In this study rooting frequency increased with increase in auxin concentration and highest percentage (91%) of rooting was achieved in 1.5 mg^{-l} IBA containing medium (Fig. 2). Similarly, Ouma *et al.,* (2004) reported that, IBA treatment resulted in more shoots compared to other auxins (NAA, IAA). Well rooted plants shifted to small polythene bags containing 1:1 ratios of vermiculite and sand for primary hardening. Initially they were kept in poly house to maintain humidity and then to greenhouse conditions in bigger bags after 2 weeks. Normal flowering and boll formation was observed in all the tissue cultured plants (Fig. 1i). It all took around 90 to 100 days to regenerate completely hardened mature plants from zygotic embryo axis explants.

Conclusion

Easier isolation, good regeneration and handling of this zygotic embryo axis explants might certainly acts as a potential alternative choice of explant for regeneration and more particularly for transfer/ delivery of novel agronomical important gene(s) either with *Agrobacterium* or biolistic transformation methods. To our knowledge this is the first report showing highest number of shoots with zygotic embryo axis explant. Finally, in this article we have reported a robust plant regeneration protocol which can be exploited for transformation studies in cotton.

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Authors Contribution

Author V.N.J. contributed to the acquisition of data by conducting different experiments and manuscript preparation. GKM has made substantial contribution in critically drafting and editing the manuscript. ACS and MG contributed in designing and critical revision of the manuscript.

References

1. Abdellatef E, M.M Khalafalla, "Adventitious shoot and plantlet formation in medium staple cotton cultivar (*Gossypium hirsutum* L. cv. Barac [67] B)". Int. J. Agric. Biol. 9 (2007): 913-916.

2. Banerjee, A.K, D.C Agarwal, S.M Nalawade, S. Hazra, "Multiple shoot induction and plant regeneration from embryo axes of six cultivars of *Gossypium hirsutum"*. Biol. Plant. 47 (2003): 433-436.

3. Chakravarthy V.S.K, "Rapid production of multiple shoots from cotyledonary node explants of an elite cotton (*Gossypium hirsutum* L.) variety". Research in Plant Biology. 3 (2013): 06-13.

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

5. Divya K, A.T Swathi, S.K Jami, P.B Kirti, "Efficient regeneration from hypocotyls explants in three cotton cultivars". Biol. Plant. 52 (2008): 201-208.

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

7. James Clive, "Global status of commercialized Biotech/GM Crops". (2012): ISAAA Brief No.44, Ithaca, New York, USA. http://www.isaaa.org/resources/publications/briefs/44/ download /isaaa-brief-44-2012

8. John M.E, "Cotton crop improvement through genetic engineering". Crit. Rev. Biotech. 17 (1997): 185-208.

9. Juturu V.N, G.K Mekala, P.B Kirti, "Current status of tissue culture and genetic transformation research in cotton (Gossypium spp)". Plant. Cell. Tissue and Organ. Cult. 120 (2015): 813-839.

10. Morre J.L, H.R Permingeat, M.V Romagnoli, C.M Heisterborg, H.R Vallejos, "Multiple shoot induction and plant regeneration from embryonic axes of cotton". Plant. Cell. Tissue. Organ. Cult. 54(1998): 131-136

11. Mushke R, T. Sultana, P.K Pindi, "High frequency regeneration and multiple shoot induction in Indian cotton (*Gossypium hirsutum* L.) cultivar". Res. J. Agri. Sci. 3 (2012): 1109-1112.

12. Naik S.K, P.K Chan, "Silver nitrate and aminoethoxy venyl glycene promotes in vitro adventitious shoot regeneration of Pomegranate (*Punica granatum* L.)". Journal of Plant Physiology. 160 (2003): 423-430.

13. Obembe O.O, T. Khan, J. Popoola, "High frequency multiple shoots induction and plant regeneration in six elite Indian cotton cultivars". Can. J. Pure. Appl. sci. 5(2011): 1385-1389.

14. Ouma J.P, M.M Young, N.A Reichert, "Rooting of in vitro regenerated cotton (*Gossypium hirsutum* L.) is influenced by genotype, medium composition, explants type and age". Afr. J. Biotechnol. 3 (2004): 313-318.

15. Ozudogru E, Y. Ozden-Tokatli, A. Akcin, "Effect of silver nitrate on multiple shoot formation of Virginia-type peanut through shoot tip culture". In Vitro Cellular and Development Biology-Plant. 41 (2005): 151-156.

16. Palmer, C.E, "Enhanced shoot regeneration from *Brassica campestris* by silver nitrate". Plant. Cell. Reports. 11 (1992): 541-545.

17. Pestana M.C, C. Lacorte, V.G Defreitas, D.E Oliveira, E. Mansur, "In vitro regeneration of peanut (*Arachis hypogaea* L.) through organogenesis: Effect of culture temperature and silver nitrate". In vitro Cellular and Developmental Biology-Plant. 35 (1995): 214-216.

18. Rahman M, T. Shaheen, N. Tabbasam, M.A Iqbal, M. Ashraf, Y. Zafar, A.H Paterson, "Cotton genetic resources: A review". Agron. Sustain. Dev. 32 (2012): 419-432.

19. Rauf S, M. Usman, B. Fatima, A. Khan, "In vitro regeneration and multiple shoot induction in upland cotton (*Gossypium hirsutum* L.)". Plant. Tiss. Org. Cult. 15 (2005): 75-81.

20. Satyavathi V.V, V. Prasad, G.B Lakshmi, S. Lakshmi, "High efficiency transformation protocol for three Indian cotton varieties via *Agrobacterium tumefaciens*". Plant. Sci. 162 (2002): 215-223.

21. Singh N.D, L. Sahoo, B.S. Neera, P.K Jaiwal, "The effect of TDZ on organogenesis and somatic embryogenesis in *pigeon pea* (*Cajanus cajan* L. Millsp)". Plant. Sci. 164 (2003): 341-347.

22. Tripathy S. and G.M Reddy, "A study on the influence of genotype, medium and additives on the induction of multiple shoots in indian cotton cultivars". Asian. J. Microb. Biotech. Env. Sci. 4 (2002): 515-519.

23. Yang X.Y, X.L Zhang, L.L Fu, L. Min, G.Z Liu, "Multiple shoot induction in wild cotton (*Gossypium bickki*) through organogen-esis and the analysis of genetic homogeneity of the regenerated plants. Bilogia". 65 (2010): 496-503.

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