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Melastomataceae: Inherent Economical Values Substantiating Potential Transgenic Studies in the Family

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ABSTRACT

Melastomataceae is one of the largest families of flowering plants. It is comprised of approximately 4,500 species in less than 200 genera, distributed in tropical and sub-tropical regions around the world. Limited fossil records have resulted in different hypothetical viewpoints on the biogeographical history of this family. Despite uncertainties in the monophyly of this family, the most obvious synapomorphy is the acrodromous leaf venation. Members of this family consist of diverse vegetative forms: from a few centimeters tall plant to woody creepers, to shrubs and even to several meters tall tree. Even though it has vast members, widely distributed worldwide, this family is one of the least studied or exploited. For those members fortunate enough to gain the attention of scientists worldwide, the outcomes have shown that members of this family have diverse valuable properties: ornamental, medicinal, herbal, phytoremediative, hinting that there might be others with new values that have yet to be explored in this huge family. Despite a great deal of research has been carried out to improve plant traits via genetic engineering in the plant kingdom, this technology has barely scratched the surface of Melastomataceae. A lack of critical information and detailed studies on the molecular aspects of this family might have hindered the progress in this aspect. This minireview focuses on the limited transgenic work that has only recently been explored in this family, with suggestions for future research, and also reviews the biochemical studies that have been conducted extensively on members of this Melastomataceae family throughout the decades.

Keywords: herbal, medicinal, ornamentals, phytoremediator, transformation

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INTRODUCTION

The Melastomataceae is one of the largest families of flowering plants that favour sun-touched areas throughout the world. Members of this family are easily recognized through their leaves featuring pairs of primary lateral veins that run in parallel converging at the base and leaf apex. Even though varied forms (creepers to woody trees) are featured in this family, their economical importance is still insignificant. The majority of the literature reported has focused on the biogeographical and evolutionary aspects of this huge family (quoting a few recent studies by Clausing *et al*. 2000; Renner *et al*. 2001; Clausing and Renner 2001; Morley and Dick 2003). A literature list of works conducted on this family can be obtained from http://www.flmnh.ufl.edu/natsci/herbarium/melastomes/melastome_literature_table.htm (compiled by Susanne Renner and Karsten Meyer and last updated in 2004). Another aspect of this family that has gained the attention of the scientific world is the biochemical factor, with studies focusing on the isolation of secondary metabolites and analyses of the isolated compounds for their medicinal values on animal cell cultures as well as *in vivo* studies in rats and mice (Andreo *et al*. 2006; Susanti *et al*. 2007). The application of modern medicines using compounds isolated from this family is getting popular. However, more studies and trials are required to support all the beneficial aspects as claimed. The aluminium accumulating property has also been studied in some members of this family and is a potential area to exploit for environmental clean-up of such chemical contamination that poses a threat to the growth of other vegetations (Watanabe *et al*. 1998). Interestingly the herbal aspect of this family has gained wide application in folk medicines even until today; the knowledge has been imparted verbally throughout the generations and only a limited amount of the information has been fortunate enough to be recorded (Jones 1993; Za-

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karia and Ali Mohd 1994; Ong and Nordiana 1999; Mat-Salleh and Latiff 2002). Surprisingly some of the uses reported since the early twentieth century remained unchanged until today. Recently the concept of transformation was applied on two species in this family and the result showed a potential of applying this technology to promote the family for other useful economical values. However, the literature about this transformation aspect and other areas not mentioned above in this family is still scarce. It is interesting to explore the kaleidoscopic values this huge family possesses. The aim of this mini-review is to integrate the available information gleaned from reported works and explore other potential uses that can be sourced out from this family and with special focus on using transformation technology to promote the economical value of this family.

