OPTIMIZING *Agrobacterium*-MEDIATED TRANSFORMATION PARAMETERS OF *Melastoma decemfidum* ROXB EX JACK

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OPTIMIZING *Agrobacterium*-MEDIATED TRANSFORMATION PARAMETERS OF *Melastoma decemfidum* ROXB EX JACK

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ABSTRACT

Melastoma decemfidum is a tropical ornamental plant belongs to family of Melastomataceae and widely used in traditional medicine. The plant was reported to contain bioactive flavonoids that exhibited antioxidant and anticancer effects. To date, there is no report on transformation system for *M. decemfidum*. In this study, the effects of transformation parameters on the transformation efficiency of *Agrobacterium*-mediated transformation of *M. decemfidum* were investigated. Parameters such as bacterial concentration, infection time and acetosyringone concentration were optimized using histochemical GUS assay. Results showed that the highest transformation efficiency (96.7%) for *M. decemfidum* with an average of 479.6 ± 10.2 blue spots per explant was obtained when leaf explants were treated with bacterial concentration of OD_{600nm} = 0.6. Forty-five minutes of infection time gave the highest percentage (96.7%) of positive transformants and an average of 316.9 ± 7.6 blue spots per explant. Addition of 100 µM acetosyringone was optimum (100%) for transforming *M. decemfidum* with an average of 509.8 ± 26.7 blue spots per explant. In conclusion, an efficient *Agrobacterium*-mediated transformation protocol for *M. decemfidum* was successfully established. Thus, the developed protocol will facilitate the delivery of desirable genes into *M. decemfidum* via *Agrobacterium*-mediated transformation.

ABSTRAK

Melastoma decemfidum merupakan sejenis tumbuhan daripada keluarga Melastomataceae di kawasan tropika. Tumbuhan ini digunakan secara meluas dalam perubatan tradisional. *M. decemfidum* telah dilaporkan mengandungi bioaktif flavonoid yang mempamerkan aktiviti antioksa dan antikanser. Sehingga kini, tidak ada laporan mengenai system pemindahan genetik untuk *M. decemfidum*. Dalam kajian ini, pemindahan genetik untuk *M. decemfidum* melalui kaedah *Agrobacterium tumefaciens* telah dilaksanakan. Parameters seperti kepekatan bakteria, jangka masa infeksi dan kepekatan asetosiringon telah dioptimumkan dengan menggunakan β-glucuronidase (GUS) sebagai penanda. Keputusan menunjukkan bahawa kecekapan yang tertinggi (96.7%) untuk pemindahan gen secara *Agrobacterium* ke dalam daun *M. decemfidum* dicapai oleh kepekatan bakteria pada OD_{600nm} = 0.6 dengan menghasilkan 479.6 ± 10.2 titik biru per eksplan secara purata. Jangka masa infeksi yang 45 minit memberi peratusan yang paling tinggi (96.7%) kepada transforman positif untuk *M. decemfidum* dan sebanyak 316.9 ± 7.6 titik biru per eksplan. Penambahan asetosiringon 100 μM adalah optimum (100%) untuk pemindahan gen ke dalam *M. decemfidum* dengan memberikan jumlah purata 509.8 ± 26.7 titik biru per eksplan. Kesimpulannya, protokol pemindahan genetik yang optimum untuk *M. decemfidum* telah berjaya dihasilkan. Protokol yang dibangunkan akan memudahkan kemasukan gen yang diinginkan ke dalam *M. decemfidum* melalui kaedah *Agrobacterium*.

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LIST OF SYMBOLS

%	-	Percent
=	-	Equal to
<	-	Less than
≤	-	Less than or equal to
℃	-	Degree Celsius
α	-	Alpha
β	-	Beta
μmol/m^2/s	-	Micromoles of light per square meter per second
μM	-	Micromolar
cm	-	Centimeter
g	-	Gram
h	-	Hour
L	-	Liter
m	-	Meter
M	-	Molar
mg	-	Milligram
min	-	Minute
mL	-	Milliliter
mM	-	Millimolar
nm	-	Nanometer
pH	-	Power of Hydrogen
v/v	-	Volume per volume
w/v	-	Weight per volume

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
BAP	-	6-Benzylaminopurine
bp	-	Base pair
cfu	-	Colony-forming unit
dH_2O	-	Distilled water
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid
GFP	-	Green fluorescent protein
GUS	-	Beta-glucuronidase
LB	-	Luria Bertani
LUC	-	Luciferase
MCF-7	-	Michigan Cancer Foundation-7
MS	-	Murashige and Skoog
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
OD	-	Optical density
rpm	-	Revolutions per minute
SEM	-	Standard error of mean
spp	-	Species
T-DNA	-	Transfer-deoxyribonucleic acid

