

## CONTENTS

1. <b>Introduction</b>	1-6
2. <b>Literature Review</b>	7-58
3. <b>Materials and methods</b>	59-84
3.1. Plant material	
3.1.1. Collection	59
3.1.2. Propagation	59
3.1.3. Plantation	59
3.1.4. Maintenance	59
3.1.5. Selection of genotypes	62
3.2. Seed treatment	
3.2.1. Chemical treatment of seeds	62
3.2.2. Seed bacterization	62
3.3. Temperature treatment	
3.3.1. Seeds	64
3.3.2. Seedlings	64
3.4. Heat- acclimation treatment	64
3.5. Foliar application of chemicals	
3.5.1. Salicylic acid (SA)	64
3.5.2. Abscisic acid (ABA)	65
3.5.3. Calcium chloride (CaCl <sub>2</sub> )	65
3.6. Tolerance index (TI) determination	65
3.7. Determination of plant growth promoting activity of <i>Bacillus megaterium</i>	65
3.8. Protein analysis	66
3.8.1. Extraction of soluble proteins	66
3.8.2. Estimation	66
3.8.3. SDS-PAGE analysis	67

3.8.3.1.	Preparation of stock solution	68
3.8.3.2.	Preparation of gel	69
3.8.3.3.	Sample preparation	69
3.8.3.4.	Electrophoresis	69
3.8.3.5.	Fixing and staining	69
3.9.	Extraction of enzymes from seedlings	70
3.9.1.	Peroxidase	70
3.9.2.	Ascorbate peroxidase	70
3.9.3.	Catalase	71
3.9.4.	Superoxide dismutase	71
3.9.5.	Glutathione reductase	71
3.10.	Assay of enzyme activities	
3.10.1.	Peroxidase	71
3.10.2.	Ascorbate peroxidase	71
3.10.3.	Catalase	72
3.10.4.	Superoxide dismutase	72
3.10.5.	Glutathione reductase	72
3.11.	Isozyme analysis by PAGE	73
3.11.1.	Peroxidase	75
3.11.2.	Catalase	76
3.12.	Extraction and quantification of non-enzymatic antioxidants	
3.12.1.	Ascorbate	76
3.12.2.	Carotenoids	76
3.13.	Determination of peroxidation of membrane lipids	77
3.14.	Determination of cell membrane thermostability	77
3.15.	Extraction and quantification of chlorophyll	77
3.16.	Determination of Hill activity	
3.16.1.	Extraction	78
3.16.2.	Assay of chloroplast activity by Hill reaction	78
3.17.	Extraction and estimation of free proline	
3.17.1.	Extraction	79

3.17.2. Estimation	79
3.18. Extraction and estimation of total and reducing sugar	
3.18.1. Extraction	79
3.18.2. Estimation	79
3.19. HPLC analysis of phenols from the seedlings	80
3.20. <i>In vitro</i> callus formation	81
3.21. Treatment of calli	81
<b>4. Experimental</b>	<b>85-157</b>
4.1. Screening of thermotolerant and susceptible genotypes	85
4.1.1. Tolerance index (TI)	85
4.1.2. Cell membrane thermostability	86
4.2. Analysis of the effects of temperature and pre-treatment on seed germination and seedling growth of <i>Cicer arietinum</i> and determination of lethal temperature	87
4.2.1. Lethal temperature determination	87
4.2.2. Seed germination	88
4.2.3. Seedling growth	96
4.3. Induction of thermotolerance in <i>Cicer arietinum</i> by pre-treatments	98
4.4. Effect of pre-treatments and elevated temperature treatments of seeds and seedlings on proteins	99
4.4.1. Protein content	99
4.4.2. Protein profile	108
4.5. Studies on antioxidative enzymes in pre-treated seedlings of chickpea following lethal temperature treatment	114
4.5.1. Changes in enzymatic activity	114
4.5.1.1. Peroxidase	114
4.5.1.2. Ascorbate peroxidase	116
4.5.1.3. Catalase	122
4.5.1.4. Superoxide dismutase	124

4.5.1.5.	Glutathione reductase	124
4.5.2.	Changes in isozyme profile	129
4.5.2.1.	Peroxidase	129
4.5.2.2.	Catalase	129
4.6.	Variations in levels of non-enzymatic antioxidants as a consequence of lethal temperature exposure	132
4.6.1.	Ascorbate	132
4.6.2.	Carotenoids	133
4.7.	Changes in lipid peroxidation of membranes following lethal temperature treatment	136
4.8.	Effect of elevated temperatures and pre-treatments on cell membrane thermostability	139
4.9.	Effect of pre-treatment of seedlings and lethal temperature treatment on chlorophylls and Hill activity	139
4.9.1.	Chlorophyll content	139
4.9.2.	Hill activity	139
4.10.	Changes in free proline in pre-treated seedlings of <i>Cicer arietinum</i> in response to lethal temperature treatment	144
4.11.	Effect of pre-treatment of seedlings and temperature treatment on carbohydrates of chickpea	145
4.11.1.	Total sugar	145
4.11.2.	Reducing sugar	149
4.12.	Changes in phenolic profile following pre-treatments and exposure to lethal temperature	149
4.13.	Analysis of the effect of elevated temperatures and pre-treatments on growth of calli <i>in vitro</i>	154
4.13.1.	Elevated temperature treatment	154
4.13.2.	Pre-treatments	156
5.	<b>Discussion</b>	158-171
6.	<b>Summary</b>	172-176
7.	<b>References</b>	177-198