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Adventitious Shoot and Callus Formation *in Vitro* from Young Leaves of *Melastoma affine*

Guohua Ma[1*] • Yong Li[2] • Genlin Jiao[2] • Xiaoping Fu[2] • Yourun Lin[1]

[1] South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou, 510650, China
[2] Fairylake Botanical Garden, Shenzhen 518004, China

Corresponding author: * magh@scib.ac.cn

ABSTRACT

The genus *Melastoma* contains about 100 species and is distributed mainly in South Asia, the Pacific Ocean and Australia. In China, there are nine species and one variety. They are mainly distributed in areas South of the Changjiang River. Among these, most species in the genus *Melastoma* have varying foliage and beautiful flowers that are used for ornamental and horticultural purposes. In this study, we established an *in vitro* propagation system for *Melastoma affine*. Young leaf lobes from *in vitro* plantlets were induced to form callus on MS medium containing 1.0 mg.l^{-1} thidiazuron or 1.0 mg.l^{-1} thidiazuron in combination with 0.5 mg.l^{-1} 6-benzyladenine (BA). Thereafter, adventitious shoots developed directly from callus on the same medium. The adventitious shoots can develop multiple-shoots on the propagation medium containing 0.2 mg.l^{-1} BA + 0.02 mg.l^{-1} NAA and root formation and plantlets regeneration were achieved on ½MS medium with 0.1 mg.l^{-1} IBA. An efficient *in vitro* propagation and plant regeneration system was successfully established.

Keywords: Shoot organogenesis, plant regeneration, propagation
Abbreviations: 2,4-D, 2-4-dichlorophenoxyacetic acid; **BA**, 6-benzyladenine; **IBA**, 3-indolebutyric acid; **NAA**, α-naphthaleneacetic acid; **TDZ**, thidiazuron

INTRODUCTION

The genus *Melastoma* contains about 100 species and is distributed mainly in South Asia, the Pacific Ocean and Australia. In China, there are nine species and one variety. They are mainly distributed in areas South of the Changjiang River (Chen 1984). Almost all species flower vigorously and is used for ornamental purposes. Among the genus, the species *Melastoma malabathricum* has a high Aluminum resistance and absorption (Watanabe and Osaki 2001, 2002; Sulaiman *et al*. 2004; Watanabe *et al*. 2005) and thus it can be used in soil environmental control and improvement. Some species have anti-microbial activity and the flavonoid content can be used as Chinese medicine and for food pigments (Chen *et al*. 2003; Zhang and Fang 2003; He *et al*. 2005; Su *et al*. 2005; Janna *et al*. 2006). Palynological studies, molecular phylogenetics, embryological studies, breeding, chemical manipulation of flowering, tissue culture and gene transformation have been reported in some species of *Melastoma* (Subramanyam 1948; Gross 1993; Chantaranothai 1997; Abdullah *et al*. 1998; Clausing *et al*. 2000; Ma *et al*. 2000; Clausing and Renner 2001; Yong *et al*. 2006).

Melastoma affine is a small shrub with attractive mauve-purple flowers. It is a pioneer shrub in Southeast Asia and in tropical Australia (Gross and Mackay 1998; Ma *et al*. 2000). *M. affine* has great usage as an ornamental and landscape plant. It usually flowers successively over 3 months from March-May in Guangdong Province, China. For its better exploitation and use, it is essential to introduce, propagate and domesticate the species. Breeding and biotechnology are also needed to improve its genetic traits. In the past, we reported on its propagation by cuttage (Ma *et al*. 2001). However, adventitious shoot induction from young leaf lobes has not yet been reported in this genus. In this report, we successfully induced adventitious shoot formation from young leaf lobes, and investigated some effects of different plant growth regulators on the induction of callus and adventitious shoots.

MATERIALS AND METHODS

Establishment of an *in vitro* shoot propagation system

Plants of *M. affine* grow in South China Botanical Garden, Guangzhou. Young, axillary shoots with leaves in 5 cm long were collected from the mother plant as explants. After sterilization in 70% (v/v) alcohol for 30 s and 0.1% (w/v) mercuric chloride for 10 min, they were rinsed in sterilized distilled water 3 times, then cut the axillary explants into 1 cm long and inoculated on MS basal media (Murashige and Skoog 1962) containing 0.1 mg.l^{-1} NAA + 1.0 mg.l^{-1} BA. Culturing jars in 10 cm high and 8 cm basal diameter were placed in a growth chamber at 26 ± 1°C and cultured in a 14-h photoperiod with 80 μmol.m^{-2}.s^{-1} fluorescent light. As new shoots grew out, they were transferred to a shoot propagation medium containing 0.02 mg.l^{-1} NAA + 0.2 mg.l^{-1} BA for multiple shoot propagation. For propagation of multiple shoots, the shoots were cut into several clumps with 1 cm high and every clump contain 4-6 shoots and sub-cultured on the above propagation medium every 1-2 months. All the media contained 30 g.l^{-1} sucrose and were adjusted to pH 5.8 and solidified with 0.7% (w/v) agar (Guangdong Huankai Microbiol Sci. Com, Guangzhou City, China), then autoclaved at 121°C for 16 min.