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In Malaysia, Melastomataceae spp., or locally known as ‘Senduduk’, are identified as potentially important flowering ornamentals and they are used for commercial purposes (Yong *et al.*, 2006). The leaves and roots of these plants can be used for the treatment of various ailments such as diarrhea, dysentery (Wiart, 2006), gastric ulcers, epilepsy, arthritis, rheumatism (Lohezic-Le *et al*., 2002) and jaundice (Libman *et al*., 2006). *Melastoma decemfidum*, or formerly known as white petal *Melastoma malabathricum* (locally known as ‘Senduduk Putih’), belongs to the family Melastomataceae and they usually grow in open fields of lowlands and mountain forest (Sarju *et al.*, 2012). It has been reported that leaf extracts of *M. malabathricum* contain characterized hydrolysable tannin. Several tannins isolated from dry leaves of light pink-magenta petal *M. malabathricum* were oligomers such as nobotannin B, nobotannins G, nobotannins H, nobotannins J, malabathrins B, malabathrins C and malabathrins D, and monomers such as 1,4,6-tri-*O*-galloyl-ß-D-glucose, 1,2,4,6-tetra-*O*-galloyl-ß-D-glucose, strictinin, casuarictin, pedunculagin, nobotanin D, pterocarinin (Yoshida *et al.*, 1992). Whereas leaves of white petal *M. malabathricum* contains four flavonoids, including naringenin, kaempferol, kaempferol-3-O-D-glucoside and

kaempferol-3-O-[2',6'-di-O-p-trans-coumaroyl] glucoside (Susanti *et al.*, 2007). Among these flavonoids, naringenin and kaempferol-3-O-[2',6'-di-*O*-*p*-trans-coumaroyl] glucoside were found to exhibit anti-proliferative effect against MCF-7, a human breast cancer cell line.

To improve the quality of plants and develop new varieties, genetic transformation is recommended for introduction of useful genes into a variety of plants because it has opened new avenues to the modification of characteristics, such as flower color, fragrant, longevity, shape and size (Yong *et al.*, 2010). There are several approaches available for transferring desired genes into plant genome, such as *Agrobacterium*-mediated method, microprojectile bombardment and electroporation. Among these approaches, *Agrobacterium*-mediated is the most commonly used method as no costly equipment is required and the simplicity of the plant transformation protocols (Yong *et al.*, 2006; Yong *et al*., 2008). This method involves the transferring of well-defined DNA from the *Agrobacterium* tumor-inducing (Ti) plasmid to the host-cell genome (Tzfira and Citovsky, 2006).

In this study, *Agrobacterium tumefaciens*-mediated transformation system was established for *M. decemfidum* by using *β*-glucuronidase (GUS) gene as a marker. To date, there was no report of genetic transformation carried out on this plant species. The effects of parameters such as bacterial concentration, infection time and acetosyringone concentration on transformation efficiency were investigated.

1.2 Problem Statement

M. decemfidum has been shown to contain flavonoids which exhibit anticancer effect (Susanti *et al*., 2007). This beneficial effect of flavonoids for

human health may increase their demand in dietary supplements and drug discovery. Therefore, improvement of flavonoid productivity in *M. decemfidum* is necessary. Some strategies such as introduction of useful genes into *M. decemfidum* can be adopted to increase the flavonoid synthesis of the plants.

Agrobacterium tumefaciens-mediated transformation is an evolved process involving genetic determinants for both the bacteria and the host plant cells. The gene transfer and its integration into the plant genome are governed by various *Agrobacterium* and plant tissue-specific factors, such as plant genotype, type of explant, plasmid vector, bacterial strain, bacterial concentration, composition of culture medium, wounding types, co-cultivation period and selection markers (Ziemienowicz, 2013). Although *Agrobacterium*-mediated transformation protocols are now available for some *Melastoma* species such as *M. malabathricum*, the protocols are applicable within each species to only a few genotypes. The transformation protocol for *M. malabathricum* may not be successful in *M. decemfidum* transformation. Therefore, the main focus of this research is to study the effects of transformation factors on *M. decemfidum* with regard to bacterial concentration, infection time and acetosyringone concentration in order to establish a transformation protocol for *M. decemfidum.* Optimization of transformation parameters are needed in order to produce an efficient *A. tumefaciens*-mediated transformation protocol for *M. decemfidum*.

1.3 Objectives of Study

The objectives of this study were:

1. To determine the effect of bacterial concentration on transformation efficiency of *M. decemfidum* using histochemical GUS assay.

2. To determine the effect of infection time on transformation efficiency of *M. decemfidum* using histochemical GUS assay.
3. To determine the effect of acetosyringone concentration on transformation efficiency of *M. decemfidum* using histochemical GUS assay.

1.4 Scope of Study

This study was mainly focused on the optimization of *Agrobacterium*-mediated transformation parameters for *M. decemfidum*. Three parameters assessed were *Agrobacterium* concentration, infection time and acetosyringone concentration which are known to influence the transformation efficiency in various plant species. *M. decemfidum* was used as the source of explant in this study. *In vitro* tissue culture was carried out for the maintenance of *M. decemfidum* and preparation of explants. *A. tumefaciens* strain LBA4404 carrying GUS reporter gene was used for *Agrobacterium*-mediated transformation. Histochemical GUS assay was performed to determine transient GUS expression. The transformation efficiency was determined based on the percentage of positive transformed explants as well as the number of blue spots per explant.

1.5 Research Significance

M. decemfidum has been widely used as herbal for treatment of various ailments. Besides, it was found to contain flavonoids which exhibit cytotoxic effects. These flavonoids can be isolated from *M. decemfidum* and potentially used in pharmaceutical industry. However, the mechanism underlying cytotoxic activity of the flvonoids is still unknown. Thus, establishment of a genetic transformation

protocol for *M. decemfidum* might be necessary. With an efficient transformation protocol, the desirable genes can be efficiently introduced into the plants and the useful genetically engineered characteristics can be expressed. The mechanisms of action of bioactive compounds in *M. decemfidum* can, therefore, be investigated. Increased production of flavonoids might also be achieved by transforming *M. decemfidum* with related genes. Moreover, the functions of other genes in *M. decemfidum* can be discovered.

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