BIOCHEMICAL STUDIES ON PRODUCTION OF SECONDARY METABOLITES FROM DIFFERENT PLANTS BY USING TISSUE CULTURE TECHNIQUES

By

HEBA MAHMOUD MOHAMED MAHMOUD
B.Sc. Agric., Sci., (Biochemistry), Fac. of Agric., Cairo Univ., 2007.

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCES

In

Agricultural Sciences
(Agricultural Biochemistry)

Department of Agricultural Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

2016

APPROVAL SHEET

BIOCHEMICAL STUDIES ON PRODUCTION OF SECONDARY METABOLITES FROM DIFFERENT PLANTS BY USING TISSUE CULTURE TECHNIQUES

M. Sc. Thesis
In
Agric. Sci. (Biochemistry)

By

HEBA MAHMOUD MOHAMED MAHMOUD
B.Sc. Agric., Sci., (Biochemistry), Fac. of Agric., Cairo Univ., 2007.

Approval Committee

Dr. FOUAD MOHAMED ABDEL-LATIF……
Professor of Fruit Science, Fac. of Agri., Banha Univ.

Dr. EMAM ABD-ELMOBDY ABD EL-REHIM……
Professor of Biochemistry, Fac. of Agri., Cairo Univ.

Dr. OSAMA KONSOWA AHMED ……...
Professor of Biochemistry, Fac. of Agri., Cairo Univ.

Date: / /

SUPERVISION SHEET

BIOCHEMICAL STUDIES ON PRODUCTION OF SECONDARY METABOLITES FROM DIFFERENT PLANTS BY USING TISSUE CULTURE TECHNIQUES

M. Sc. Thesis
In
Agric. Sci. (Biochemistry)

By

HEBA MAHMOUD MOHAMED MAHMOUD

B.Sc. Agric., Sci., (Biochemistry), Fac. of Agric., Cairo Univ., 2007.

SUPERVISION COMMITTEE

Dr. OSAMA KONSOWA AHMED
Professor of Biochemistry, Fac. of Agri., Cairo Univ.

Dr. EMAN AHMED HANAFY
Lecturer of Biochemistry, Fac. of Agri., Cairo Univ.

Dr. WAFAA TAWFIK SAEED MOUHAMED
Professor and Head Researcher of Olive and Fruit of Semi Arid Zone, H.R.I, A. R. C.

DEDICATION

I dedicate this work to whom my heart felt thanks; ***my God, my mother, my sisters , my brother*** *for all the support they lovely offered along the period of my post graduation, as well as to my husband* ***Mohammed and my sons Ahmed and Hamza*** *for their help and patience.*

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to ***Dr. Osama konsowa Ahmed,*** *Professor of Biochemistry, Biochemistry Department, Faculty of Agriculture, Cairo University, for his supervision and help throughout this investigation.*

I am also greatly indebted to ***Dr. Eman Ahmed Hanfy,*** *Lecturer of Biochemistry, Biochemistry Department, Faculty of Agriculture, Cairo University, for her supervision, valuable assistance and suggestions, useful advice and guidance help throughout this investigation.*

Also many thanks to ***Dr. Wafaa Tawfik Saeed,*** *Head Researcher of olive and fruit of semi arid zone Department, Horticulture Research institute, Agriculture Research Center,, for her supervision, help and advice throughout the preparation of the thesis.*

I am deeply grateful to my colleagues in Horticulture Research institute, Agriculture Research Center also, many thanks to Biochemistry Department, Faculty of Agriculture, Cairo University.

Name of candidate: Heba Mahmoud Mohamed Mahmoud
Degree: M.Sc.
Title of Thesis: Biochemical studies on production of secondary metabolites from different plants using tissue culture techniques
Supervisors: Dr. Osama Konsowa Ahmed
Dr. Eman Ahmed Hanafy
Dr. Wafaa Tawfik Saaed

Department : Agricultural Biochemistry

Approval:

ABSTRACT

The present study was carried out in Tissue Culture Research Laboratory, Horticulture Research Institute, A.R.C., Giza, Egypt, during 2011 to 2014. Because of the importance of *Ginkgo biloba* and industrial needs of ginkgo secondary metabolites significantly directed this study towards elicitation using tissue culture for over production of ginkgo metabolites via using sodium nitroprusside (SNP) which utilized as the donor of nitric oxide (NO) and using micro algal elicitors (*Anabaena Sp*. and *Spirulina Sp*.) to investigate its effect as elicitor on phenols, flavonoids, ginkgo flavone glycosides and ginkgolides accumulation in *G. biloba* cell suspension cultures and the effect of these elicitors on cells growth. Moreover, study the effects of different hormone combinations on callus induction for production the best callus in *Ginkgo biloba* cell cultures.

