BIOLOGIA PLANTARUM 49 (3): 415-417, 2005

BRIEF COMMUNICATION

An efficient and rapid *in vitro* regeneration system for metal resistant cotton

E. BIÇAKÇI* and A.R. MEMON**,[1]

*Yildiz Technical University, Department of Biology, Istanbul, Turkey**
*Research Institute for Genetic Engineering and Biotechnology, TUBITAK, P.O. Box 21, 41470 Gebze, Kocaeli, Turkey***

Abstract

In this report we describe the most suitable protocol for callus formation and plant regeneration for cotton. We screened 15 cotton (*Gossypium hirsutum* L.) genotypes for metal resistance and two of them, Nazilli M-503 (M503) Nazilli 143 (N-143) selected as Cd, Cu and Ni resistant. The cotyledonary nodes from these genotypes were the best explants for regeneration of shoots (more than 90 %) and roots (50 to 70 %). Shoot apex also gave good shoot regeneration (more than 90 %) but their root regeneration efficiency was low (35 %). These results show that Murashige and Skoog (MS) media containing 0.44 μM naphthaleneacetic acid (NAA) and 0.98 μM indole-3-butyric acid (IBA) was the most suitable recipe for getting high shoot and root regeneration from cotyledonary nodes of N-143 and M503 cotton genotypes.

Additional key words: callus, *Gossypium hirsutum*, heavy metal tolerant plants, plant regeneration.

Heavy metal contamination can significantly reduce cotton yield and quality. Additionally irrigation and continuous heavy application of fertilizers and pesticides worsen this situation. Metal toxicity is becoming increasingly important growth limiting factor for cotton cultivation (Memon *et al.* 2000). One of the main reasons for this work is to regenerate metal resistant cotton from suitable plant parts of selected metal tolerant genotypes.

Plant regeneration *via* organogenesis has crucial importance in the application of *in vitro* methods for cotton improvement (Aydin *et al.* 2004). The regeneration of cotton is reported to be difficult and regeneration frequency is dependent on genotype used (Saeed *et al.* 1997, Zapata *et al.* 1999). Problems with cotton tissue culture include slow growth of callus tissue and production of some pigments inhibitory to callus growth, which can interfere with *Agrobacterium* transformation system (Rani and Bhojwani 1976). Because most of the difficulties in obtaining transgenic cotton are associated largely with regeneration, it will be useful to improve the trans-formation efficiencies so that large number of independent callus lines can be obtained from which good regenerative lines can be selected. These regenerative lines could also be used for efficient micro-propagation.

In this work we have examined several factors including different plant parts (cotyledonary node, hypocotyl, shoot apex and leaf) and different hormonal ratios that can significantly affect the efficiencies of regeneration.

Two metal tolerant cotton (*Gossypium hirsutum* L.) genotypes were selected (M503 and N-143) from our previous screening experiments to optimize regeneration efficiencies for transformation (unpublished data). Their seeds were obtained from Nazilli Cotton Research Institute. As cotton seeds are highly contaminated (due to

Received 15 August 2003, *accepted* 3 November 2004.
Abbreviations: 2,4 D - 2,4-dichlorophenoxyacetic acid; BAP - 6-benzylaminopurine; GA - gibberellic acid; IBA - indole-3-butyric acid; MS medium - Murashige and Skoog medium; NAA - naphthaleneacetic acid.
Acknowledgements: The help given by Omer Kacar in these experiments is highly appreciated. This work was supported by the Textile Industry grant No. F000301 given to A.R. Memon.
[1] Author for correspondence; fax: (+90) 262 6463929, e mail: armemon@rigeb.gov.tr

hairy surface) they were first treated with concentrated H_2SO_4 for 15 min and then were rinsed for 3 times with distilled H_2O for 3 min each. They were bubbled in H_2O for overnight and were treated with 70 % EtOH containing *Tween-20* (two drops per 100 cm^3) for 1 - 2 min. Later they were shaken in 20 % bleach containing *Tween-20* for 30 min and seeds were washed with sterile H_2O for several times (Saeed *et al*. 1997, Hemphill 1998, Zhang *et al*. 2001).

Surface sterilized and uncoated seeds (7 seeds planted into each *Magenta* jar) were transferred to germination medium containing half-strength Murashige and Skoog (MS) salts, 10 g dm^{-3} sucrose, 1 cm^3 dm^{-3} MS vitamin solution (*Sigma-M3900*, USA) and 3 g dm^{-3} *Phytagel* as gelling agent and pH was adjusted to 7.0 (Hemphill 1998). Plants were grown for 7 d under 16-h photoperiod with 96 μmol m^{-2} s^{-1} irradiation, air humidity 60 - 70 %, and day/night temperature of 25/23 °C. The explants (cotyledonary node, shoot apex, cotyledonary leaf and hypocotyls) were cultured in the same MS media as mentioned previously but contained 20 g dm^{-3} sucrose and different concentrations of several plant growth regulators (Table 1). Plant growth regulators were added before autoclaving and pH of the media was adjusted to 5.8 (Hemphill 1998, Morre *et al*. 1998).

