



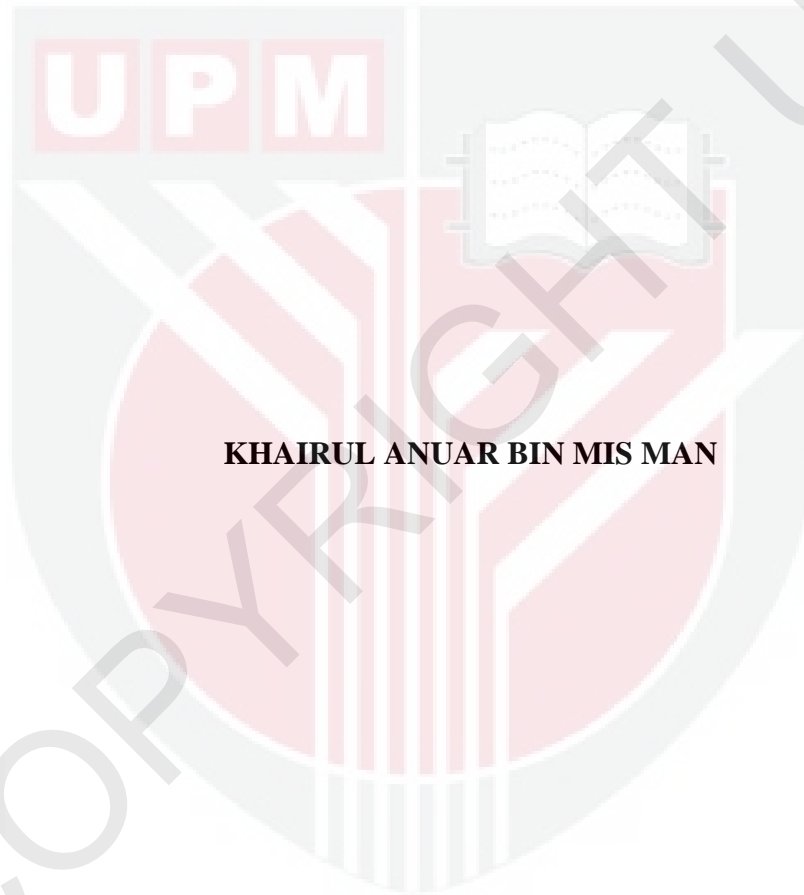
**UNIVERSITI PUTRA MALAYSIA**

**COMPARISON OF THE GROWTH OF TWO *ARABIDOPSIS THALIANA*  
ECOTYPES AND TRANSFORMATION EFFICIENCY IN UPM'S  
TRANSGENIC HOUSE**

**KHAIRUL ANUAR MIS MAN**

**FP 2015 144**

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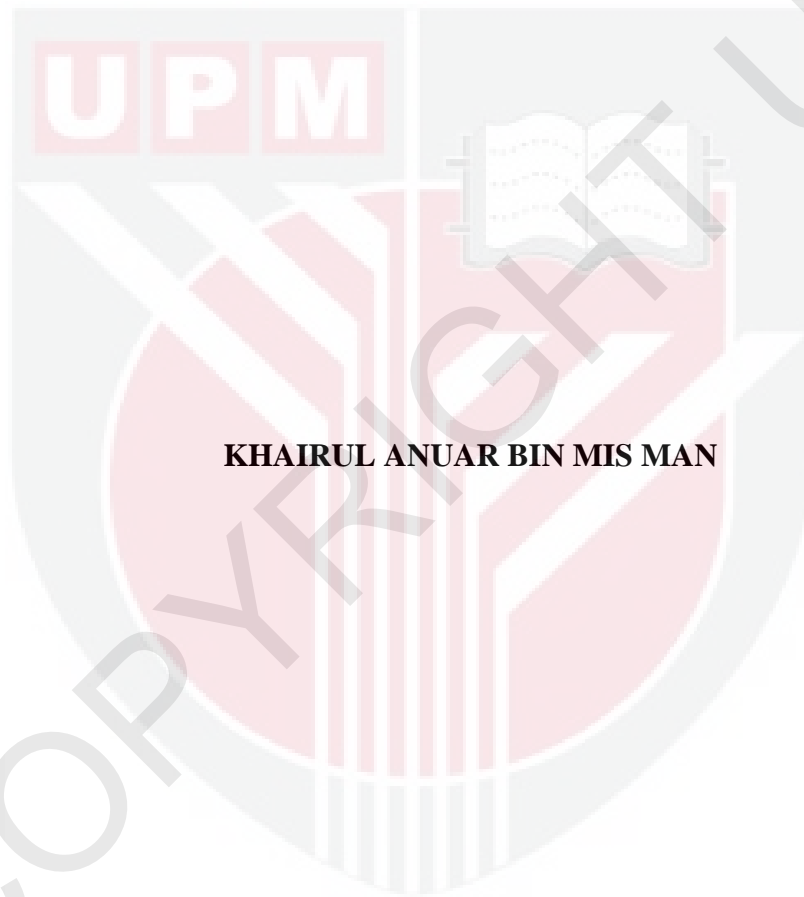
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**BY  
KHAIRUL ANUAR BIN MIS MAN**