BIOCHEMICAL STUDIES AND CURRENT SCENARIO OF ITS EXPLOITATION

The biochemical exploration in the Melastomataceae family has been conducted for several decades, but it is still a second to biogeographical and evolutionary studies in terms of extensiveness. Early interests in identifying the chemical compounds in members of this family probably stemmed from searches for natural sources of food colours. Among the plant pigments, anthocyanins are commonly explored. Anthocyanins are water-soluble pigments that are responsible for the colours of flowers, fruits and leaves. They are beneficial to health as they possess antioxidant properties (Tsai *et al.* 2002), are non-toxic or mutagenic and possess positive therapeutic properties (Bridle and Timberlake 1996). The showy dark blue flowers of some species in this family are attractive candidates for pigments isolation. Harborne (1964) identified malvidin-3,5-diglucoside in *Tibouchina semidecandra*, and Francis *et al.* (1982) revealed malvidin-3-(di-*p*-coumaroyl xyloside)-5-glucoside and malvidin-3-(*p*-coumaroyl xyloside)-5-glucoside in *Tibouchina granulose*. This was followed by peonidin-3-sophoroside and malvidin-3,5-diglucoside identification in *Tibouchina grandiflora* (Bobbio *et al.* 1985). Some compounds were, however, too minute to be able to confirm their identification. Following up on this line of thought of natural colourants, a study on the stability of crude anthocyanins extracted from *T. semidecandra* was conducted (Janna *et al.* 2007) for possible applications in food as well as in non-food items. The study showed that the anthocyanin crude extracts are stable in acidic condition and are sensitive to heat and light.

Other phytochemical explorations include studies on the polyphenolic constituents of Melastomataceous plants. The isolation and structural elucidation of hydrolysable tannin oligomers were well established for several species in this family as described in Yoshida *et al.* (1992, 1994, 1999, 2005) and Isaza *et al.* (2004). The Melastomataceous plants analysed revealed a unique polymerization pattern of the oligomers and this was regarded as a significant chemotaxonomic trait, which was exploited for the determination of new oligomeric structures isolated from new members. The presence of these polyphenolics, tannins, has also been linked to their beneficial contribution to the traditional medicinal values exhibited by members in this family, as described in subsequent sections in this review.

Exploitation for environment restoration

It is a challenging prospect to use plants for environmental restoration. Unlike organic compounds, metals cannot be degraded. Soils polluted with certain metals may hinder the growth of other vegetations. An interesting trait that prevails in the Melastomataceous family is the capacity to accumulate a large amount of aluminium. Jansen *et al.* (2002) surveyed across members of this family and found at least 127 species have the capacity to accumulate aluminium, which is useful for phylogenetic studies. *Melastoma affine* was reported to uptake and accumulate aluminium up to 9932 mg kg^{-1} in the leaves from the soil in an abandoned tea plantation (Xie *et al.* 2001). Aluminium toxicity is often the primary factor affecting crop productivity. However, *Melastoma malabathricum* growth was reported to improve when supplemented with aluminium at 0.5 mM in the growth medium (Watanabe *et al.* 2005) and this plant has high Al intake (exceeding 7 mg g^{-1} of aluminium in young leaves or more than 10000 mg kg^{-1} in mature leaves) (Watanabe *et al.* 1997, 1998; Watanabe and Osaki 2001). Besides aluminium, *M. malabathricum* was reported able to accumulate arsenic from soil (Visoottiviseth *et al.* 2002). Looking at the criteria used in selecting plants for phytoremediation, *M. malabathricum* proves to be an ideal candidate. It is a pioneer shrub, possessing a high propagation rate and is widespread in cleared lands (Kochummen and Ng 1977; Hashimotio *et al.* 2000). Besides *Melastoma*, *Pternandra* and *Dissochaeta* might also be potential candidates as phytoremediators as they have high dispersal capacity (Brearley *et al.* 2004; Jankowska-Blaszczuk and Grubb 2006; Shono *et al.* 2006). More studies, however, are needed to test the versatility of these members as metal accumulators. The inherent pioneering nature of members in the Melastomataceae family is beneficial enough to overcome any lack of versatility in accumulating all types of metal because the candidate plant species can be genetically engineered or custom-made to target at specific metal of interest. Drake *et al.* (2002) demonstrated the possibility of engineering *Nicotiana* plants by expressing antibodies with specific targets for potential use in phytoremediation. An understanding of the biological mechanisms is necessary if this phytoremediation technology is to be practiced on a large scale and turned into an economically sound investment for large metallurgical industries. It would be interesting to see how this family could be exploited to absorb tons of metallurgical industrial wastes, clean up masses of contaminated agricultural soils and make them viable again. Klumpp *et al.* (2000) reported the potential of using *Tibouchina pulchra* Cogn. as a bioindicator plant to map polluted zones in industrial areas. The study showed that *T. pulchra* suffered metabolic disturbances, when exposed to polluted air and soil, despite it being known as a natural air pollution resistant plant.

