The Effect of 2,4-D on Callus Induction of *Melia azedarach* L.

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Abstract

Plant growth regulator 2,4-D has an important role on callus induction and growth of cultured plant tissue. In this experiment, embryonic axes and cotyledons of *Melia azedarach* L. were cultured on MS medium with different concentrations of 2,4-D (0, 1 and 5 µM). The highest callus induction percentage was obtained in the medium enriched with 1 µM 2,4-D for embryonic axes and 5 µM for cotyledons. Embryonic axes and cotyledons did not produce callus in the medium without 2,4-D. Embryonic axes produced plantlets on medium without 2,4-D. Callus has a proper potential for plant regeneration through indirect organogenesis and embryogenesis.

Keywords: cotyledons, embryonic axes, micropropagation, Persian lilac, plant regeneration, woody plants

Introduction

Chinaberry or Persian lilac (*Melia azedarach* L., Meliaceae) is an Asiatic multipurpose tree of worldwide cultivation with economic importance (Sharry et al., 2006). It is a fast-growing species with long lasting wood, used as a component of agro-forestry systems (Sharry et al., 2006). Chinaberry is resistant to locust and ants and is a good source of natural compounds with potent insecticide and antimicrobial action (Sharry et al., 2006). The *in vitro* culture techniques are suitable for micropropagation and the establishment of disease free stock materials. Micropropagation deals with the propagation of plants, *in vitro*, has many advantages over conventional vegetative propagation. Micro propagation of woody/ tree/ forest plant is feasible (Haissinget al., 1997). Micropropagation, especially by indirect organogenesis and embryogenesis is a hard process for most types of woody perennials. However, with some exceptions, traditional *in vitro* methods are not as yet practical or commercially viable for most forest trees (Rathore et al., 2004). Callus is a good source for suspension culture, production of secondary metabolites, clonal propagation through somatic embryogenesis and organogenesis, also, the study of cell division, elongation and differentiation process and genetic transformation. Production of the secondary metabolites through plants in natural conditions is limited by several environmental, ecological and climatic conditions (Praveen and Murthy, 2011). Moreover, the production of secondary metabolites is tissue-specific (Nasim et al., 2010). In recent years, plant cell, tissue and organ culture technology has been efficiently utilized in the production of secondary metabolites (Praveen et al., 2010; Piekoszewska et al., 2010). More than three hundred compounds have been isolated from various parts of *M. azedarach* L. (Kumar et al., 1996). Among all culture methods, cell suspension culture (obtained via callus) represents the best system for producing secondary metabolites because of their fast growth rate (Chan et al., 2010; Praveen and Murthy, 2011). Plant growth regulators (PGRs) have important role in callus induction and growth. The 2,4-D has the most application for this purpose (Jain and Häggman, 2007). Type of explants plays important role in the success of callus induction consequently micro propagation. There are limited reports on tissue culture of *M. azedarach* L. Thus, the possibility of callus induction of *M. azedarach* L. by 2,4-D using embryonic axes and cotyledons explants was investigated in this study.

Materials and Methods

Plant Materials

Embryonic axes and cotyledons of *Melia azedarach* L. were isolated from the seeds of mature fruits collected from Mazandaran province in the northern part of Iran.

Disinfection Procedures

Seeds of *M. azedarach* L. were disinfected in 70% ethanol (v/v) for 1 min followed by 1% sodium hypochlorite (w/v) for 15 min and then rinsed three times in sterile water. Embryonic axes were excised from the cotyledons and placed in culture medium for experiments.

Culture Media and Callus Induction

Excised embryonic axes and cotyledons of *M. azedarach* L. were placed in the culture medium for the experiments. All experiments were done in MS (Murashige and Skoog, 1962) medium (basal salt mixture and vitamins) supplemented with 3% sucrose and 0.7% Agar-agar. Also, 2 mg L^{-1} glutamine was added. In order to induce callus on explants (embryonic axes and cotyledons), they were cultured on media containing 0, 1 and 5 µM 2,4-D. All culture media were adjusted to pH 5.7 and autoclaved at 121°C and 1.2 kg cm^{-2} for 20 min. The cultures were incubated at 24°C with a photoperiod of 16/8 h.

Statistical Analysis

In every experiment, approximately 12 embryonic axes and cotyledons of *M. azedarach* L. were treated for each of three replicates. Data were subjected to analysis of variance (ANOVA) and significant differences between treatments were determined by Duncan's Multiple Range Test (DNMRT) using the statistical package for social science (SPSS ver. 14).