**A project submitted to Faculty of Agriculture, Universiti Putra Malaysia, in  
fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of  
the degree of Bachelor of Agriculture Science**

**FACULTY OF AGRICULTURE  
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**2014/2015**

This project paper entitled “**Comparison of the Growth of Two *Arabidopsis thaliana* Ecotypes and Transformation Efficiency in UPM’s Transgenic House**” is prepared by Khairul Anuar Bin Mis Man and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science

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## ABBREVIATION

DNA	deoxyribonucleic acid
EB	elution buffer
UV	ultraviolet
TAE	Tris-Acetate -EDTA
PCR	Polymerase Chain Reaction
TE	Tris-EDTA
MS	Murashige and Skoog
g	gram
ml	milliliter
nm	nanometre
bp	base pair
μl	microliter
°C	degree Celsius
mg/kg	milligram/kilogram
%	percentage

## ABSTRACT

*Arabidopsis thaliana* is a small flowering plant in the mustard family (Brassicaceae). It is used to study the genetic improvement of plants and is a model organism in agricultural biotechnology. Therefore, the identification of suitable varieties for genetic experiments including the Columbia (Col-O) ecotype and Landsberg (Ler-0) ecotype is important. The objective of this experiment is to compare the growth rate of Columbia (Col-O) and Landsberg (Ler-0) *A. thaliana* ecotypes and the transformation efficiency in UPM's Transgenic Green House. The parameters used to measure the growth rate were size of leaf and height of plant. The Columbia (Col-O) and Landsberg (Ler-0) took around eight weeks to finish the life cycle. The Columbia (Col-O) has been reported to have very robust growth and average growth characteristic compared to Landsberg (Ler-0) which has shorter stems and smaller siliques. It is expected that the two ecotypes will also vary in the growth rate and transformation efficiency when grown in the UPM's Transgenic Greenhouse. *Agrobacterium tumefaciens* strain LBA4404 that harbour the binary vector pMDC32 will mediate transformation of *A. thaliana* which was performed on the Columbia (Col-0) ecotypes and the transformation efficiency was compared subsequently after culturing on regeneration medium with containing hygromycin at 60 mg/litre. The transformation was successful where the percentages seeds germinated on MS media are 56.67% with the addition of the antibiotic.