The results showed that the optimal medium for inducing leaf-derived callus and the reculture of callus was MS media with 2.0 mg/L naphthalene acetic acid (NAA) + 2.0 mg/L 6-benzylaminopurine (6-BA). It was found that using SNP at concentrations of 25 and 50 ppm showed the highest increase in biomass of cultured *Ginkgo biloba* on day 13 which was 0.599 and 0.58 g FW, respectively from the initial inoculums of 0.5 g FW. Whereas the highest accumulation of phenols and flavonoids content with 150 ppm SNP on day 22 showed an increase of 2.8 and 2.2 fold relative to control. Similarly, *Anabaena Sp*. extract at concentration of 150 ppm which showed the highest accumulation of phenols and flavonoids content (2.5 and 2 fold on day 22 of the control, respectively). On the other hand, *Anabaena sp*. extract at concentration of 150 ppm also influenced the highest increase in biomass *G. biloba* cultured cells on day 13 day which was 0.69 g FW. Moreover, *G. biloba* cultures treated with *Spirulina Sp*. extract at concentrations of 150 ppm observed the maximum increase in biomass on day 13 over the control which was 0.705 g FW. Also, the highest accumulation of phenols and flavonoids content increased to 1.7 and 2.4 fold, respectively during day 22 in cultures treated with 100 ppm of *Spirulina Sp*. extract. In addition, the highest contents of ginkgo flavone glycosides and ginkgolides compounds found in *G. biloba* cell suspension culture was 1.9 and 1.4 fold of the control by using 150 ppm *Anabeana Sp*. extract and the highest contents of the content of these compounds were 1.9 and 2.3 fold with 150ppm *Spirulina Sp*. extract, respectively. While, the highest content of ginkgo flavone glycosides and ginkgolides was increased to 2.3 and 3.2 fold relative to control with 150 ppm SNP elicitation.

Key words: *Anabaena*, callus, elicitor, *Ginkgo biloba*, nitric oxide, phenols, *Spirulina*.

CONTENTS

INTRODUCTION 1

REVIEW OF LITERATURE 5

1. Plant biotechnology and tissue culture **12**

2. Elicitors and elicitation and production of secondary metabolite by plant cell cultures. **18**

a. Nitric oxide elicitor. 22

b. Cyanobacterial elicitors. 27

1. *Spirulina Sp*. 29

2. *Anabaena Sp*. 30

MATERIALS AND METHODS **32**

MATERIALS **32**

METHODS **32**

Tissue culture technique **32**

1. Culture media 32

2. Leaves sterilization 33

3. Callus initiation 33

a. Medium types used for callus induction 33

b. Initiation of suspension cultures 34

c. Measurement of cell suspension culture growth curve 34

4. Chemical analysis 35

a. Chemical characterization of *Ginkgo biloba* leaves and its callus 35

1. Determination of moisture 35

2. Determination of crude protein 35

3. Determination of ash 35

4. Determination of total carbohydrates..... 35
b. Elicitation of secondary metabolites production..... 35
Preparations and addition of elicitors 35
Nitric oxide elicitor 35
Algal extract preparation..... 36
c. Determination of total phenolic compounds 36
d. Determination of total flavonoids content..... 37
e. Extraction and quantification of ginkgo flavone glycosides and ginkgolides..... 37
Statistical analysis 38
RESULTS AND DISCUSSION 39
1. Callus induction of *Ginkgo biloba* 39
a. Effect of medium type on callus fresh weight (CFW)..... 39
2. Measurement of *Ginkgo biloba* callus and cell suspension growth..... 43
3. Chemical composition of *Ginkgo biloba* fresh leaves and callus (g / 100 g dw) 44
4. Effects of different types of elicitors (*Anabaena Sp*., *Spirulina Sp*., Nitric oxide) with different concentrations on cell suspension cultures growth and metabolite accumulation of *Ginkgo biloba* culture cells 45
a. Effects of *Anabaena Sp*. elicitor at (25, 50, 100and 150 ppm) on cell suspension cultures growth and metabolite accumulation of *Ginkgo biloba* culture cells..... 45
b. Effects of *Spirulina Sp*. elicitor at (25, 50, 100and 150 ppm) on cell suspension cultures growth and metabolite accumulation of *Ginkgo biloba* culture cells..... 52