

EFFECTS OF SALINITY ON THE  $\alpha$ -TOCOPHEROL,  
ASCORBIC ACID AND CAROTENOID CONCENTRATION IN  
*Cryptocoryne ciliata* CULTURES

NIK NADIAH RAMIZ ET YANZZ

FAKULTAS SAINS DAN TEKNOLOGI  
UNIVERSITI MALAYSIA TERENGGANU  
2007



EFFECTS OF SALINITY ON THE  $\alpha$ -TOCOPHEROL, ASCORBIC ACID AND  
CAROTENOID CONCENTRATION IN *Cryptocoryne ciliata* CULTURES

By

Nik Nadiyah Ramiz binti Yaaziz

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

1100051148

This project should be cited as:

Nik Nadiah Ramiz, Y. 2007. Effects of Salinity on the  $\alpha$ -Tocopherol, Ascorbic Acid and Carotenoid Concentration in *Cryptocoryne ciliata* cultures. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may 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**

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

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **EFFECTS OF SALINITY ON THE  $\alpha$ -TOCOPHEROL, ASCORBIC ACID AND CAROTENOID CONCENTRATION IN *Cryptocoryne ciliata* CULTURES** oleh **NIK NADIAH RAMIZ BINTI YAAZIZ**, no. matrik: **UK10603** telah diperiksa dan semua pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**, Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

Penyelia Utama / *Main Supervisor*  
Nama: **NORHAYATI BINTI YUSUF**  
Pensyarah  
Cop Rasmi: **Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu.**

Tarikh: 15/5/07

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

Nama: **DR. AZIZ AHMAD**  
Cop Rasmi: **Pensyarah  
Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu.**

Tarikh: 15/5/07

Ketua Jabatan Sains Biologi / *Head, Department of Biological Sciences*

Nama:  
Cop Rasmi: **DR. AZIZ BIN AHMAD**  
Ketua  
**Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu**

Tarikh: 15/5/2007

## ACKNOWLEDGEMENTS

I would like to express my gratefulness to Allah S.W.T. upon the successful completion of my thesis because without His will and help, I couldn't have done this far and go through this much.

My special thanks are extended to Puan Norhayati binti Yusuf as my supervisor. With her guidance and taught, I'm able to finish the lab works and submitted the thesis. I really appreciate her kindness and willingness to help me complete the work. Not forgotten Dr. Aziz Bin Ahmad as my co-supervisor, for his support and all the laboratory assistants of biochemistry and biotechnology labs.

My deepest gratitude to Dr. Noraznawati, as the coordinator for the final year project for her tolerance. This acknowledgement also goes to all my friends who helped me a lot to complete my thesis, Nor Rohana Abdul Rahim, Lim Wei Chun and Nur Aishah Ariffin. Thanks a lot for lending me a hand and for the moral support. I would like to take this opportunity to convey my appreciation to my parents and family for their encouragement.



## TABLE OF CONTENTS

	<b>Page</b>
<b>LIST OF FIGURES</b>	v
<b>LIST OF ABBREVIATIONS</b>	vi
<b>LIST OF APPENDICES</b>	vii
<b>ABSTRACT</b>	viii
<b>ABSTRAK</b>	ix
<b>CHAPTER 1 INTRODUCTION</b>	
1.1 Study Background	1
1.2 The Objective of Study	3
<b>CHAPTER 2 LITERATURE REVIEW</b>	
2.1 <i>Cryptocoryne</i>	4
2.1.1 <i>Cryptocoryne ciliata</i>	4
2.2 Salt Tolerance and Salinity Effects On Plants	6
2.3 Oxidative Stress In Plants	7
2.4 Plant Defense Mechanisme	9
2.4.1 $\alpha$ -Tocopherol (Vitamin E)	9
2.4.2 Ascorbic acid (Vitamin C)	10
2.4.3 Carotenoid	11
<b>CHAPTER 3 METHODOLOGY</b>	
3.1 Plant Materials	13
3.2 Preparation of Culture Medium	13
3.3 Preparation of Treatment Medium	13
3.4 Determination of Antioxidants	
3.4.1 Determination of $\alpha$ -Tocopherol	14
3.4.2 Determination of Ascorbic Acid	14
3.4.3 Determination of Carotenoid	15
3.5 Statistical Analysis	15
<b>CHAPTER 4 RESULT</b>	
4.1 The Effects on $\alpha$ -Tocopherol	16

