



Original Article

Agrobacterium tumefaciens-mediated transformation of *Dendrobium lasianthera* J.J.Sm: An important medicinal orchid

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ABSTRACT

A protocol for genetic transformation mediated by *Agrobacterium tumefaciens* and production of transgenic *Dendrobium lasianthera* has been developed for the first time. The 8-week-old protocorm explants were used as target of transformation with *Agrobacterium tumefaciens* strain LBA4404 carrying plasmid pG35SKNAT1. Several parameters such as infection period, *Agrobacterium* density, concentration of acetosyringone, and co-cultivation period were evaluated for the transformation efficiency. The data were analyzed using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with $p < 0.05$. Subsequently, KNAT1 gene expression was confirmed by polymerase chain reaction (PCR) analysis. The highest efficiency of transformation (70%) obtained from protocorm explants infected with *Agrobacterium* culture was at the OD₆₀₀ concentration of 0.6 for 30 min, and co-cultivated with acetosyringone 100 μM for 5 days. The results of confirmation by PCR analysis show that the KNAT1 gene has been integrated and expressed in the genome of *Dendrobium lasianthera* transgenic.

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1. Introduction

Currently, orchid has become a significantly commercial commodity in Indonesia. Despite being a major part of cut flower industry, orchid specifically genus *Dendrobium* has been known as traditional medicine. In fact, traditional medicines sourced from orchid have long been circulated in China [1]. Multiple bibenzyls secondary metabolite, fluorenones and gigantol have been isolated from *Dendrobium nobile* which has a higher antioxidant activity than vitamin C [2]. Extracts from leaf, stem, root and pseudobulb of *Dendrobium crumenatum* have an anti-microbial activity [3]. New compounds of dendroside D, dendroside E, dendroside F and dendroside G have been discovered in *Dendrobium nobile* and indicated immunomodulatory activity [4]. One of orchid's species in Indonesia that has anticancer activity is *Dendrobium lasianthera* J. J.Sm.

Three vegetative organs (root, stem and leaf) of *D. lasianthera* J.J. Sm, are toxic and have anticancer activity, however, the most toxic organ with the highest breast anticancer activity T47D is stem with

LC50 (μg/mL) = 117 ± 6.35. Owing to its notable potential of becoming raw material for medicine and producing cut flowers, *Dendrobium lasianthera* is of high economic value and is promising to be cultivated.

The main problems in the development of orchid plant to be used as raw material for medicine are: the technique mass propagation is relatively difficult, too long vegetative phase in its life cycle (1–2 years), and genetic stability of the plant. To increase orchid production, genetic engineering is applied by inserting foreign gene into genome of *Dendrobium lasianthera* mediated by *Agrobacterium tumefaciens*.

The insertion of foreign genes into the genome of plants mediated by *Agrobacterium tumefaciens* is an effective and reproducible method and has been successfully applied to various plants such as *Artemisia carvifolia* [5], *Woodfordia fruticosa* [6], *Solanum trilobatum* [7], *Withania somnifera* [8], *Vanda kasem's* [9], and *Erycina pusilla* [10].

The genetic transformation by inserting *KNAT1* (*KNOTTED1* like *Arabidopsis thaliana*) gene into *Phalaenopsis amabilis* Blume has been done by Semiarti et al. which resulted in the formation of multiple shoots from one protocorm [11]. Recently, more success of genetic transformation in medicinal plants has been reported [7,12–13]. However, gene transformation of *KNAT1* into

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