Results and Discussion

Excised embryonic axes and cotyledons explants of *M. azedarach* L. were applied for the induction of callus. No callus formation occurred from embryonic axes and cotyledons explants cultured on medium without 2,4-D (Figure 1). After a week, embryonic axes cultured on medium without 2,4-D started to growth and after a month, plantlets produced (Figure 1). Up to 90% callus induction was observed when 2,4-D was applied at 1 and 5 µM. Maximum callus induction and callus mass was obtained on embryonic axes explants grown on medium containing 1 µM 2,4-D. Callus mass produced in this medium was higher than that of produced on medium containing 5 µM 2,4-D (Figure 1). After amonth, callus was produced on cotyledons cultured on medium supplemented with 1 and 5 µM 2,4-D (Figure 2). More than 90% callus induction was observed when 2,4-D was used at 1 and 5 µM. Maximum callus induction and callus mass was obtained on cotyledons explants grown on medium containing 5 µM 2,4-D. Callus mass produced in this medium was larger than that of produced on medium containing 1 µM 2,4-D (Figure 2). Callus produced on medium enriched with 5 µM was more uniform than that of produced on medium enriched with 1 µM (Figure 2).

The success of *in vitro* culture is related to the correct choice of explants material (George et al., 2008). In current study, both of embryonic axes and cotyledons of *M. azedarach* L. showed suitable potential for production of callus. Study of Sharryet al. (2006) on *M. azedarach* L. showed that the callus was formed from cotyledons on the 93% of the explants after 20 days. These researchers revealed that 2,4-D had a positive effect on callus formation. Al-Mallah and Salih (2006) demonstrated that 2,4-D and KIN can be used in callus initiation on leaves and petioles of *M. azedarach* L. since callus was continuously induced at ratio of 60.0%. These researchers revealed that increasing NAA level inhibited callus formation from 82.5% to 6.4%. Results of current work confirm this findings, because maximum callus induction and callus mass was obtained on embryonic axes explants grown on medium containing 1 µM 2,4-D. Callus mass produced in this medium was higher than that of produced on medium containing 5 µM 2,4-D. These results confirm that embryonic axes have enough levels of endogenous hormones and does not require the addition of high levels of exogenous PGRs (Wala and Jasrai, 2003; Al-Mallah and Salih, 2006). Current study showed that the concentration of 2,4-D in embryonic axes is higher than that of cotyledons, because maximum callus mass was calculated on

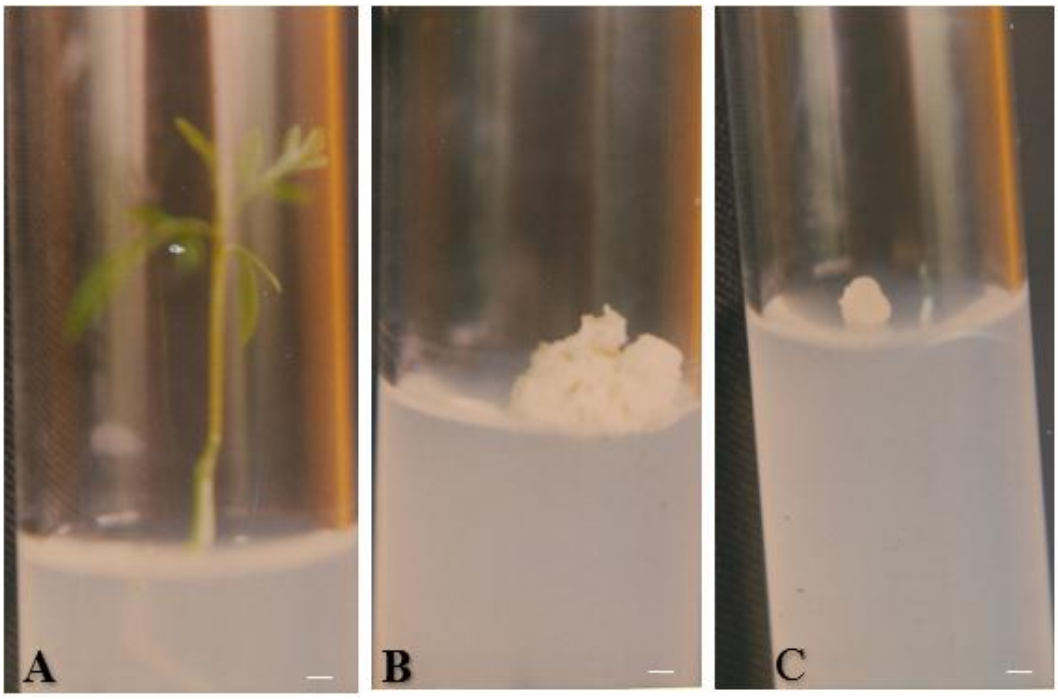

Figure 1 The effect of different concentrations of 2,4-D on embryonic axes of *Melia azedarach* L.: A) Without 2,4-D; B) 1.00 µM 2,4-D and C) 5.00 µM 2,4- D (Bar=5 mm).

