UNIVERSITI TEKNOLOGI MARA

IN VITRO REGENERATION OF Barringtonia racemosa

NURUL HANANI BINTI KAMARUDIN

Thesis submitted in fulfillment of the requirements for the degree of Master of Science (Applied Biology)

Faculty of Applied Sciences

June 2018

ABSTRACT

Barringtonia racemosa is a type of medicinal plant belongs to Lecythidaceae family. The plants have numerous biological activities such as anti-tumour, anti-bacterial and anti-fungal. However, in Singapore, this species had been acknowledged as endangered species and classified as under-utilized crops in Malaysia. Therefore, the efforts to conserve this threatened species are crucial to ensure its survivability by the implementation of plant tissue culture technique. The objectives of this study were to identify the most effective surface sterilization technique for leaf, stem and shoot tip explants, to identify the most responsive explant for in vitro regeneration of B. racemosa, to determine the optimum concentration of plant growth hormones for in vitro regeneration of B. racemosa and to determine stomatal density of in vitro and in vivo grown B. racemosa leaves. Different types and concentrations of plant growth hormones were tested to induce adventitious shoot from various explants (young leaf, stem and shoot tip). Stomatal density (SD) on abaxial and adaxial surfaces between in vitro and in vivo grown B. racemosa was determined using Field Emission Scanning Electronic Microscopy (FESEM). The results indicated that BAP was superior to KIN in promoting adventitious shoot formation. The highest shoot formation (100%) was achieved on MS medium containing 0.4 mg/L NAA + 0.5 mg/L BAP for shoot tip explants, while MS medium supplemented with 0.2 mg/L NAA + 1.0 mg/L BAP was the best for stem explants. However, leaf explants failed to produce any shoot. All explants cultured on MS media supplemented with NAA + KIN became necrotic at all concentrations tested. The results from FESEM micrographs indicated that the stomata of in vitro leaf are round in shape and larger size, whereas the stomata of in vivo leaf are elliptical shape and smaller size. It was observed that SD of in vivo grown B. racemosa was higher than in vitro for both adaxial and abaxial leaf surfaces. The in vitro leaf from stem explant exhibited the lowest mean number of SD. The shape of stomata found on in vivo leaf were anomocytic, whereas in in vitro leaf were paracytic. Glandular and non-glandular trichomes were also observed on in vitro and in vivo leaves. Thus, somaclonal variation occurred in in vitro plantlets.

ACKNOWLEDGEMENT

Firstly, I wish to thank Allah for giving me the opportunity to embark on my Master and for completing this long and challenging journey successfully. I would like to express the deepest appreciation to my supervisor, Dr Azani Saleh, who always taught and guide me on how to produce a good research. She always supports and gives me positive words along my journey to complete my final year project. Furthermore, my appreciation goes to Prof. Madya Dr Norrizah Jaafar Sidik for her guidance and assistance during sampling.

A big thanks I would address to a few staff in Institute of Bioscience, Universiti Putra Malaysia for giving me a chance to do sampling there. Special thanks to my colleagues and friends for helping me with this project.

Finally, this thesis is dedicated to my beloved family especially my father, Kamarudin bin Mohamed and my mother, for their everlasting support and courage in terms of financial and emotional. They always give courage and strengths to me to overcome the difficult hardships I'm facing as a student. I also want to thank my brothers Muhammad Haziq Kamarudin and Muhammad Hafizuddin bin Kamarudin and also my sister Nurul Hanis Maisarah Kamarudin who always supports and helping me throughout my journey to complete this research.

This piece of victory is dedicated to all of you. Alhamdulillah.

TABLE OF CONTENTS

		P	age					
CONFIRMATION BY PANEL OF EXAMINERS								
AUTHOR'S DECLARATION								
ABSTRACT								
ACKNOWLEDGEMENT TABLE OF CONTENTS LIST OF TABLES								
					LIST	OF FIG	GURES	X
					LIST	OF SY	MBOLS	xii
LIST OF ABBREVIATIONS								
LIST	LIST OF NOMENCLATURES							
СНА	PTER (ONE: INTRODUCTION	1					
1.1	Resear	rch Background	1					
1.2	Proble	em Statement	3					
1.3	Object	tives	3					
1.4	Signif	icance of Study	3					
1.5	Scope	and Limitation of Study	4					
СНА	PTER T	TWO : LITERATURE REVIEW	5					
2.1	The O	rigin and Taxonomy of Barringtonia racemosa	5					
2.2	Morph	Morphology of Barringtonia racemosa						
2.3	Plant '	Plant Tissue Culture Technique						
	2.3.1	In Vitro Regeneration	8					
	2.3.2	Tissue Culture of Baringtonia Species	9					
	2.3.3	Advantages and Disadvantages of Plant Tissue Culture Technique	10					
2.4	Factor	rs Affecting Plant Tissue Culture	11					
	2.4.1	Plant Growth Hormones	11					
	2.4.2	Explants	13					

	2.4.3	Media Composition	14
	2.4.4	Murashige and Skoog medium	15
	2.4.5	Chu (N6) medium	16
	2.4.6	Gamborg B5 medium	17
2.5	Micro	morphological Studies	18
СНА	PTER T	THREE: RESEARCH METHODOLOGY	20
3.1	Materials		
	3.1.1	Raw Materials	20
	3.1.2	Chemicals	20
	3.1.3	Apparatus	20
3.2	Methods		
	3.2.1	Hormone Stock Preparation	20
	3.2.2	Media Preparation	21
	3.2.3	Surface Sterilization of Explants	23
	3.2.4	Inoculation of Explants for In Vitro Shoots Induction	23
	3.2.5	Measurement of Shoots Produced	23
	3.2.6	Microscopic Studies on Stomatal Density and Stomatal Behavior	24
3.3	Statist	ical Analysis	24
СНА	PTER I	FOUR: RESULTS AND DISCUSSION	25
4.1	Seeds	and Seedlings Collection from the Field	25
4.2	Maintenance Inside the Greenhouse		
4.3	Optimization of Explant Sterilization Technique		
4.4	Effects of BAP and NAA on Shoots Induction of B. racemosa		
4.5	Micromorphological Observation 4		