To optimize the indirect regeneration system, cotyledonary leaf and hypocotyl tissues were used. Leaf samples were divided (about 0.25- 0.5 cm^2) and placed in the media in such a way that abaxial sides of the leaves were in touch with the culture medium. Explants from hypocotyl were divided about 0.5 - 1 cm in length and wounded tips were dipped in the culture medium. Callus formation were observed and scored. Subcultures were performed every after one-month.

Shoot apex from 7-d-old plants was removed as described by Gould *et al*. (1998) and cotyledonary nodes were cut off about 9 - 16 mm. Plant parts were placed into 10 different culture media in upright position and

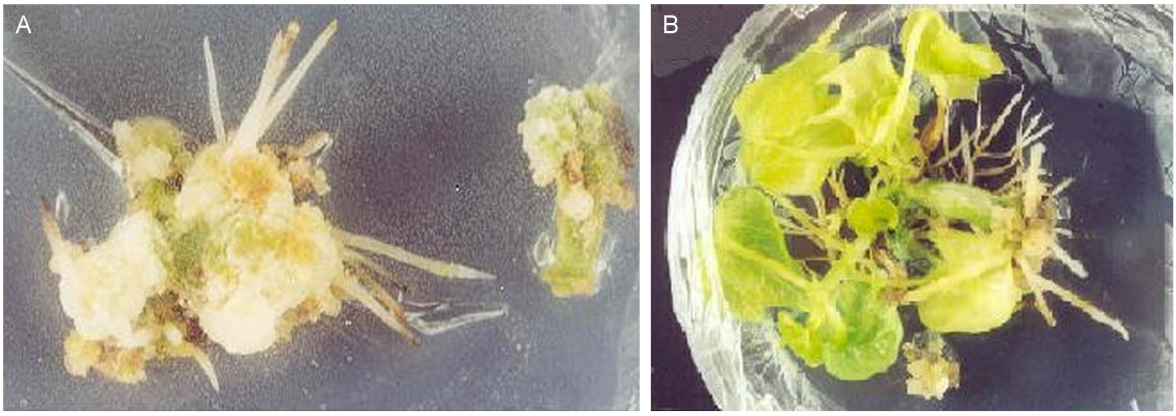

Fig. 1. *A* - Root regeneration on callus tissue developed from hypocotyl (M603 genotype) cultured in MS medium containing 9.12 μM zeatin. *B* - Direct regeneration from cotyledon node of N-143 genotype cultured in the MS medium containing 4.44 μM NAA + 0.98 μM IBA.

Table 1. Regeneration of shoots and roots in N-143 and M503 genotypes as affected by explant type and medium composition. Mean of three replications ± S.D. Each replication contained 20 explants.

Phytohormones [μM]	N-143				M503			
	node		apex		node		apex	
	shoot [%]	root [%]	shoot [%]	root [%]	shoot [%]	root [%]	shoot [%]	root [%]
0.44 NAA	74.65±23.0	72.43± 8.0	78.05± 2.7	9.52±1.3	75.00±17.7	45.00± 7.1	78.30±16.5	11.65± 2.3
0.89 NAA	88.20± 0.3	0.00± 0.0	91.65±11.8	7.75±3.2	97.91± 2.9	50.83±13.0	85.00±21.2	20.00±14.1
2.22 NAA	94.00± 8.5	55.00± 7.7	84.40± 6.2	7.75±3.2	97.80± 3.1	20.00± 2.8	90.00±14.1	26.65± 9.4
4.45 NAA	90.00±14.1	23.30± 4.7	86.50±19.1	16.65±2.3	96.65± 4.7	20.00± 0.0	100.00± 0.0	20.00±14.1
8.90 NAA	96.87± 4.4	53.97±12.0	46.25± 4.7	5.00±0.7	92.50± 3.5	20.00± 0.0	90.00±14.1	35.00± 7.1
0.98 IBA	100.00± 0.0	13.04± 1.8	87.50±17.7	3.30±4.7	95.00± 7.1	7.50± 1.6	96.87± 4.4	0.00± 0.0
0.44 NAA + 0.98 IBA	90.00±14.1	60.00± 5.6	60.00±28.3	8.00±1.1	86.25±19.6	50.55±14.0	77.50±17.7	5.00± 7.1
0.44 NAA + 0.49 IBA	95.00± 7.1	2.25± 3.18	96.65± 4.7	5.00±7.1	97.50± 3.5	10.00± 0.0	100.00± 0.0	0.00± 0.0
0.44 NAA + 0.44 BAP	87.50±15.5	0.00± 0.0	78.90±14.9	0.00±0.0	92.35± 3.3	0.00± 0.0	91.65± 2.3	0.00± 0.0
0.89 NAA + 2.28 GA	87.50±10.6	7.50± 3.5	78.00±17.0	0.00±0.0	16.65±23.5	6.65± 4.9	67.50±31.8	5.00± 7.1

regeneration performances were scored after 1 month (Table 1). Regenerated plantlets were transferred to MS medium without plant growth regulators. After two months plantlets were transferred to autoclaved soil and sand mixture (1:1) in pots and high humidity was maintained by covering the pots with plastic bags. Three weeks later pots were transferred to normal growth chamber conditions as described earlier.