4.2	The Effects on Ascorbic Acid	17
4.3	The Effects on Carotenoid	17
<b>CHAPTER 5 DISCUSSION</b>		20
<b>CHAPTER 6 CONCLUSION AND RECOMMENDATION</b>		24
<b>REFERENCES</b>		25
<b>APPENDICES</b>		32
<b>CURRICULUM VITAE</b>		64



## LIST OF FIGURES

Figure		Page
2.1	<i>C. ciliata</i> as ornamental plants in aquarium.	5
2.2	<i>C. ciliata</i> in natural habitat.	5
2.3	<i>In vitro</i> cultures of <i>C. ciliata</i>	5
2.4	A close up of <i>C. ciliata</i> cultures.	5
4.1	Effects of NaCl stress on $\alpha$ -tocopherol concentration (mg/g fwt) in <i>C. ciliata</i> cultures.	18
4.2	Effects of NaCl stress on ascorbic acid concentration ( $\mu$ g/g fwt) in <i>C. ciliata</i> cultures.	18
4.3	Effects of NaCl stress on carotenoid concentration ( $\mu$ g/g fwt) in <i>C. ciliata</i> cultures.	19

## LIST OF ABBREVIATIONS

APX	-	ascorbate peroxidase
BAP	-	benzylaminopurine
CAT	-	catalase
fw	-	fresh weight
GR	-	glutathione reductase
g/L	-	gram per liter
HCl	-	hydrochloric acid
H <sub>2</sub> O <sub>2</sub>	-	hydrogen peroxide
M	-	molar
mg/l	-	milligram per liter
ml	-	milliliter
mM	-	millimolar
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
nm	-	nanometer
OH <sup>·</sup>	-	hydroxyl radical
O <sub>2</sub> <sup>·-</sup>	-	superoxide radical
O <sub>2</sub>	-	oxygen
PSII	-	photosystem II
ROS	-	reactive oxygen species
rpm	-	revolution per minute
SE	-	standard error
SOD	-	superoxide dismutase
v/v	-	volume per volume
<sup>1</sup> O <sub>2</sub>	-	singlet oxygen
°C	-	degree celcius
μl	-	microliter
μg/ml	-	microgram per liter

## LIST OF APPENDICES

Appendix		Page
1	Standard curve for $\alpha$ -tocopherol	32
2	Standard curve for ascorbic acid	32
3	Effects of NaCl stress on $\alpha$ -tocopherol concentrations (mg/g fwt) in <i>C. ciliata</i> cultures	33
4	Effects of NaCl stress on ascorbic acid concentrations ( $\mu$ g/g fwt) in <i>C. ciliata</i> cultures	33
5	Effects of NaCl stress on carotenoid concentrations ( $\mu$ g/g fwt) in <i>C. ciliata</i> cultures	33
8	Anova 1-Way and Tukey Test for $\alpha$ -tocopherol (Day 1)	34
9	Anova 1-Way and Tukey Test for $\alpha$ -tocopherol (Day 2)	36
10	Anova 1-Way and Tukey Test for $\alpha$ -tocopherol (Day 7)	38
11	Anova 1-Way and Tukey Test for $\alpha$ -tocopherol (Day 14)	40
12	Anova 1-Way and Tukey Test for $\alpha$ -tocopherol (Day 28)	42
13	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 1)	44
14	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 2)	46
15	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 7)	48
16	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 14)	50
17	Anova 1-Way and Tukey Test for Ascorbic Acid (Day 28)	52
18	Anova 1-Way and Tukey Test for Carotenoid (Day 1)	54
19	Anova 1-Way and Tukey Test for Carotenoid (Day 2)	56
20	Anova 1-Way and Tukey Test for Carotenoid (Day 7)	58
21	Anova 1-Way and Tukey Test for Carotenoid (Day 14)	60
22	Anova 1-Way and Tukey Test for Carotenoid (Day 28)	62