Exploitation in folk or traditional herbal medicine

Traditional medicine is a practice in healthcare that has been going on for generations and the knowledge is passed on verbally and in written forms as well. The revival of traditional medicine as an alternative cure for ailments and some diseases came about due to the failures of modern medicines or fear of side effects from synthetic medicines. Phytochemicals are no exception to producing side effects from overdose or abuse usage. For example, tannins are reported to have anti-microbial activity (reviewed by Scalbert 1991) but can also cause neoplasia (Wiart 2002). In addition, sometimes modern scientific data developed from modern well-equipped laboratories contradict traditional reports. Uawonggul *et al.* (2006) in their screen for scorpion venom antidotes revealed that several herbal plants showed negative results, contradicting previous traditional reports. Another problem persisting in folk medicine is the establishment of records. Sometimes there are overlapping records or confusion in recorded data because of the usage of different names for the same species in different areas in a country or in different countries. For example, the local names of *M. malabathricum* L. in different parts of Malaysia are “Akar keduduk hitam”, “Senduduk”, Sekenduduk”, “Kodok”, “Keduduk”, and “Sikaduduk” (Zakaria and Ali Mohd 1994). In Indonesia, the local names for the same species above are “Halendong”, “Sendudok” and “Mua e bong” (Dévéhat *et al.* 2002). This species is also known as Singapore Rhododendron (Jones 1993). Despite the contrary results, it is always wise to be cautious in the use of any plant materials for internal applications until it is scientifically proven safe for use. The risk assessment of inherent plant toxicants (plant metabolites) in use as plant food additives is reviewed by

Essers *et al*. (1998).

Over the years, reports have shown varied traditional usage of decoctions of different vegetative or floral parts of some members of Melastomataceae in treating daily ailments. *M. malabathricum* L. always tops the list by having the most reports on its usages. For example, decoctions of the leaves were found to be able to cure diarrhea, dysentery, to treat mouth ulcers, piles and gastric ulcers, eliminate flatulence and for treating leucorrhea, and pounded leaves are applied to wounds to accelerate healing; the roots are boiled and the water can be used for gargling to relieve toothache, to relieve rheumatism, to prevent car sickness, to cure food poisoning and to prevent epilepsy; the fruits can be applied to treat dry or cracked lips (Zakaria and Ali Mohd 1994; Ong and Nordiana 1999; Ong and Norzalina 1999; Sharma *et al*. 2001; Dévéhat *et al*. 2002; Mat-Salleh and Latiff 2002). The leaves of *Dissochaeta gracilis* (Jack) Bl. have also been reported to be able to cure diarrhea (Grosvenor *et al*. 1995). The leaves of *Miconia willdenowii*, reported to contain 0.2% caffeine, were dried and used as tea leaves for drinking (Lewis and Elvin-Lewis 1977). *Mouriri pusa* Gardn. was also reported to be used to treat gastrointestinal ailments like ulcer and gastritis (Andreo *et al*. 2006). The ethanolic extract of *M. malabathricum* was shown to exhibit antinociceptive effect in male Balb/C mice (Sulaiman *et al*. 2004). Muhamad *et al*. (2000) tested the aqueous extract of the same species above in Spraque-Dawley rats and demonstrated it possessed hypotensive properties.

