

## TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS</b>	<b>II</b>
<b>ABSTRACT</b>	<b>III</b>
<b>ABSTRAK</b>	<b>V</b>
<b>LIST OF TABLES</b>	<b>VII</b>
<b>LIST OF FIGURES</b>	<b>VIII</b>
<b>LIST OF PLATES</b>	<b>IX</b>
<b>LIST OF APPENDICES</b>	<b>XI</b>
<b>CHAPTER 1 : INTRODUCTION</b>	
1.1 : Importance of Seaweeds.....	1
1.2 : Economic Importance of Agar and <i>Gracilaria</i> .....	3
1.3 : Tissue and Protoplast Cultures.....	5
1.4 : Significance of Research.....	6
1.5 : Aims and Objectives of Research.....	7
<b>CHAPTER 2 : LITERATURE REVIEW</b>	
2.1 : Rhodophyta – The Red Algae.....	8
2.2 : <i>Gracilaria changii</i> Abbott, Zhang and Xia.....	8
2.3 : Conventional Culture Techniques for <i>Gracilaria</i> species..	9
2.4 : Important Factors Controlling Tissue Culture.....	10
2.4.1 : Surface Sterilisation of Tissue.....	10
2.4.2 : Callus Formation and Frond Regeneration.....	12
2.4.3 : Media and Growth Substances.....	13
2.4.4 : Growth and Maintenance of Culture.....	16
2.5 : Tissue Culture of Seaweeds.....	17

2.5.1 :	Tissue Culture of <i>Gracilaria</i> .....	19
2.6 :	Important Factors Controlling Protoplast Culture.....	20
2.6.1 :	Structure and Organisation of Marine Algal Cell Wall....	20
2.6.2 :	Cell Wall-degrading Enzymes.....	21
2.6.3 :	Source of Protoplasts.....	24
2.6.4 :	Incubation Conditions During Protoplast Isolation.....	24
2.7 :	Protoplast Culture of Seaweeds.....	26
2.7.1 :	Protoplast Culture of <i>Gracilaria</i> species.....	29

**CHAPTER 3 : MATERIALS AND METHODS**

3.1 :	<i>Gracilaria changii</i> Abbott, Zhang and Xia .....	31
3.2 :	Collection of <i>Gracilaria</i> .....	33
3.2.1 :	Maintenance of <i>Gracilaria changii</i> in the Laboratory...	33
3.3 :	Sterilisation Techniques.....	34
3.3.1 :	Treatment 1.....	34
3.3.2 :	Treatment 2.....	34
3.3.3 :	Treatment 3.....	34
3.3.4 :	Treatment 4.....	35
3.3.5 :	Treatment 5.....	35
3.3.6 :	Treatment 6.....	35
3.3.7 :	Sterility Test.....	36
3.4 :	Methods of Tissue Culture.....	37
3.4.1 :	Source and Preparation of Sterile Seawater.....	38
3.4.2 :	Tissue Culture on Solid MS and PES Media.....	39
3.4.2.1 :	Murashige and Skoog Basal Salt Medium (MS) .....	40
3.4.2.2 :	MS Basal Medium with Added Vitamins.....	41

3.4.2.3 :	Provasoli's Enriched Seawater Medium (PES).....	41
3.4.3 :	Effect of Solid MS and PES media with Growth Substances.....	42
3.4.4 :	Tissue Culture in Semi-solid MS and PES Media.....	43
3.4.4.1 :	Semi-solid Media with Growth Substances.....	43
3.4.5 :	Liquid MS and PES Media.....	44
3.4.5.1 :	Liquid Media with Growth Substances.....	45
3.5 :	Methods of Protoplast Culture.....	45
3.5.1 :	Determination of Mannitol (osmoticum) Concentration.....	45
3.5.2 :	Protoplast Isolation.....	46
3.5.3 :	Determination of the Effect of Individual Enzyme Concentration and Incubation Time on the Amount of Protoplast Isolated.....	46
3.5.4 :	Effect of a Combination of Enzymes on the Amount of Protoplast Isolated at Various Times of Incubation.....	47

**CHAPTER 4 : RESULTS**

4.1 :	Sterilisation of Explants.....	49
4.2 :	Tissue Culture of <i>Gracilaria changii</i> Abbott, Zhang and Xia.....	52
4.2.1 :	Solid Media.....	52
4.2.2 :	Semi-solid Media.....	54
4.2.3 :	Liquid Media.....	56
4.2.4 :	Effects of Liquid Media with Growth Substances.....	57
4.3 :	Determination of Optimal Mannitol (osmoticum) Concentration.....	63
4.4 :	Protoplast Isolation Using Single Enzyme and Combination of Enzymes.....	66

<b>CHAPTER 5 :</b>	<b>DISCUSSION</b>	
5.1 :	Sterilisation of <i>Gracilaria changii</i> Explants.....	72
5.2 :	Tissue Culture of <i>Gracilaria changii</i> Abbott, Zhang and Xia.....	74
5.2.1 :	Effects of the forms of Culture Medium.....	74
5.2.2 :	Branching or Shoot Formation.....	76
5.2.3 :	Regeneration of Callus-like Structures (CLS).....	78
5.3 :	Optimal Mannitol Concentration.....	82
5.4 :	Protoplast Isolation Using Single and Combination of Enzymes.....	83
<b>CHAPTER 6 :</b>	<b>CONCLUSION</b> .....	87
<b>BIBLIOGRAPHY</b> .....		91
<b>APPENDICES</b> .....		100