## ABSTRAK

*Arabidopsis thaliana* adalah tumbuhan berbunga kecil dalam keluarga mustard (Brassicaceae). Ia digunakan untuk mengkaji penambahbaikan genetik tumbuhan dan merupakan model organisma dalam bioteknologi pertanian. Oleh itu, pengenalpastian jenis yang sesuai untuk ujikaji genetik termasuk Columbia (Col-0) ecotype dan Landsberg (Ler-0) ecotype adalah penting. Objektif eksperimen ini adalah untuk membandingkan kadar pertumbuhan pokok *A. thaliana* varieti Columbia (Col-O) dan Landsberg (Ler-0) ecotypes dan kecekapan transformasi di rumah hijau transgenik UPM. Parameter yang digunakan untuk mengukur kadar pertumbuhan adalah saiz daun dan ketinggian tumbuhan. Varieti Columbia (Col-O) dan Landsberg (Ler-0) mengambil masa lapan minggu untuk menamatkan satu kitaran hidup. Varieti Columbia (Col-O) telah dilaporkan mempunyai pertumbuhan yang sangat teguh dan purata pertumbuhan yang lebih baik berbanding Landsberg (Ler-0) yang mempunyai lebih batang yang pendek dan siliques yang lebih kecil. Kedua-dua varieti menunjukkan bahawa pertumbuhan varieti juga akan berubah-ubah dalam proses pembesaran setelah ditanam di rumah hijau transgenik UPM. *Agrobacterium tumefaciens* LBA4404 jenis pMDC32 merupakan vektor binari yang digunakan untuk transformasi *A. thaliana* dan telah dijalankan pada varieti Columbia (Col-0) dan kecekapan transformasi telah dibandingkan selepas dicambahkan pada medium pertumbuhan semula dengan kepekatan hygromycin sebanyak pada 60 mg/liter. Kecekapan Transformasi adalah berjaya kerana peratus percambahan biji benih sebanyak 56.67% pada MS media mengandungi antibiotik tersebut.

# CHAPTER 1

## INTRODUCTION

*Arabidopsis thaliana* has been most studied and becomes a model organism for agricultural biotechnology. It is a small flowering plant in the Brassicaceae (mustard) family. *Arabidopsis* plays the role of mice and fruit flies (*Drosophila*) in animal biology, and provides information on the way that gene function can affect commercial crop production. It has no inherent agricultural value and is even considered a weed. The *Arabidopsis* growth and development analysis will provides an identifying and interpreting phenotypic framework of methodology for differences in plants resulting from genetic variation and environmental stress (Boyes *et al.*, 2001).

The variety or ecotypes of *Arabidopsis thaliana* have many different species. Each plant type has specific properties, such as a leaf, maximum growth height, or number of seeds produced. The named of varieties are made usually after the plant location were first found. The Columbia and Landsberg ecotype are considered to be the wild type or the plant type found in nature (Cotter, 2005). The *Arabidopsis thaliana* was grown successfully in the greenhouse for one complete cycle as expected and reported by Crist *et al.*, in 2006 and it was also expected there is several significant different and effect between the variety growth.

Plant transformation technology offers opportunities for basic scientific research and for modification of food and fiber crops. Transgenic plants are typically produced by complex methods that require careful preparation of plant cells or tissues, introduction of DNA using *Agrobacterium tumefaciens* or particle bombardment, selection of transformed cell lines, and regeneration of plants (Christou,1996). *Agrobacterium*-mediated transformation has several advantages, such as higher transformation efficiency and minimal re-arrangement of transferred DNA. *Agrobacterium tumefaciens*- mediated method is commonly used in rice genetic transformations (Lin *et al.*, 2009).

The floral-dip method for *Agrobacterium*-mediated transformation of *Arabidopsis* allows efficient plant transformation without need for tissue culture (Hansen *et al.*, 1999). Generating and identifying transformants is essential for many studies of gene function. In *Arabidopsis thaliana* study, a revolutionary protocol termed floral dip was now the most widely used transformation method. It was reported by Amanda *et al.*, in 2009, although robust it involves a number of relatively time-consuming and laborious steps, including manipulating an *Agrobacterium tumefaciens* culture and aseptic procedures for the selection of plant lines harboring antibiotic-selection markers to be used in transformation *Agrobacterium* in *Arabidopsis thaliana*.

The objective of this experiment was to make comparison of the height and the length of leaves between two ecotypes *Arabidopsis thaliana* plant between Columbia (Col-0) and Landsberg (Ler-0) ecotypes in one complete life cycle. The second objective is to determine the transformation efficiency of *Agrobacterium tumefaciens* mediated transformation seeds of *Arabidopsis thaliana* plant on selection transformant media.



There was expected that the plant *Arabidopsis thaliana* Columbia (Col-0) ecotype has very robust growth and average growth characteristic, so it widely established in specific used in *Agrobacterium tumefaciens* mediated transformation as reported by Cotter in 2005. While, the Landsberg (Ler-0) ecotype has shorter stems and smaller siliques than Columbia. There was a successful transformed seeds containing DNA insertion of *Agrobacterium tumefaciens* using hygromycin antibiotics in *Arabidopsis thaliana* plant to get transformation efficiency.

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