Exploitation in extraction of bioactive compounds and modern medicines

Increasing stress and unhealthy lifestyle of modern day activities are the root cause for the rise in many modern day ailments. Extensive search on plant systems to look for alternative cures are growing. Recorded practices of folk or traditional medicine often lay the foundation for modern medicines in search for more scientific proofs of the medicinal values that some plants possess. Phytochemical analyses of some of the Melastomataceous plants revealed the presence of varied bioactive compounds that can be isolated using different solvent systems. He *et al*. (2005) reported on gallic acid determination in *M. dodecandrum* using RP-HPLC (Polaris C18 column) with tetrahydrofuran-methanol-phosphoric acid as the mobile phase. Ethyl acetate extract of *M. malabathricum* L. yielded three compounds, naringenin, kaempferol and kaempferol-3-*O*-D-glucoside, while methanolic extraction gave kaempferol-3-*O*-(2″,6″-di-*O*-*p*-trans-coumaroyl)-glucoside and kaempferol-3-*O*-D-glucoside. These compounds were found to be active radical scavengers and capable of inhibiting the proliferation of MCF-7 cell lines (Dévéhat *et al*. 2002; Susanti *et al*. 2007). Andreo *et al*. (2006) reported the presence of tannins, flavonoids and epicatechin in the methanolic extract of *Mouriri pusa* that exhibited anti-ulcerogenic activity in male Swiss mice and male Wistar rats. Hydrolysable tannins (casuarinin, casuarictin, pedunclagin and nobotannin B) in the whole plant extract of *Melastoma dodecandrum* Lour. were found to inhibit nitric oxide production by the murine macrophage-like cell line, RAW264.7 (Ishii *et al*. 1999). Nobotannin B was earlier reported to be a poly-(ADP-ribose) glycohydrolase inhibitor (Aoki *et al*. 1993). This tannin is widely distributed in some genera of the Melastomataceae such as *Tibouchina* (Yoshida *et al*. 1999) and *Melastoma* (Yoshida *et al*. 1992) and might have contributed to their anti-oxidant property. Lee *et al*. (2001) isolated four flavonoids, quercitrin, isoquercitrin, rutin and quercetin from the leaves of *Melastoma candidum* and demonstrated that the four potent compounds exhibited radical scavenging activity and inhibited monoamine oxidase type B activity. Castalagin, procyanidin B-2 and helichrysoside, isolated from the leaves of *M. candidum*, were found to be antihypertensive when injected intravenously into spontaneously hypertensive rats (Cheng *et al*. 1993). Hexane, methylene chloride and ethanol extracts of *Miconia rubiginosa*, which contained ursolic acid, oleanoic acid and triterpenes, were found to significantly inhibit acetic acid-induced abdominal writhing in mice and rats (Spessoto *et al*. 2003). The mechanisms underlying each of the activities propounded by the isolated bioactive compounds above are unknown. Knowledge in this area would be useful as the bioactive compounds isolated in this family are also common beneficial secondary metabolites found in other plant families. Genetic engineering studies would help to target and profile the mechanisms of action of each potential compound involved.

Exploitation for ornamental values

The showy flowers of *T. semidecandra* and *Melastoma decemfidum* make them excellent candidates for ornamental purposes. Nurseries in Malaysia, Singapore and Thailand propagated them using cuttings and sold them as landscaping plants for borders and foundation planting. However, constant pruning is required to maintain them at suitable heights. Abdullah *et al*. (1998) reported using chemicals to control the growth and flowering capacity of potted *M. decemfidum* and *T. semidecandra*. Their results showed that flurprimidol was more effective than paclobutrazol where 50 mg L^{-1} is sufficient to reduce the plant height, shorten the flowering time and increase the number of flowers produced. The use of the above chemicals might not be economical or environmentally friendly in the long run. This is because without the continuous application of the chemical, growth resumes back to normal. Another disadvantage is that run-off from the chemicals might contaminate surrounding areas or groundwater. The side-effects of soil contaminated with those chemicals have yet to be ascertained. So, an alternative route for controlling the plant growth for useful economical purpose might be to genetically engineer the plants. This approach might be more economical in the long run because it is long lasting for as long as the transgene remains in the genome and is expressed.

Tissue culture technique is a primary pre-requisite for genetic transformation. The plant's part (single cell callus or multiple cells tissue) is the first item required in a transformation protocol. The totipotency of the plant materials to recover from the transformation events and regenerate into whole plant would spark the success or failure of an attempt to introduce a foreign gene into a plant genome. Poospooragi (2005) has successfully propagated *T. semidecandra*, *M. malabathricum*, *M. dodecandrum* and *M. decemfidum* using tissue culture technology. In the study, Poospooragi reported that shoot initiation was optimal using the shoot tip as explant for *M. malabathricum*, *M. dodecandrum* and *M. decemfidum* cultured on full strength Murashige and Skoog (MS) medium supplemented with 30 µM 6-benzylaminopurine (BAP), while nodal explants of *T. semidecandra* responded better in full strength MS medium supplemented with 20 µM BAP. Following shoot initiation, shoots multiplication was achieved in half strength MS medium supplemented with 6 µM for *T. semidecandra*, 9 µM for *M. malabathricum* and 12 µM for *M. decemfidum*, while *M. dodecandrum* responded to quarter strength medium supplemented with 3 µM BAP. *In vitro* rooting of the plantlets was carried out in MS medium without any hormonal supplements but different species were found to require different medium strengths: full strength for *T. semidecandra* and *M. decemfidum*, half strength for *M. malabathricum* and quarter strength for *M. dodecandrum*. All the *in vitro* plants were maintained at 25 ± 2°C with a 16-h light / 8-h dark photoperiod. One major problem in plant tissue culture is the great losses often encountered during the transfer of the tissue cultured plantlets from the culture room to the glasshouse. In the acclimatization of the four aforementioned species to glasshouse conditions, each plantlets was transferred to pot containing a mixture of sterile soil, perlite and sand (1:1:3 v/v) after their removal from the culture flasks and maintenance in distilled water for one week. The acclimatization process showed great success with 91% of the *M. dodecan-*

