

**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 Botany has 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

Bijaya Pant, PhD

Professor

Central Department of Botany

Tribhuvan University, Nepal.

.....

Head of Department

Mohan Siwakoti, PhD

Professor

Central Department of Botany

Tribhuvan University, Nepal

.....

External Examiner

Sanu Devi Joshi, PhD

Professor

Academician, NAST

.....

Internal Examiner

Ms. Shreeti Pradhan

Assistant Professor

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 juniorSubhadraPudasaini for 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 GA₃, CW, BAP, BAP plus 6% sucrose and NAA. Among them, quarter strength MS medium supplemented with 0.5 mg l⁻¹ BAP was found to be effective for shoot multiplication, MS medium supplemented with 1.5 mg l⁻¹ BAP plus 6% sucrose was found to be effective for shoot bud development and quarter strength of MS medium supplemented with 0.5 mg l⁻¹ NAA favored the root formation. Higher number of leaves was found in quarter strength of MS medium supplemented 1.5mg l⁻¹ GA₃ and longest leaf length was found in quarter strength MS medium supplemented with 0.5 mg l⁻¹ GA₃. 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

RECOMMENDATION	I
LETTER OF APPROVAL	II
ACKNOWLEDGEMENT	III
ABSTRACT	IV
ABBREVIATIONS AND ACRONYMS	V
TABLE OF CONTENTS	VI
LIST OF FIGURES	VII
CHAPTER I	1-6
INTRODUCTION	1
1.1 Background	1-2
1.1.1 Tissue Culture Practice in Orchids	2-3
1.1.2 Taxonomic Description of Plant	3
1.1.3 Medicinal and other uses	3-4
1.2 Hypothesis	5
1.3 Objectives	5
1.3.1 General objective	5
1.3.2 Specific objectives	5
1.4 Rationale	5-6
CHAPTER II	7
LITERATURE REVIEW	7-10
CHAPTER III	11-17
MATERIALS AND METHOD	11
3.1 Plant materials	11
3.2 Methodology	11
3.2.1 Preparation of stock solutions	11
3.2.1.1 Preparation of stock solutions for MS medium	11-13
3.2.2 Hormones used for the experiments	13-14
3.2.3 Preparation of Hormones stock solution	14
3.2.4 Preparation of nutrient medium	14-15
3.2.5 Method of sterilization	15

3.2.5.1 Sterilization of glass wares and metallic instruments	15
3.2.5.2 Sterilization of inoculation chamber	15
3.2.5.3 Sterilization of media and distilled water	15
3.2.6.3 Inoculation of protocorm	16
3.2.7 Inoculation of protocorms for shooting	16
3.2.8 Rooting of shoots	16
3.2.9 Data recording	16
3.2.10 Statistical Analysis	16-17
CHAPTER IV	18-31
RESULTS	18
4.1 Shoot multiplication	18-19
4.1.1 Shoot multiplication in different concentration of GA ₃	20
4.1.2 Shoot multiplication in different concentration of coconut water	21
4.1.3 Shoot multiplication in different concentration of BAP + 6% sucrose	22
4.1.4 Shoot multiplication in different concentration of NAA	23
4.1.5 Shoot multiplication in different concentration of BAP	24
4.1.6 Shoot multiplication in different concentration of NAA plus 0.5 BAP	25
4.2 Rooting of Shoots	26
4.2.1 Root formation in IAA	26
4.2.2 Root formation in IBA	27
4.2.3 Root formation in NAA	28
CHAPTER V	32-34
DISCUSSION	32
5.1 Protocorm formation in different strength of MS medium	32
5.2 Shoot Multiplication	32-33
5.3 Rooting of Shoots	33-34
CHAPTER VI	35
CONCLUSION	35
CHAPTER VII	36
RECOMMENDATIONS	36
REFERENCES	37-45
ANNEXES	46-52

LIST OF TABLES

Table 1. Composition of Murashige and Skoog (1962) medium	11-13
Table 2: Effect of different MS medium on <i>in vitro</i> growth of protocorm of <i>Vanda tessellata</i> (Roxb.)Hook.ex G. Don (Observation taken in weeks)	18

LIST OF FIGURES

Figure 1: Image of <i>Vanda tessellata</i>	3
Figure 2: Effect of different strength of MS media on initiation of PLBs, shoot and leaf proliferation observed in weeks	19
Figure 3: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented GA ₃ in different concentrations.	20
Figure 4: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented 5% and 10% coconut water.	21
Figure 5: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented BAP and 6% sucrose in different combinations.	22
Figure 6: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented NAA with different concentrations.	23
Figure 7: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented BAP with different concentrations.	24

Figure 8: Average number of shoot, shoot bud number, shoot length, leaf number and leaf length produced per responsive protocorm culture in ¼ MS medium supplemented NAA and BAP with different combinations.	25
Figure 9: Average number of shoot, shoot bud number, shoot length, leaf number, leaf length, root number and root length produced per responsive protocorm culture in ¼ MS medium supplemented IAA with different concentrations.	26
Figure 10: Average number of shoot, shoot bud number, shoot length, leaf number, leaf length, root number and root length produced per responsive protocorm culture in ¼ MS medium supplemented IBA with different concentrations.	27
Figure 11: Average number of shoot, shoot bud number, shoot length, leaf number, leaf length, root number and root length produced per responsive protocorm culture in ¼ MS medium supplemented NAA with different concentrations.	28
Figure 12: Photoplates of <i>Vanda tessellata</i> : Growth of protocorm in MS, ½ MS and ¼ MS and shoot multiplication.	29
Figure 13: Photoplates of <i>Vanda tessellata</i> : Shoot multiplication	30
Figure 14: Photoplates of <i>Vanda tessellata</i> : Root formation	31

ABBREVIATIONS AND ACRONYMS

°C	:	degree centigrade
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
<i>et al.</i>	:	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