Induction of callus and adventitious shoots

Multiple shoots were cultured on shoot propagation media as defined above for 1-2 weeks in light, then the 2-3 top young leaves from one shoot were selected as explants. They were cut transversally into two pieces, and inoculated on different induction media in the jars (**Table 1**) for induction of callus or shoot organogenesis. After culturing for 3-5 weeks in the dark, induction of callus and adventitious shoots was investigated. Data derived from

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Short Communication

Table 1 Adventitious shoot formation from young leaves of *Melastoma affine* after culturing for 28 d on different media.

PGR combination in media ($mg.l^{-1}$)	Observation results		
	Callus with shoots (%)	Root formation (%)	Shoot number per explant
NAA 1.0	0	43.3	0 a
2.4-D 1.0	0	0	0 a
BA 1.0	10	0	3.6 b
TDZ 1.0	23.3	0	8.4 c
NAA 1.0 + BA 0.5	16.7	13.3	5.3 b
TDZ 1.0 + BA 0.5	30	0	9.6 c

PGR = plant growth regulator. Values followed by the same lower-case letter in the same column showed no significant difference by the LSD ($P \leq 0.05$) test.

randomly designed experiments with 40 explants each. The experiments were repeated twice in 1 week. Data were statistically analyzed using the LSD test ($P \leq 0.05$).

Root formation and plant transplanting

The co-induced callus with adventitious shoots was transferred together into propagation medium containing 0.2 $mg.l^{-1}$ BA + 0.02 $mg.l^{-1}$ NAA for shoot development. Half a month later, when the adventitious shoots grew 1- 2 cm high in the jars, then the adventitious shoot clumps with callus were transferred to rooting medium containing half strength MS basal medium with 0.1 $mg.l^{-1}$ IBA for root formation. After one month of culture, the plantlets were removed from the jars and agar was rinsed off with water, and then transplanted to sand in plastic pots in 30 cm × 40 cm.

RESULTS AND DISCUSSION

Establishment of *in vitro* shoot propagation system

Shoots developed from young, axillary shoots and in general developed into multiple-shoots on propagation medium containing 0.01 $mg.l^{-1}$ NAA + 0.2 $mg.l^{-1}$ BA during light culture. After 1-2 months of culture, the shoots could be cut shorter to 1 cm long and the multiple-shoot clumps could be divided into several smaller clumps for subculture. The propagation coefficient in one month of culture was 4.2, indicating a higher propagating ability (**Fig. 1A**).

Induction of adventitious shoot formation

As the young leaf lobes were cultured on different induction media with a different combination of plant growth regulators, different growth reactions occurred as outlined in **Table 1** and summarized below:

On induction medium containing 1.0 $mg.l^{-1}$ NAA, little red callus was induced. Otherwise, most leaf explants could directly induce adventitious root formation from leaf veins. However, no adventitious shoots were visible.

One $mg.l^{-1}$ 2,4-D could induce callus at the cut surface of leaf explants. However, the callus general turn brown. Even when it was transferred to medium containing 1.0 $mg.l^{-1}$ TDZ, the callus could not dedifferentiate into shoots.

One $mg.l^{-1}$ BA could induce very little callus from leaf explants. At earlier stages of culture, some adventitious shoot primordia developed on the surface of the callus (**Fig. 1B**). With a prolonged culture period to 4-5 weeks, some single or multiple adventitious shoots developed directly on the surface of the callus (**Fig. 1C, 1D**).

Leaf explants could induce callus on medium containing 1.0 $mg.l^{-1}$ TDZ. The callus was divided into two types. One type of callus was loose and could not develop into adventitious shoots. Another type of callus was greenish and compact. Prolonging culture to 4-5 weeks, some adventitious shoots developed directly on the callus (**Fig. 1C, 1D**).

A combination of 1.0 $mg.l^{-1}$ NAA and 0.5 $mg.l^{-1}$ BA in the medium could induce callus and then adventitious shoot formation. Few roots formed on the veins of leaf explants.

Most leaf explants could induce callus and adventitious shoots on induction medium containing 1.0 $mg.l^{-1}$ TDZ and 0.5 $mg.l^{-1}$ BA. No root formation was observed.