drum plantlets survived, 80% for *T. semidecandra*, 75% for *M. malabathricum* and 67% for *M. decemfidum*. Janna *et al.* (2005) reported a higher survival percentage in the acclimatization of one of the tissue cultured species above to glasshouse conditions. In the study, rooted *in vitro M. malabathricum* plantlets were acclimatized to glasshouse conditions either by leaving them in open jars in distilled water or maintained in soil (3 parts peat: 2 organic matter: 1 sand v/v) in a covered aquarium for 7 to 14 days. Survival of the tissue cultured plantlets reached 85 to 98% using those cheap, simple techniques. This success has opened up opportunity for genetically modifying the species above for useful economical traits and boost up the value of members of this Melastomataceae family, which is still regarded as economically insignificant.

GENETIC ENGINEERING ASPECTS

Application of genetic engineering

Genetic engineering is a powerful tool for improvement of plant traits. To date, there are many reports on genetic transformation of a wide variety of plant species in order to improve the qualities of the plants (Teixeira da Silva 2006).

There are several approaches available to achieve successful genetic transformation of different plant species as reviewed by Rakoczy-Trojanowska (2002). These include *Agrobacterium*-mediated method, microprojectile bombardment, electroporation, protoplast fusion, microinjection and silicon carbide whisker-mediated method. Among these methods, *Agrobacterium*-mediated is the most commonly used system which involved the capability of delivering DNA from *Agrobacterium* plasmid into wide variety of plant cells, with or without the assistance of sonication. Despite reported successes in the plant kingdom, this technology has barely scratched the surface of the Melastomataceae.

Agrobacterium-mediated transformation of *Melastomataceae* spp.

In Malaysia, some *Melastomataceae* spp. are identified as potentially important flowering ornamentals. However, their limited flower colours, mainly pink to purple, reduced their commercial value. Therefore, genetic engineering is an avenue to develop new varieties. To date, there is no other report of genetic manipulation work carried out on *Melastomataceae* except recently one was carried out on the optimization of *Agrobacterium*-mediated transformation system of two *Melastomataceae* species (*M. malabathricum* and *T. semidecandra*) using GFP as a reporter (Yong *et al.* 2006b). Parameters such as bacterial strain, bacterial concentration, pre-culture period, co-cultivation period, immersion time, acetosyringone concentration and wounding type known to influence the transformation efficiency were assessed and the results obtained were based on the percentage of GFP expression which was observed three days post-transformation.

Previous research indicated that each particular strain of *Agrobacterium* showed different levels of virulence to the plant species (Bauer *et al.* 2002). Hakrabarty *et al.* (2002) indicated that the superiority of *Agrobacterium* strain GV2260 over LBA4404, A208 and EHA105 in *Brassica oleracea* transformation. Padilla *et al.* (2006) also stated that different strains of *Agrobacterium* affected transgene expression on *Prunus persica* transformation. For *Melastomataceae* spp. studied by Yong *et al.* (2006b), strain LBA4404 showed the highest virulence on *M. malabathricum* transformation while EHA105 gave similar result on *T. semidecandra*. Analyses also showed that different bacterial concentrations had different effects on transformation efficiency as similarly reported by de Bondt *et al.* (1994). The assessment showed that 1×10^7 cfu mL^{-1} (OD$_{600nm}$ 0.8) of LBA4404 and EHA105 gave the highest transformation efficiency for *M. malabathricum* and *T. semidecandra*, respectively.

In *Melastomataceae* spp. transformation carried out by Yong *et al.* (2006b), four days of pre-culture and two days of co-cultivation were optimum for *M. malabathricum*, while three days of pre-culture and co-cultivation were observed for *T. semidecandra*. Yong *et al.* (2006b) revealed that 60 minutes of immersion and addition of 200 μM acetosyringone gave the highest percentage of positive transformants for both *M. malabathricum* and *T. semidecandra*. Mild wounding of the explants with a scalpel was assessed by Yong *et al.* (2006b) and found to significantly increase the efficiency of *M. malabathricum* transformation but not of *T. semidecandra*.

