

C O N T E N T S

	PAGE No.
CHAPTER I. <u>INTRODUCTION</u>	1
CHAPTER II. <u>MATERIALS AND METHODS</u>	20
1. Plant Material	20
2. Culture Media	21
A) Preparation of the media	
B) Culture vessels	
C) Sterilization of media and culture vessels	
3. Aseptic Techniques	25
4. Culture Techniques	25
A) Initiation of seedling cultures	
B) Stock cultures	
5. Measurements of Growth	27
A) Fresh weights	
B) Dry weights	
6. Chemical Analysis	28
A) Extraction and estimation of phenolic compounds	
7. Enzyme Assays	29
A) Peroxidase	
B) Indoleacetic acid oxidase (IAA oxidase)	

C)	Ammonia-lyases : Phenylalanine ammonia-lyase and tyrosine ammonia-lyase	
D)	p-Coumarate : CoA ligase (Cinnamyl CoA synthetase)	
E)	Transaminases : phenylalanine transaminase and tyrosine transaminase	
8.	Polyacrylamide Disc Gel Electrophoresis	37
9.	Molecular Weight Estimation by Gel Filtration	38
10.	PAL subunits	39
11.	Determination of Protein	40
12.	Photomicrography	40
CHAPTER III.	<u>RESULTS</u>	41
A.	<u>CHANGES IN GROWTH, POLYPHENOLS AND THE DEVELOPMENT OF PEROXIDASE, IAA OXIDASE, PAL AND TAL ENZYMES DURING GERMINATION IN CROTALARIA</u>	43
B.	<u>INITIATION AND GROWTH OF SEEDLING CALLUS OF CROTALARIA</u>	55
1.	Initiation of Callus and Suspension cultures	55
2.	Effect of Hormones on Callus Growth	56
3.	Growth curve of Crotalaria Callus and Suspension Cultures	61

C.	<u>NUTRITIONAL STUDIES ON GROWTH AND POLYPHENOL ACCUMULATION IN CROTALARIA CALLUS CULTURES</u>	66
	1. Effect of Different Sugars on Growth and Polyphenol Production in <u>Crotalaria</u> Callus Cultures	67
	2. Effect of Nitrogen Sources on Growth and Polyphenol Accumulation in Callus Cultures of <u>Crotalaria</u>	70
	A) Effect of different levels of nitrogen	
	B) Inorganic nitrogen sources	
	C) Inorganic nitrogen sources in different combinations	
	D) Organic nitrogen sources	
	E) Effect of different concentrations of urea.	
	3. Effect of L-Phenylalanine and L-Tyrosine on Growth and Polyphenol Synthesis in Callus Cultures of <u>Crotalaria</u>	84
	4. Effect of Phenolic acids on Growth and Polyphenol Production in <u>Crotalaria</u> Callus Cultures	87
D.	<u>PROGRESSIVE CHANGES IN GROWTH, ACCUMULATION OF PHENOLIC MATERIALS AND THE DEVELOPMENT OF RELATED ENZYME ACTIVITIES UNDER DIFFERENT CULTURAL CONDITIONS</u>	95
	1. Effect of Sucrose Concentrations on Growth, Polyphenol Synthesis and the Development of Peroxidase, IAA Oxidase, PAL and TAL Enzyme Activities in <u>Crotalaria</u> Callus Cultures	96

2.	2,4-D Concentration Effect on Growth, Polyphenol Production and the Development of Peroxidase, IAA Oxidase, PAL and TAL Activities in Presence and Absence of Light	103
3.	NAA Effect on Growth, Polyphenol Synthesis and the Development of Peroxidase, IAA Oxidase, PAL and TAL in Presence and Absence of Light	115
4.	GA ₃ Effect on Growth, Polyphenol Synthesis and the Development of Peroxidase, IAA Oxidase, PAL and TAL Activities in Presence and Absence of Light	126
5.	Effect of Kinetin on Growth, Polyphenol Accumulation and the Development of Peroxidase, IAA Oxidase, PAL and TAL Activities	137
6.	Effect of Cycloheximide on Growth, Polyphenol Synthesis and the Development of Peroxidase, IAA Oxidase, PAL and TAL Activities	143
7.	Changes in p-Coumaryl:CoA Ligase, Phenylalanine Transaminase and Tyrosine Transaminase During the Course of Growth Cycle	150
E.	<u>IN VIVO AND IN VITRO L-PHENYLALANINE AMMONIA-LYASE FROM CROTALARIA JUNCEA L. : PURIFICATION, SUBUNIT STRUCTURE AND KINETIC PROPERTIES</u>	158

1. Purification of Enzyme	159
2. General Properties	164
3. Substrate Specificity	164
4. Effect of Carbonyl Reagents	165
5. Effect of Sulphydryl Reagents	165
6. Effect of Aromatic Compounds	169
7. Requirements for Enzyme Assay	169
8. Kinetic Analysis of PAL	171
9. Molecular Weight and Subunit Structure	172
CHAPTER IV. <u>GENERAL DISCUSSION</u>	175
<u>SUMMARY</u>	226
<u>BIBLIOGRAPHY</u>	236