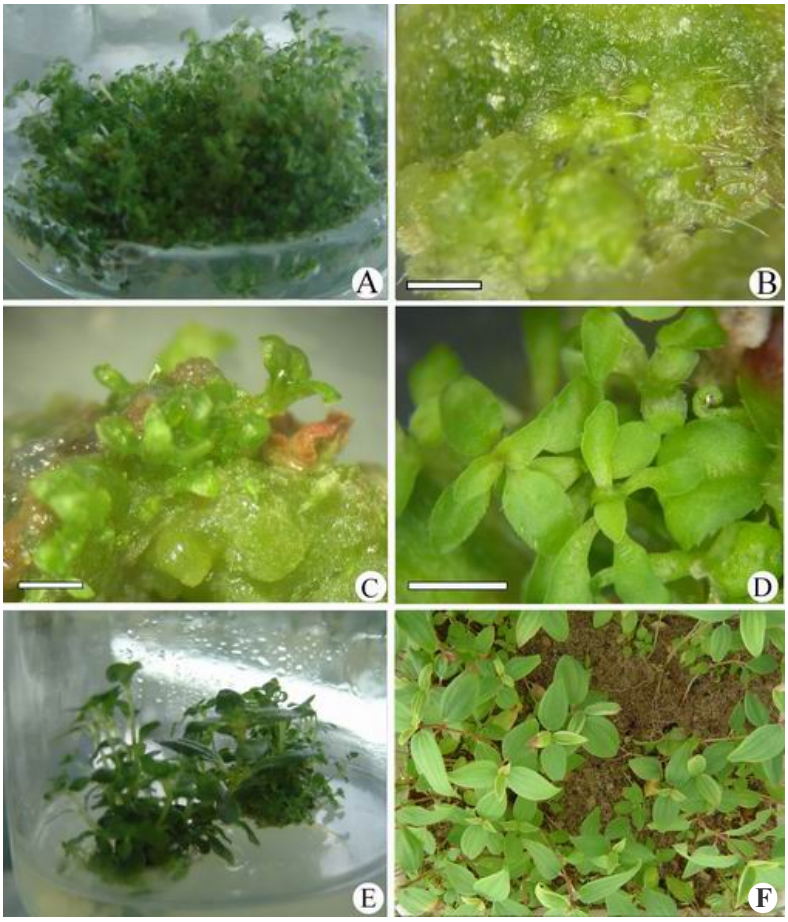

Fig. 1 Callus and adventitious shoot formation and plant regeneration in *Melastoma affine*. (**A**) Mass propagation of multiple shoots *in vitro*; (**B**) Callus with adventitious shoot primordia induced on the callus surface after cultured for 21 days; (**C**) Multiple adventitious shoots developed on the callus after culturing for 28 days; (**D**) Multiple adventitious shoots developed on callus after culturing for 35 days; (**E**) Callus with adventitious shoots was transferred to propagation medium; (**F**) Plantlet development and rooting in a growing medium *ex vitro*. Scale bars = 2 mm.

Root formation and plantlet transplantation

As the callus with adventitious shoots were transferred to propagation medium for shoot development, multiple-shoots were easier to establish within one month (**Fig. 1E**). Root formation was observed when shoots were cultured on rooting medium for 2 weeks. After total of one month in culture, all *in vitro*-grown plants developed vigorous roots. As the plantlets were transferred to a sand-based growing medium, 87% of plantlets survived after 30 d and continued to grow (**Fig. 1F**).

Our results revealed that cytokinin (TDZ or BA) was necessary for the induction of callus and adventitious shoots from young leaf explants. However, auxin (2,4-D or NAA) did not seem to play any role on the induction of callus or adventitious shoots. Otherwise, NAA could induce roots from young leaf explants. TDZ obviously induced more adventitious shoots than BA, indicating that TDZ plays a greater role in the induction of shoot organogenesis than BA, observations that have been reported for the other plants (sycamore, strawberry, begonia, coffee, etc.) to induce shoot organogenesis by TDZ or BA from explants of sepals, zygotic embryos, root and petiole indicated that TDZ also play similar role (Wilhelm 1999; Debnath 2005; Génève 2005; Nhut *et al*. 2005; Mohamed *et al*. 2006).

