Micropropagation of *Vanda tessellata* (Roxb.) Hook. ex G. Don

A Dissertation submitted for the partial fulfillment of the requirements of Master's Degree in Botany

By

Bijaya Bahadur Malla

Plant Biotechnology Unit

TU Regd. No. 5-1-48-2909-2004

Exam Roll No. 18235

Batch: 2068/70

Central Department of Botany

Tribhuvan University

Kirtipur, Kathmandu, Nepal

2016

RECOMMENDATION

Kirtipur, Ktm.
Nepal
27th Aug 2016

This is to certify that the dissertation entitled **'Micropropagation of *Vanda tessellata* (Roxb.)Hook.ex G. Don'** submitted by Mr. Bijaya Bahadur Malla for the partial fulfillment of the requirement of Master's Degree in Botanyhas been carried out under my supervision and guidance. The result of this

work has not yet been submitted for any other degree.

Therefore, we recommend his work for approval and acceptance.

……

Supervisor

Bijaya Pant, PhD

Professor

Central Department of Botany

Tribhuvan University, Nepal.

LETTER OF APPROVAL

The dissertation work submitted by **Mr. Bijaya Bahadur Malla** entitled **"Micropropagation of *Vanda tessellata*(Roxb.) Hook.ex G. Don"** has been accepted for the partial fulfillment of M.Sc. in Botany.

Expert Committee

……	……..
Supervisor	**Head of Department**
Bijaya Pant, PhD	**Mohan Siwakoti, PhD**
Professor	Professor
Central Department of Botany	Central Department of Botany
Tribhuvan University, Nepal.	Tribhuvan University, Nepal
……	…….
External Examiner	**Internal Examiner**
Sanu Devi Joshi, PhD	**Ms. Shreeti Pradhan**
Professor	Assistant Professor
Academician, NAST	Central department of Botany

Date of Examination: 27th August, 2016

ACKNOWLEDGEMENTS

It is my great pleasure to express my gratitude to my supervisor Prof. Dr. Bijaya Pant (Prof., C.D.B., T.U.) for her noble guidance, continuous encouragement, best propositions, convenient criticism and reliable solutions and inspirations during my research work. I am also thankful to Ms. Shreeti Pradhan (Asst. Prof. C.D.B., T.U.) for her valuable suggestions and guidance. I would also like to express my deep gratitude to Prof. Dr. Mohan Siwakoti (HoD, C.D.B.) for providing me administrative and laboratory facilities during my research work.

I'd like to express my sincere gratitude to Dr. ChitraBahadurBaniya for his valuable guidance and support during my research work. I am grateful to Mr. Shailendra Kumar Singh (lab officer) and Mr. SambhuBista for continuous support and help. Similarly I am thankful to all teaching staff and non teaching staffs of C.D.B., T.U. for their help.

I highly acknowledge my seniors Mukti Ram Poudel (Phd. Scholar) for helping me analyzing the data and Ashok Kumar Patel and MukeshBabu Chand for their kind help during my research work. I am also thankful to my besties Anil Joshi, PrakashGairhe, SashiKafle, Srijanasharma, Sabitri Sharma, SabitaDhungana, TilKumariChhetri and SamjhanaDahal for their continuous support and suggestions. I am also thankful to my juniorSubhadraPudasainifor her support during lab work.

I am indebted to my parents for their regular encouragement and patient. I'd like to express my gratitude to my sister BinitaMalla, Brother Bishal Malla and my beloved Susheela Devi Gurung for their valuable support and encouragement during my research work.

At last but not least I'd like to remember all my friends who have helped, supported and encouraged me directly or indirectly.

Bijaya Bahadur Malla

ABSTRACT

Vanda tessellata (Roxb.)Hook.ex G. Don is a monopodial orchid and epiphytic in habitat with leafy stem of medium size plant body. It is renowned for its horticultural and high medicinal value as well. The aim of its study was to develop an efficient protocol for micropropagation of *Vanda tessellata* Roxb. In the present study the protocorms were first tried in full MS, half MS and quarter MS medium without addition of any plant hormones and other organic additives. Quarter strength of MS medium was found to be effective medium for the earlier development of shoots and shootbuds from protocorm. Therefore further experiment was carried out in this medium. Shoot multiplication was carried out in different concentrations and combinations of GA_3, CW, BAP, BAP plus 6% sucrose and NAA. Among them, quarter strength MS medium supplemented with 0.5 mgl^{-1} BAP was found to be effective for shoot multiplication, MS medium supplemented with 1.5 mgl^{-1}BAP plus 6% sucrose was found to be effective for shoot bud development and quarter strength of MS medium supplemented with 0.5 mgl^{-1} NAA favored the root formation. Higher number of leaves was found in quarter strength of MS medium supplemented 1.5mgl^{-1} $GA_3$and longest leaf length was found in quarter strength MS medium supplementedwith 0.5 mgl^{-1} GA_3.This present study reveals that this species favors BAP for shoot multiplication as compared to other hormones and organic additives used. This protocol might be useful for *in vitro* shoot bud formation and multiplication from protocorms for mass propagation and *ex situ* conservation of this valuable plant that is used as medicinal and horticultural aspects.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

°C	:	degree centrigrade
µM	:	micromolar
2,4-D	:	2,4- Dichlorophenoxyacetic acid
a.s.l.	:	above sea level
BA	:	Benzyl Adenine
BAP	:	6-Benzylaminopurine
CITES	:	Convention on International Trade in Endangered Species of Wild Floraand Fauna
cm	:	centimeter
df	:	Degrees of freedom
EDTA	:	Ethylene Diamine Tetra Acetate
et al.	:	and others
etc		etcetera
eg		as an example
Fig.	:	Figure
g	:	gram
GA_3	:	Gibberellic acid
HCl	:	Hydrochloric acid
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
KN	:	Kinetin
Kn C	:	Knudson C (1946)
l		liter
Lindl.	:	John Lindley
mg	:	milligram
ml	:	milliliter
MS	:	Murashige and Skoog (1962)
N		Normality
NAA	:	α-Naphthalene acetic acid

NaOCl	:	Sodium hypochloride
NaOH	:	Sodium hydroxide
NP	:	New Phalaenopsis
PLBs	:	Protocorm-like bodies
PM	:	Phytamax
ppm	:	parts per million
psi	:	pound per square inch
Sig.	:	Significance level
SPSS	:	Statistical Package for Social Sciences
T.U.	:	Tribhuvan University
viz	:	namely