

SERIKAT SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
CAMPUS SCIENTIA ET TECNICA

NOR ROHAYA BINTI ABDUL RAHMAN

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
2007

CH: 1636

1100051155 Perpustakaan Sultanah Nur Zahirah (UMT)
Universiti Malaysia Terengganu

LP 42 FST 2 2007



1100051155

Effects of salinity on a-Tocopherol, ascorbic acid and carotenoid contents of *Cryptocoryne elliptica* cultures / Nor Rohana Abdul Rahim.



PERPUSTAKAAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1100051315

1000E

4200081158

Figure 1. A schematic diagram of the experimental setup. The sample was placed in a glass tube and positioned in the center of the magnetic field. The magnetic field was generated by a superconducting magnet.

1100051155

Lihat sebelah

HAK MILIK
PERPUSTAKAAN UMT

EFFECT OF SALINITY ON THE α -TOCOPHEROL, ASCORBATE AND
CAROTENOID CONTENT OF *Cryptocoryne elliptica* CULTURES

By

Nor Rohana Binti Abdul Rahim

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
UNIVERSITI MALAYSIA TERENGGANU
2007

1100051155

This project should be cited as:

Nor Rohana, A.R. 2007. Effect of Salinity on the α -Tocopherol, Ascorbic Acid and Carotenoid Content of *Cryptocoryne elliptica* Cultures. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu. 57p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in form of phonographic recording, nor may be it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor (s) of the project.



JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: EFFECT OF SALINITY ON THE α -TOCOPHEROL, ASCORBATE AND CAROTENOID CONTENT OF *Cryptocoryne elliptica* CULTURES oleh Nor Rohana Binti Abdul Rahim, no. matrik: UK10642 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

Penyelia Utama / Main Supervisor

NORHAYATI BINTI YUSUF
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 6/5/07

Penyelia Kedua (jika ada) / Co-Supervisor (if applicable)

Nama:

DR. AZIZ AHMAD
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh:

Ketua Jabatan Sains Biologi /Head, Department of Biological Sciences

Nama:

DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 6/5/2007

ACKNOWLEDGEMENT

Firstly, I would like to express my very gratefulness to Allah SWT for His blessing; my final year project was successfully completed due to the time given.

Special thanks are extended to Puan Norhayati Binti Yusuf my beloved supervisor, for all her taught on my project since the project was started till the end of my thesis writing. Her brilliant idea and supervised also her helpfulness made my project and the thesis writing out of my expectations. Not forgotten to Dr. Aziz Bin Ahmad as my co-supervisor for his support on my project.

I would like to take this opportunity to convey my special appreciation to Dr. Chuah Tse Seng from Fakulti Agroteknologi dan Sains Makanan (FASM), lab assistants from Biotechnology Lab, Biochemistry Lab and Microbiology Lab and also all my friends for their commitment and guidance in helping me to complete my project.

Lastly, my special thanks also go to my father and my family for their support and encouragement in completing my final year project.

TABLE OF CONTENTS

	Pages
ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vi
LIST OF APPENDICES	vii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Importance of Study	3
1.3 Objective of Study	3
 CHAPTER 2 LITERATURE REVIEW	4
2.1 <i>Cryptocoryne elliptica</i>	4
2.2 Salinity Stress	6
2.3 Production of Reactive Oxygen Species (ROS)	8
2.4 Antioxidative Defense Mechanism	11
2.4.1 α -Tocopherol	12
2.4.2 Ascorbic Acid	15
2.4.3 Carotenoid	16
 CHAPTER 3 MATERIALS AND METHODS	19
3.1 Plant Materials	20
3.2 Preparation of Culture Medium	20
3.3 Preparation of Treatment Medium	20
3.4 Antioxidant Assays	
3.4.1 Determination of α -Tocopherol	20
3.4.2 Determination of Ascorbic Acid	21
3.4.3 Determination of Carotenoid	22
3.5 Statistical Analysis	22
 CHAPTER 4 RESULTS	23
4.1 The Effects on α -Tocopherol	23

4.2	The Effects on Ascorbic Acid	23
4.3	The Effects on Carotenoid	25
CHAPTER 5 DISCUSSION		27
CHAPTER 6 CONCLUSION AND RECOMMENDATION		31
REFERENCES		32
APPENDICES		38
CURRICULUM VITAE		57

LIST OF FIGURES

Figure		Page
2.1	<i>C.elliptica</i>	4
4.1	Effects of NaCl stress on α -tocopherol concentration ($\mu\text{g/g fwt}$) in <i>C.elliptica</i> cultures.	24
4.1	Effects of NaCl stress on ascorbic acid concentration ($\mu\text{g/g fwt}$) in <i>C.elliptica</i> cultures.	24
4.2	Effects of NaCl stress on carotenoid concentration (mg/g fwt) in <i>C.elliptica</i> cultures.	26

LIST OF ABBREVIATIONS

ATP	-	adenine triphosphate
APX	-	ascorbate peroxidase
BAP	-	benzylaminopurine
CO ₂	-	carbon dioxide
°C	-	degree of celcius
DNA	-	deoxyribonucleic acid
fwt	-	fresh weight
g/L	-	gram per liter
HCl	-	hydrochloric acid
H ₂ O ₂	-	hydrogen peroxide
OH ⁻	-	hydroxyl radical
µg/ml	-	microgram per liter
µl	-	microliter
mg/L	-	milligram per liter
mM	-	millimolar
NaOH	-	natrium hydroxide
O ₂	-	oxygen
%	-	percentage
PSII	-	photosystem II
ROS	-	reactive oxygen species
rpm	-	revolution per minute
¹ O ₂	-	singlet oxygen
NaCl	-	sodium chloride
SE	-	standard error
SOD	-	superoxide dismutase
O ₂ ⁻	-	superoxide radical