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REFERENCES

Abdullah T, Maleh AA, Ahmad SH (1998) Chemical manipulation of growth and flowering in potted *Melastoma decemfidum* and *Tibouchina semidecandra*. *Acta Horticulturae* **454**, 297-302

Chantaranothai P (1997) Palynological studies in the family Melastomataceae from Thailand. *Grana* **36**, 146-159

Chen C (1984) Melastomataceae. *Flora Reipublicae Popularis Sinicae* (Science Press, Beijing) **53**, 135-293

Chen HF, Xing FW, Liu DM, Yi QF (2003) Chinese medicine plant resources of Melastomaceae in Guangdong province. *Journal of Chinese Medicinal Materials* **26**, 323-325

Clausing G, Meyer K, Renner SS (2000) Correlations among fruit traits and evolution of different fruits within Melastomataceae. *Journal of the Linnean Society of Botany* **133**, 303-326

Clausing G, Renner SS (2001) Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. *American Journal of Botany* **88**, 486-498

Debnath SC (2005) Strawberry sepal: Another explant for thidiazuron-induced adventitious shoot regeneration. *In Vitro Cellular and Developmental Biology – Plant* **41**, 671-676

Génève RL (2005) Comparative adventitious shoot induction in Kentucky coffee tree root and petiole explants treated with thidiazuron and benzylaminopurine. *In Vitro Cellular and Developmental Biology – Plant* **41**, 489-493

Gross CL (1993) The breeding system and pollinators of *Melastoma affine* (Melastomataceae); a pioneer shrub in tropical Australia. *Biotropica* **25**, 468-474

Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biological Conservation* **86**, 169-178

He X, Li Y, Liu L, Lan Y (2005) Determination of gallic acid in *Melastoma dodecandrum* by RP-HPLC. *China Journal of Chinese Materia Medica* **30**, 180-181

Janna OA, Khairul A, Maziah M (2006) Flower pigment analysis of *Melastoma malabathricum*. *African Journal of Biotechnology* **5**, 170-174

Ma GH, Lin YR, Jian SG, Liu N (2000) Tissue culture and plantlet regeneration of *Melastoma candidum* and *M. Dodecandrum*. *Plant Physiology Communications* **36**, 233-234

Ma GH, Lin YR, Jian SG, Hu YJ, Liu N (2001) Collection and propagation of wild flower plants in Melastomataceae of South China. *China Wild Plant Resources* **20**, 72-73

Mohamed SV, Sung JM, Jeng TL (2006) Organogenesis of *Phaseolus angularis* L.: high efficiency of adventitious shoot regeneration from etiolated seedlings in the presence of N^6-benzylaminopurine and thidiazuron. *Plant Cell, Tissue and Organ Culture* **86**, 187-199

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

Nhut DT, Hai NT, Huyen PX, Huong DTQ, Hang NTT, Teixeira da Silva JA (2005) Thidiazuron induces high frequency shoot bud formation from *Begonia* petiole transverse thin cell layer culture. *Propagation of Ornamental Plants* **5**, 151-157

Su Y, Zhou S, Liu J (2005) Determination of anti-microbial activity and the content of flavonoids of *Melastoma affine*. *Journal of Plant Resources and Environment* **14**, 56-57

Su YP, Chen ZB, Zhang L, Chen BH, Liu JQ (2003) Extraction and tests on stability of red pigment from *Melastoma affine*. *Chinese Bulletin of Botany* **20**, 336-341

Subramanyam K (1948) An embryological study of *Melastoma malabathricum* L. *Journal of the Indian Botanic Society* **27**, 11-19

Sulaiman MR, Somchit MN, Israf DA (2004) Antinociceptive effect of *Melastoma malabathricum* ethanolic extract in mice. *Fitoterapia* **75**, 667-672

Watanabe T, Jansen S, Osaki M (2005) The beneficial effect of aluminium and the role of citrate in Al accumulation in *Melastoma malabathricum*. *New Phytologist* **165**, 773-780

Watanabe T, Osaki M (2002) Role of organic acids in aluminum accumulation and plant growth in *Melastoma malabathricum*. *Tree Physiology* **22**, 785-792

Watanabe T, Osaki M (2001) Influence of aluminum and phosphorus on growth and xylem sap composition in *Melastoma malabathricum* L. *Plant and Soil* **237**, 63-70

Whiffin T, Tomb AS (1972) The systematic significance of seed morphology in the neotropical capsular-fruited Melastomataceae. *American Journal of Botany* **59**, 411-422

Wilhelm E (1999) Micropropagation of juvenile sycamore maple via adventitious shoot formation by use of thidiazuron. *Plant Cell, Tissue and Organ Culture* **57**, 57-60

Yong WTL, Abdullah JO, Mahmood M (2006) Optimization of *Agrobacterium*-mediated transformation parameters for *Melastomataceae* spp. using green fluorescent protein (GFP) as a reporter. *Scientia Horticulturae* **109**, 78-85

Yoshida T, Nakata F, Hosotani K, Nitta A, Okuda T (1992) Dimeric hydrolysable tannins from *Melastoma malabathricum*. *Phytochemistry* **31**, 2829-2833

Zhang C, Fang YX (2003) Studies on the chemical constituents of Chinese herb *Melastoma dodecandrum*. *China Journal of Chinese Materia Medica* **28**, 429-431