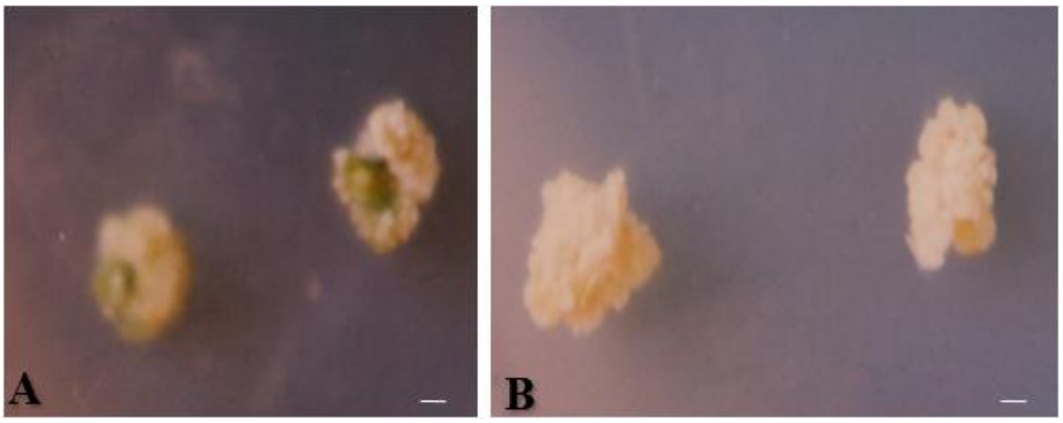

Figure 2 The effect of different concentrations of 2,4-D on cotyledons of *Melia azedarach* L.: A) 1.00 µM 2,4-D and B) 5.00 µM 2,4-D (Bar=5 mm).

cotyledons explants grown on medium containing 5 µM 2,4-D not medium containing 1 µM 2,4-D. Callus mass produced in this medium was larger than that of produced on medium containing 1 µM 2,4-D. Nirmalakumari et al. (1996) indicated that MS medium containing 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} KIN promoted callus formation from cotyledons and leaves explants. It was observed that there are different factors influencing tissue growth and differentiation. These factors include explant source and the addition of PGRs (Mihaljevi et al., 2002; Al-Mallah and Salih, 2006). The importance of auxins (especially 2,4-D) for callus induction in various plants was demonstrated by many researchers (Jain and Ochatt, 2010; Jain and Häggman,2007). My library study on hundreds papers revealed that 2,4-D is the most widely applied for callus formation among all PGRs (Data not published). Ponsamue et al. (1996) showed that auxins tested at higher levels produced only non-embryogenic callus from cotyledon explants of *M. azedarach* L. and at lower levels did not show any

morphogenetic response. Vieitez and Barciela (1990) revealed that 2,4-D, the auxin most widely used for embryonic cultures, induced callus. It was obvious that increasing NAA level inhibited callus formation from 82.5% to 6.4%.These results confirm that this plant have enough levels of endogenous hormones and does not require the addition of high levels of exogenous PGRs (Walaand Jasrai, 2003). Callus induction from explants depends strongly on genotype (George et al., 2008). Study of Mulanda et al. (2012) on *Melia volkensii* Gurke showed that callus induction occurred at a very high frequency (96.7% to 100%) in all treatments including the control, suggesting that callogenesis in mature embryos may be independent of exogenous application of PGRs. The high frequency of callus induction on control media was unexpected. This suggests that endogenous levels of PGRs in mature zygotic embryos of *M. volkensii* may be adequate for callus induction. This is not in agreement with present report showing no callus formation occurred from embryonic axes and cotyledons explants cultured on medium without 2,4-D.

Conclusions

Callus has a suitable potential for plant regeneration through indirect organogenesis and embryogenesis. 2,4-D has an important role in callus induction of explants cultured *in vitro*. The content of PGR especially 2,4-D in embryonic axes of *Melia azedarach* L. are higher than those of cotyledon. The best concentration of 2,4-D for callus induction was 1 µM 2,4-D for embryonic axes and 5 µM for cotyledons.

References

Al-Mallah, Sh. and S.M. Salih. 2006. A protocol for shoot regeneration from leaves petiols tissue culture of neem trees (*Meliaazedarach*). Mesopotamia J. Agric. 34: 1-8.