To optimize the regeneration system for M503 and N-143 cotton genotypes, several plant parts were tested in MS based different media containing several combinations of plant growth regulators (9.12 μM zeatin, 4.56 μM zeatin, 2.28 μM zeatin, 0.45 μM zeatin, 0.045 μM zeatin, 0.89 μM naphthaleneacetic acid (NAA) + 9.12 μM zeatin, 8.9 μM NAA, 4.45 μM NAA, 2.22 NAA). Hypocotyls from both genotypes gave good callus formation in most of the media. Callus from cotyledonary leaf explant of N-143 was formed only in the media containing either 0.045 μM zeatin or 0.89 μM NAA together with 9.12 μM zeatin. Callus formation frequency from the cotyledonary leaves of M503 was also relatively good (50 %) when media was supplemented with 2.28 μM zeatin but these callus died at the 3rd week. Root initiation on callus tissue was observed from both leaves and hypocotyls of N-143 and M503 when cultured on media containing 9.12 μM zeatin (Fig 1*A*).

Cotyledonary nodes from both genotypes (M503 and N-143) were most suitable explants for regeneration of shoots (more than 90 %) and roots (50 to 70 %) (Fig. 1*B*). Shoot regeneration from apex was also successful and more than 90 % shoots were regenerated but their root regeneration efficiencies were low (Table 1). Although several hormonal concentrations gave high regeneration efficiencies with individual plant parts, 0.44 μM NAA and 0.49 μM IBA combination was shown to be the best one for regenerating cotyledonary nodes and shoot apex of both genotypes. The explants of these two genotypes exhibited different regeneration frequencies when cultured in different media (Table 1). Cotyledonary node of N-143 genotype gave 100 % shoot regeneration in the media containing 0.98 μM IBA but their root regeneration efficiency in the same media was very low (13 %). Similarly shoot apex of M503 showed 100 % shoot regeneration in the media containing 4.45 μM NAA but its root regeneration efficiency in the same media was around 20 % (Table 1).

These regenerated plants from both cotyledonary node and shoot apex of tolerant genotypes M503 and N-143 were transferred in the loamy soil. At the beginning of growth period, relative humidity of the pots culture was kept constant (~70 %). Mature plants with cotton bolls were obtained in 3 - 4 months (data not shown).

In conclusion our results show that node is the most suitable plant part for getting high efficiency in regeneration of both roots and shoots. Both genotypes showed good shoot and root regeneration efficiencies when their nodes were supplied with 0.44 μM NAA and 0.98 μM IBA.

References

Aydin, Y., Ipekci, Z., Talas-Oğraş, T., Zehir, A., Bajrovic, K., Gozukirmizi, N.: High frequency somatic embryogenesis in cotton. - Biol. Plant. **48**: 491-495, 2004.

Gould, J.H., Cedeno, M.M.: Adaptation of cotton apex culture to *Agrobacterium*-mediated transformation. - Plant mol. Biol. Rep. **16**: 1-10, 1998.

Hemphill, J.K., Maier, C.G.A., Chapman, K.D.: Rapid *in vitro* plant regeneration of cotton. - Plant Cell Rep. **17**: 273-178, 1998.

Memon, A.R., Özdemir, A., Cevher, B., Bıçakçı, E., Kumbaracı, M., Gencer, O.: Characterisation and Expression of Heavy Metal Induced Genes in Cotton Grow Southern Region of Turkey. - The Inter-Regional Cooperative Research Network on Cotton, Adana 2000.

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

Murashige, T., Skoog, F.: A revised medium for rapid growth of and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Rani, A., Bhojwani, S.S.: Establishment of tissue cultures of cotton. - Plant Sci. Lett. **7**: 163-169, 1976.

Saeed, N.A., Zafer, Y., Malik, K.A.: A simple procedure of *Gossypium* meristem shoot tip culture. - Plant Cell Tissue Organ Cult. **51**: 201-207, 1997.

Zapata, C., Srivatanakul, M., Park, S.H., Lee, B.M., Salas, M.G., Smith, R.H.: Improvement in shoot apex regeneration of two fiber crops: cotton and kenaf. - Plant Cell Tissue Organ Cult. **56**: 185-191, 1999.

Zhang, B.H., Feng, R., Liu, F., Wang, Q.: High frequency somatic embryogenesis and plant regeneration of an elite Chinese cotton variety. - Bot. Bull. Acad. sin. **42**: 9-16, 2001.