## ABSTRACT

Salinity induces wide range of responses in plants. Since the *Cryptocoryne ciliata* can tolerate salinity by using its defense mechanism, the resistance level of this plant was studied in order to see how far they can survive with salinity. Thus, the resistant type of this plant might be produced. The objective of this experiment was to investigate the effects of different NaCl concentrations on the  $\alpha$ -tocopherol, ascorbic acid and carotenoid concentration in *C. ciliata* cultures. *C. ciliata* cultures were treated with 0, 25, 50 and 100 mM of NaCl for 28 days in solid MS medium. All the antioxidant levels were measured every 0, 1, 2, 7, 14 and 28 days of treatment periods. The NaCl initially decreased the  $\alpha$ -tocopherol concentration up to 2 days of treatment and longer incubation period tend to increase the  $\alpha$ -tocopherol concentration and decreased afterwards. At the earlier stage of treatment, the ascorbic acid and carotenoid concentration decreased up to day 1 of treatment and longer treatment period increased the ascorbic acid concentration until day 2 of treatment period and slowly decreased at the later stages of treatment. Similar result was obtained in carotenoid concentration except that the carotenoid concentration increased at the later stage of treatment period. Results indicated all treatments induced the oxidative stress in *C. ciliata* cultures where  $\alpha$ -tocopherol, ascorbic acid and carotenoid concentrations were affected differently in *C. ciliata* cultures.

**KESAN SALINITI KE ATAS KEPEKATAN  $\alpha$ -TOKOFEROL, ASID  
ASKORBIK DAN KAROTENOID DALAM KULTUR *Cryptocoryne ciliata***

**ABSTRAK**

Saliniti dapat mempengaruhi pelbagai tindakbalas di dalam tumbuhan. Memandangkan *Cryptocoryne ciliata* adalah rintang terhadap saliniti, tahap kerintangannya dikaji untuk melihat sejauh mana ia mampu menghadapi keadaan kemasinan. Oleh itu, pokok yang rintang terhadap saliniti mungkin dapat dibiakkan. Objektif eksperimen ini adalah untuk mengkaji kesan kepekatan NaCl yang berlainan terhadap kepekatan  $\alpha$ -tokoferol, asid askorbik dan karotenoid di dalam kultur *C. ciliata*. *C. ciliata* dirawat dengan 0, 25, 50 dan 100mM NaCl selama 28 hari. Semua aras antioksidan diukur pada setiap 0, 1, 2, 7, 14, dan 28 hari masa rawatan di dalam media pepejal MS. Rawatan NaCl pada awalnya merendahkan kepekatan  $\alpha$ -tokoferol sehingga hari ke-2 rawatan dan tempoh rawatan yang lebih panjang meningkatkan kepekatan  $\alpha$ -tokoferol dan merendahkannya kembali selepas itu. Pada peringkat awal eksperimen, kepekatan asid askorbik dan karotenoid menurun sehingga hari pertama rawatan dan masa rawatan yang lebih lama meninggikan kepekatan asid askorbik dan seterusnya merendahkannya secara perlahan-lahan di akhir tempoh rawatan. Keputusan yang sama juga diperoleh bagi kepekatan karotenoid kecuali di akhir masa rawatan, di mana kepekatan karotenoid telah meningkat. Keputusan menunjukkan bahawa rawatan saliniti telah merangsang tegasan oksidatif di dalam kultur *C. ciliata* di mana kepekatan  $\alpha$ -tokoferol, asid askorbik dan karotenoid memberi kesan yang berlainan di dalam kultur *C. ciliata*.