With the established and optimized *Agrobacterium*-mediated transformation system for *Melastomataceae* spp., current transformation work in our laboratory is focused on using antisense technology to improve the ornamental value of the plants. Antisense RNA technology is used to down regulate the expression of specific gene in transgenic organism by introduction of transgenes, which express RNA complementary to endogenous coding mRNA. Antisense mRNA has been found to inhibit gene expression at the level of target mRNA transcription processing transport from the nucleus and translation (Dashek 1997). The underlying mechanism is not altogether clear, but it most certainly involves the hybridization between antisense and sense copies of the RNA. Synthesis of antisense RNA in a transformed plant is an effective way of carrying out gene subtraction (Brown 1998).

The introduction of antisense constructs of pigmentation genes into a fully-coloured *Petunia* has been used to develop variants with reduced pigmentation (van der Krol *et al.* 1988). In several ornamental plants, transformation with antisense dihydroflavonol-4-reductase (DFR) genes has resulted in the production of different shades of blue flowers (Aida *et al.* 2000). DFR is the enzyme which catalyses the reduction of dihydroflavonols such as dihydrokaempferol, dihydroquercetin and dihydromyricetin to the respective leucoanthocyanidins in the anthocyanin pathway (Dooner *et al.* 1991). The leucoanthocyanidins are substrates for the next step in the biosynthesis of anthocyanins and proanthocyanidins.

Selection of putative regenerated transgenics

The ultimate goal of plant genetic transformation is to produce transformants capable of regenerating into whole plants and subsequently express the useful genetically engineered characteristics. In some particular cases, the failure in recovery of transgenic plants is due to the lack of response for regeneration rather than to the DNA delivery method (Christou 1995). Tissue culture acts as an important tool for transgenic plant regeneration after genetic transformation. It is significant that the transformed plant cells must be capable of sustained division and subsequently regenerate into whole plants. Other factors affecting the production of transgenic products are a good reporter and an effective selection system which allow early detection of the transformation event and the growth of transformed cells only.

Commonly used reporters include gene encoding chloramphenicol acetyl transferase (CAT), luciferase (LUC), β-glucuronidase (GUS) and protein involved in the regulation of anthocyanin biosynthesis. However, green fluorescent protein (GFP) from *Aequorea victoria* has recently been shown to have the characteristics of intrinsic signal which in non-invasive, non-destructive and cell autonomous (Tee *et al.* 2005). This gene proved to be an extremely useful and reliable marker in screening of putative transformants during the regeneration period of various transformed crop plants including *Brassica rapa* (Wahlroos *et al.* 2003) and *Helianthus annuus* (Weber *et al.* 2003). In a transformation event, only a fraction of plant cells exposed to foreign DNA provides the basis for regeneration of transgenic plants. Antibiotic selection is one of the factors that can increase the efficiency of transformation system by inhibit the growth of untransformed cells but enable transformants to survive and regenerate into complete transgenic plants. Poor selection system will favour the production of chimeric plants by allowing the untransformed cells to replicate, especially when the selection agent is no longer alive due to prolong period in culture. By far the most widely used antibiotic selectable marker gene has been the *npt* gene coding for neomycin phosphotransferase, which was originally isolated from the bacterial transposon Tn5 (Lal and Lal 1990). The second generally useful selection system is based on the use of a bacterial phosphotransferase coded by *hpt* gene, which inactivates hygromycin (Miki and McHugh 2004).

In our current research, shoots (two-leaf stage) and nodes (all three nodes counting from the apex) of *M. malabathricum* and *T. semidecandra* were transformed with plasmids (generous gift from Suntory Limited, Japan), harbouring a DFR gene at different orientations (sense and antisense) and a selectable marker *npt*II for kanamycin resistance. Putative transformants of *M. malabathricum* and *T. semidecandra* were selected in the presence of kanamycin with their respective optimized concentration (Yong *et al.* 2006a). During the selection of *M. malabathricum*, 9.0% shoots and 13.7% nodes survived in sense transformation. However, only 4.0% and 6.7% of them regenerated. In antisense transformation, 7.7% *M. malabathricum* shoots and 11.3% nodes survived on the selection plates whereas only 3.7% and 5.3% of them regenerated. For the selection of *T. semidecandra*, 10.3% shoots and 15.7% nodes survived in sense transformation where the percentages of their regeneration were only 5.3% and 9.3%, respectively. In antisense transformation, 9.3% *T. semidecandra* shoots and 14.7% nodes survived with only 4.7% and 8.3%, respectively, regenerated.