LIST OF APPENDICES

Appendix		Page
1	Media preparation of B5 media (Gamborg <i>et al.</i> , 1968)	38
2	Ingredients of B5 media (Gamborg <i>et al.</i> , 1968)	39
3	Standard curve	40
4	Effects of NaCl stress on non-enzymatic antioxidant concentrations in <i>C.elliptica</i> cultures	41
5	Anova 1-Way and Tukey Test for α -tocopherol (Day 1)	42
6	Anova 1-Way and Tukey Test for α -tocopherol (Day 2)	43
7	Anova 1-Way and Tukey Test for α -tocopherol (Day 7)	44
8	Anova 1-Way and Tukey Test for α -tocopherol (Day 14)	45
9	Anova 1-Way and Tukey Test for α -tocopherol (Day 28)	46
10	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 1)	47
11	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 2)	48
12	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 7)	49
13	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 14)	50
14	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 28)	51
15	Anova 1-Way and Tukey Test for Carotenoid (Day 1)	52
16	Anova 1-Way and Tukey Test for Carotenoid (Day 2)	53
17	Anova 1-Way and Tukey Test for Carotenoid (Day 7)	54
18	Anova 1-Way and Tukey Test for Carotenoid (Day 14)	55
19	Anova 1-Way and Tukey Test for Carotenoid (Day 28)	56

ABSTRACT

Tolerability of *Cryptocoryne elliptica* to salt stress was observed through the non-enzymatic antioxidants concentration produce so that it can be propagate to maintain its species. The objective of this experiment was to investigate the effect of different concentrations of NaCl on non-enzymatic antioxidant (α -tocopherol, ascorbic acid and carotenoid) content in *C. elliptica* cultures, an aquatic plant (freshwater plant). *C. elliptica* cultures were treated with 0, 25, 50 and 100mM NaCl for 28 days in B5 solid medium. α -Tocopherol, ascorbic acid and carotenoid concentrations were measured at 0, 1, 2, 7, 14 and 28 days of treatment periods. No significant differences ($p>0.05$) were observed in α -tocopherol concentration in treated and controls up to 2 days of treatment. After 2 days treatment, α -tocopherol concentration increased significantly ($p<0.05$) especially in cultures treated with 25 and 50mM NaCl. However, no significant differences ($p>0.05$) were observed in plant treated with 25 and 50mM of NaCl. Longer treatment periods significantly ($p<0.05$) lowered the α -tocopherol concentrations. Ascorbic acid concentrations were increased significantly ($p<0.05$) after 1 to 7 days of treatment with NaCl. Higher concentration of NaCl led to greater increase of ascorbic acid concentration. Longer treatment period significantly ($p<0.05$) decreased the ascorbic acid concentrations except for plant treated with 50mM NaCl where the ascorbic acid concentrations remained constant. NaCl treatments reduced the carotenoid concentrations. Higher NaCl concentration significantly ($p<0.05$) lowered the carotenoid concentration. Results indicated that α -tocopherol, ascorbic acid and carotenoid contents were affected differently in *C. elliptica* cultures when treated with NaCl.

KESAN SALINITI TERHADAP KANDUNGAN α -TOKOFEROL, ASID ASKORBIK DAN KAROTENOID DALAM KULTUR *Cryptocoryne elliptica*

ABSTRAK

Rentan *Cryptocoryne elliptica* terhadap kemasinan dikesan melalui kepekatan antioksidan bukan enzim yang dihasilkan supaya ia boleh dibiakkan untuk mengekalkan spesies ini. Objektif eksperimen ini ialah untuk mengkaji kesan kepekatan NaCl yang berlainan terhadap kandungan antioksidan bukan enzim (α -tokoferol, asid askorbik dan karotenoid) di dalam kultur *C. elliptica* sejenis tumbuhan akuatik (tumbuhan air tawar). Kultur *C. elliptica* dirawat dengan 0, 25, 50 dan 100mM NaCl selama 28 hari dalam media pepejal B5. Kandungan α -tokoferol, asid askorbik dan karotenoid diukur pada setiap 0, 1, 2, 7, 14 dan 28 hari masa rawatan. Tiada perbezaan bererti ($p>0.05$) diperhatikan dalam kepekatan α -tokoferol pada rawatan dan kawalan sehingga 2 hari rawatan. Selepas 2 hari rawatan, kepekatan α -tokoferol meningkat dengan signifikan ($p<0.05$) terutamanya dalam kultur yang dirawat dengan 25 dan 50mM NaCl. Walau bagaimanapun, tiada perbezaan bererti ($p>0.05$) diperhatikan dalam tumbuhan yang dirawat dengan 25 dan 50mM NaCl. Peningkatan masa rawatan, menyebabkan pengurangan kepekatan α -tokoferol secara signifikan. Kepekatan asid askorbik meningkat dengan signifikan ($p<0.05$) selepas 1 hingga 7 hari rawatan dengan NaCl. Kepekatan NaCl yang tinggi meningkatkan kepekatan asid askorbik. Peningkatan masa rawatan menyebabkan penurunan secara signifikan ($p<0.05$) kepekatan asid askorbik kecuali pada tumbuhan yang dirawat dengan 50mM NaCl di mana kepekatan asid askorbik adalah tetap. Rawatan NaCl mengurangkan kepekatan karotenoid. Kepekatan NaCl yang tinggi menyebabkan penurunan secara signifikan ($p<0.05$) kepekatan karotenoid. Keputusan menunjukkan kandungan α -tokoferol, asid askorbik dan karotenoid memberi kesan yang berlainan di dalam kultur *C. elliptica* apabila diberi rawatan dengan NaCl.