Chan, L.K., P.S. Lim, M.L. Choo and P.L. Boey. 2010. Establishment of *Cyperusaromaticus* cell suspension cultures for the production of juvenile hormone III. In Vitro Cell Dev.-Biol. Plant 46: 8-12.

George, E.F., M.A. Hall and J.D. Klerk. 2008. Plant Propagation by Tissue Culture. The Background, Springer 1: 65-75.

Haissig, B.E., N.D. Nelson and G.H. Kidd. 1997. Trends in the use of tissue culture in forest improvement. Biotechnol. 5: 52-87.

Jain, S.M. and H. Häggman. 2007. Protocols for Micropropagation of Woody Trees and Fruits. Springer Publication.

Jain, S.M. an S.J. Ochatt. 2010. Protocols for *In Vitro* Propagation of Ornamental Plants. Springer Protocols, Humana Press.

Kumar, C., M. Srinivas and S. Yakkundi. 1996. Limonids from the seeds of *Azadirachtaindica*. Phytochem. 43: 451-455.

Mihaljevi, S., I. Bjedov and M. Kova. 2002. Effect of explant source and growth regulators on *in vitro* callus growth of *Taxusbaccata*L. Washingtonii Food Technol. Biotechnol. 4: 299-303.

Mulanda, E.S., M.A. Ochieng, N.A. Onzere, E. Akunda and J.I. Kinyamario. 2012. High-frequency regeneration of the drought-tolerant tree *Melia volkensii* Gurkeusing low-cost agrochemical thidiazuron.

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

Nasim, S.A., B. Dhir, R. Kapoor, S. Fatima, S. Mahmooduzzafar and A. Mujib. 2010. Alliin production in various tissues and organs of *Allium sativum* grown under normal and sulphur-supplemented *in vitro* conditions. Plant Cell Tiss. Organ. Cult. 101: 59-63.

Nirmalakumari, A., N. Ramaswamy and R. Sree. 1996. Tissue culture studies in neem (*Azadirachta indica* A. Juss), pp. 982-992. In R.P. Singh, M.S. Chari, A.K.Raheja and W. Kraus, eds., Neem and Environment, Vol. 2. Science Publishers, Lebanon, New Hampshire.

Nirmalakumari, A., N. Ramaswamy and S. Rangaswamy. 1993. Tissue culture studies in neem (*Azadirachtaindica* A. Juss), pp. 981-992. In Proceedings of World Neem Conference, 24th -28th February, Bangalore, India.

Piekoszewska, A., H. Ekiert and S. Zubek. 2010. Arbutin production in *Ruta graveolens* L. and *Hypericum perforatum* L. *in vitro* cultures. Acta Physiol. Plant, 32: 223-229.

Ponsamue, J., N.P. Samson, P.S. Ganeshan, V. Sathyaprakash and Abraham, G.C. 1996. Somatic embryogenesis and plant regeneration from the immature cotyledonary tissues of cultivated tea (*Camellia sinenesis* (L.) O. Kuntze). Plant Cell Rep. 16: 210-214.

Praveen, N. and H.N. Murthy. 2011. Effects of macroelements and nitrogen source on biomass accumulation and with anolide-A production from cell suspension cultures of *Withania somnifera* (L.) Dunal. Plant Cell Tiss. Organ Cult. 104: 119-124.

Praveen, N., P.M. Naik, S.H. Manohar, A. Nayeem and H.N. Murthy. 2010. *In vitro* regeneration of brahmi shoots using semisolid and liquid cultures and quantitative analysis of bacoside A. Acta Physiol. Plant 31: 723-728.

Rathore, J.S., N.S. Vinod Rathore, R.P. Shekhawat, G. Singh, G. Liler, M. Phulwaria and H.R. Dagla. 2004.

Micropropagation of woody plants, pp. 195-205. In P.S. Srivastava, A.Narula and Sh. Srivastava, eds., Plant Biotechnology and Molecular Markers. Anamaya Publishers, New Delhi, India.

Sharry, S., J.L.C. Ponce, L.H. Estrella, R.M.R. Cano, S. Lede and W. Abedini. 2006. An alternative pathway for plant *in vitro* regeneration of chinaberry-tree *Melia azedarach* L. derived from the induction of somatic embryogenesis. Electronic J. Biotehnol. Online. DOI: 10.2225/vol9-issue3-fulltext-13.

Vieitez, A.M. and J. Barciela. 1990. Somatic embryogenesis and plant regeneration from embryonic tissues of *Camellia japonica* L. Plant Cell Tiss. Organ Cult. 21: 267-274.

Wala, B. and Y. Jasrai. 2003. Micropropagation of an endangered medicinalplant: *Curculigo orchioides* Gaertin. Plant Tiss. Cult. 1:13-19.

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