The presence of the transgenes in the plant was further verified by polymerase chain reaction (PCR). However, some of the samples showed negative results for analyses due to the possibility that the putative transformants lost the transgene when the selective pressure was gradually decreased. They were also probably escapees on the selection medium (Kuvshinov *et al.* 1999) or chimeric for the expression (Torbert *et al.* 1995). The PCR-amplified samples were sent for nucleotide sequencing and the alignment result showed high identity to the transgenes. Further southern blot analysis was carried out to verify the integration of the transgenes into the plant genome. Molecular analyses indicated that the sense and antisense DFR genes were present in putative *M. malabathricum* and *T. semidecandra* transformants. Regenerated putative transformants were subsequently acclimatized to glasshouse conditions awaiting flowering. Other works suggested to follow up on the transformants are analyses of the flower(s) produced, histochemical study to investigate the morphological changes occurring within the plants, monitoring the expression patterns of the anthocyanins and flavones in the flower, and Northern blot analysis to study the level of expression or suppression of the RNA molecules.

Recent years, plant genetic engineering has produced revolutionary results in agriculture. It has been utilized in many different ways to increase the qualitative and quantitative yield of crop plants, to enhance protection against pests, and to produce sustainable raw materials for industry and pharmaceutical purposes. With the established and optimized *Agrobacterium*-mediated transformation system for *Melastomataceae* spp., further transformation with other economically important genes such as dwarfism gene, fragrance gene, and possibly genes that control ethylene production in order to prolong the blooms on the plant are recommended in order to improve their qualities and values as ornamental plants. However, the introduction of transgenic cultivars requires a risk analysis. Each plant licensed for cultivation has to be analyzed according to strict scientific criteria as to whether the corresponding plant represents a hazard to the environment or to human health. Besides, the possibility of crossing between the released transformants and wild plants has to be examined and the potential consequences for the environment have to be investigated (Heldt and Heldt 2005).

FUTURE PROSPECTS AND CONCLUSION

Melastomataceae is a huge family with much potential yet to be discovered. As each day progresses, more findings are being revealed and the list is getting longer. The horizon is broadening in avenues such as studies of potential enemy of this family. Recently, susceptible infection by *Eucalyptus* canker pathogen, *Chrysoporthe cubensis*, was reported for *Rhynchanthera mexicana*, *Tibouchina urvilleana* and *M. malabathricum* (Gryzenhout *et al.* 2006). Earlier studies revealed that *M. malabathricum* was susceptible to *Chrysomelidae* beetle (Ooi 1987; Kamarudin and Shah 1978). The beetle is viewed as a potential biological control agent of *M. malabathricum*, which is regarded as being a noxious weed inhabiting crop plantations. Some members, such as *M. malabathricum* L. and *Tibouchina* sp., have been studied more extensively compared to other species/members in the family probably due to the availability of the plant in abundance. *M. malabathricum* especially is well studied in the Asian regions because it is widespread along roadsides and in cleared lands. Also this species is used frequently in folk medicines and in local dishes (young shoots eaten raw or added in fish curry dish). The transgenic aspect has yet to be fully exploited and there is much to be done for this family in this arena. The molecular aspects of this family are still scarce. So far, the molecular aspect established in this family is the polymerase chain reaction amplification and sequencing of *rbc*L and *ndh*F genes and *rpl*16 intron in support of the phylogenetic studies (Clausing and Renner 2001). It would be interesting to see how the isolation and manipulation of genes that control useful traits like dwarfism, ethylene synthesis, and flower colour would increase the economical value of this family. Some members in this family have great potential as natural phytoremediators or could potentially be genetically engineered to produce transgenic plants for phytoremediating any pollutant. In medicine, definitely the search for phytochemicals is still essential in the quest for finding medication for existing incurable diseases. Members in this family are rich sources of secondary metabolites responsible for some of the medicinal properties where their mechanisms of action can be elucidated using genetic engineering techniques.

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