UNIVERSITI TEKNOLOGI MARA

AGROBACTERIUM RHIZOGENES – MEDIATED HAIRY ROOTS CULTURES OF CAPSICUM ANNUUM AND CAPSICUM FRUTESCENS

NURSURIA MD SETAMAM

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

Faculty of Applied Science

March 2015

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of University Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification

I, hereby acknowledge that I have been supplied with the academic rules and regulation for Post Graduates, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	;	Nursuria Md Setamam	
Student I.D. No.	:	2011980079	
Programme	:	AS 780 (Plant Tissue Cultures)	
Faculty	:	Applied Science	
Thesis Title :		Agrobacterium rhizogenes – Mediated Hairy Roots	
		Cultures of Capsicum annuum and Capsicum	
		frutescens	
Signature of Student	;	Nursung.	
Date	::	March 2015	

ABSTRACT

Capsicum annuum and Capsicum frutescens also known as "chilies" have tremendous economic values as crops and medicinal plants. In recent years, the establishment of hairy roots cultures via Agrobacterium rhizogenes had provided numerous advantages to many plant studies including Capsicum species. Therefore, in this research, A. rhizogenesmediated hairy roots cultures were studied with the expectation to provide a better understanding and alternative solutions regarding these species. The objectives were to induce and proliferate the putative hairy roots, callus, and regenerated plantlets of C. annuum and C. frutescens with variables factors such as hormones, media and explants. The volatile compounds present in cultures samples were also identified. Initially, the cotyledon, radical and hypocotyl explants were obtained from seedlings and co-cultured with isolated strains of A. rhizogenes (ATCC 15834, ATCC 43056, ATCC 13333 and ATCC 43057). The highest induction putative hairy roots were transferred as explants into liquid MS with 0.2, 0.4, 0.6, 0.8, 1.0 mg/L of IAA for proliferation. The explants were also cultured in 0.5, 1.0, 1.5, 2.0 mg/L of each IAA, NAA, and 2,4-D for callus induction. In plant regeneration, the explants were cultured in solid MS containing 0.25, 0.5, 1.0, 1.5, 2.0 mg/L of BAP. Each treatment consisted of 20 replications and subcultured every two weeks. Proliferated hairy roots, callus, leaves, and stem from putative hairy roots explants were dried and extracted by using three types of solvents for GC-MS analysis. Findings showed that, the highest induction efficiency was achieved by using cotyledon explants which were ATCC 43056 for C. annuum and ATCC 15834 for C. frutescens. The presence of 1.0 mg/L IAA was suitable for putative hairy roots proliferation in both species. Meanwhile, 1.0 mg/ L and 1.5 mg/L of NAA for C. annuum and C. frutescens respectively, improved the callus induction. Through direct morphogenesis, the optimum concentration of BAP for plant regeneration were achieved in 1.5 mg/L for C. annuum and 1.0 mg/L for C. frutescens. Various beneficial compounds had been identified via GC-MS analysis for both C. annuum (33 compounds) and C. frutescens (37 compounds). More compounds were found in hexane extract compared to other solvents and proliferated putative hairy roots consisted more of compounds than other cultures. As a conclusion, A. rhizogenes-mediated hairy roots cultures can be used as an alternative tool to overcome recalcitrant. However, more further studies on additional factors were recommended in the future.

TABLE OF CONTENTS

AUTHOR'S DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENT	iv
TABLES OF CONTENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xvii

CHA	APTER ONE: INTRODUCTION	1
1.1	Background of Study	1
1.2	Problem Statement	2
1.3	Significance of Study	3
1.4	Objective of Study	4
1.5	Scope and Limitation of Study	4
СНА	APTER TWO: LITERATURE REVIEW	6
2.1	Capsicum annuum and Capsicum frutescens	6
	2.1.1 Taxonomy	6
	2.1.2 Morphology and Anatomy	6
2.2	Economical Value of Capsicum Species	7
	2.2.1 Agricultural Values	7
	2.2.2 Medicinal Values	9
2.3	Plantation and Biotechnology Problems	9
	2.3.1 Phytopathological Problem	9
	2.3.2 Recalcitrance Problem	10
2.4	Chemical Properties of Capsicum Species	
	2.4.1 Mechanism of Defenses	10

v

	2.4.2	Phenolic Group	11		
	2.4.3	Flavanoid Group	11		
	2.4.4	Other Compounds Group	12		
2.5	Characteristics of Plant Roots				
	2.5.1	Roots as Transportation System	12		
	2.5.2	Roots in Biosynthesis	13		
	2.5.3	Factor Affecting Roots Development	13		
	2.5.4	Types of Roots Structures	14		
2.6	Plant	Plant Tissue Culture			
2.7	Root (15			
2.8	Trans	16			
2.9	Mecha	anism of Transformation via Agrobacterium rhizogenes	17		
2.10	Prope	rties of Hairy Roots Cultures	19		
	2.10.1	Hairy Roots Structure	19		
	2.10.2	Production of Opines Group	20		
	2.10.3	rol genes	20		
2.11	Factor	rs Affecting Hairy Roots Induction	21		
	2.11.1	Different Plant Species	22		
	2.11.2	2 Types of Agrobacterium rhizogenes Strains	22		
	2.11.3	3 Types of Explants	23		
	2.11.4	Additional Factors	23		
2.12	Factor	rs Affecting Growth of Hairy Roots Cultures	23		
	2.12.1	Plants Hormones	24		
	2.12.2	2 Types of Media	24		
	2.12.3	3 Additional Factors	25		
2.13	Furthe	er Development of Hairy Roots Cultures	25		
	2.13.1	Hairy Roots Proliferation	26		
	2.13.2	2 Callus Induction of Hairy Roots	27		
	2.13.3	B Plant Regeneration	28		
2.14	Adva	ntages of Hairy Roots	28		
	2.14.1	Plant Development	29		