
List of Contents

S. No.	Title	Page No.
1.	List of abbreviation	i
2.	List of tables	iv
3.	List of figure	v
4.	Abstract	1
5.	Chapter 01 Introduction	3
1.1	Types of Stresses	3
1.2	Different types of abiotic stresses and their effects	3
1.2.1	Heat stress	3
1.2.2	Cold stress	5
1.2.3	Metal stress	5
1.2.4	Salt stress	6
1.2.5	Drought stress	7
1.3	Major effects of abiotic stresses	7
1.3.1	Effect of abiotic stresses on plant physiology	8
1.3.2	Effect on gene expression	9
1.4	Heat shock proteins (HSPs)	9
1.4.1	Role of HSPs	9
1.4.2	Types of HSPs	10
1.4.2.1	High molecular weight heat shock proteins	10
1.4.2.1.1	HSP100	10
1.4.2.1.2	HSP90	11
1.4.2.1.3	HSP70	11
1.4.2.1.4	HSP60	12
1.4.2.2	Small heat shock proteins (sHSPs)	12
1.4.3	Chloroplast small heat shock proteins (Cp-sHSPs)	13
1.4.3.1	Role of chloroplast small heat shock proteins	14
1.5	HSP gene expression	15
1.6	HSP promoters	16

1.7	Cp-sHSP promoters	18
1.8	Aims and Objectives	20
5.	Chapter 02 Analysis of <i>C. album</i> Ca-sHSP promoters for presence of putative cis-regulatory elements	22
	Abstract	22
2.1	Introduction	23
2.1.1	Promoter region of heat shock genes	24
	Table 2.1 Types and sequences of the core motifs of <i>Arabidopsis thaliana</i>	27
2.2	Materials and Methods	29
2.2.1	Promoter sequences	29
2.2.2	Alignment of Cp-sHSPs promoters	29
2.2.3	Tree construction	29
2.2.4	Analyses for putative regulatory motifs	29
2.3	Results and discussion	31
	Figure 2.1. A consensus bootstraps parsimony tree based on promoter sequences	35
	Figure 2.2. Comparisons of putative promoter regions of <i>C. album</i> (US ecotypes) chloroplast small HSPs	36
	Figure 2.3. Complete promoter sequence of <i>C. album</i> Ca-sHSP gene, NY-1 showing all putative cis-regulating elements	37
	Figure 2.4. Complete promoter sequence of <i>C. album</i> Ca-sHSP gene, NY-2 showing all putative cis-regulating elements	38
	Figure 2.5. Complete promoter sequence of <i>C. album</i> Ca-sHSP gene, MS-1 showing all putative cis-regulating elements	39
	Figure 2.6. Complete promoter sequence of <i>C. album</i> Ca-sHSP gene, MS-2 showing all putative cis-regulating elements	40
	Table 2.2. Comparisons of the HSE core motifs in Cp-sHSP promoters isolated from both <i>C. album</i> ecotypes (US variety)	41
	Figure 2.7. Promoter sequence of a representative <i>C. album</i> Ca-sHSP gene and diagrammatical representation of <i>C. album</i> Ca-sHSP gene	42

6	Chapter 03	Dual role for <i>Chenopodium album</i> chloroplast small heat shock protein: photosystem II protection from heat and metal stresses	43
		Abstract	43
	3.1	Introduction	44
	3.2	Materials and Methods	48
	3.2.1	Plant materials, growth conditions and heat/metal stresses	48
	3.2.2	Physiological parameters	48
	3.2.2.1	Chlorophyll contents	48
	3.2.2.2	Superoxide dismutase (SOD)	48
	3.2.2.3	Peroxidase (POD)	49
	3.2.3	Photosystem II efficiency	49
	3.2.4	Statistical analysis	49
	3.2.5	Identification of Cp-sHSP genes	49
	3.2.6	PCR amplification of full length Cp-sHSPs	50
	3.2.7	RNA isolation and reverse transcriptase reaction	50
	3.2.7.1	Total RNA isolation	50
	3.2.7.2	First strand cDNA synthesis	51
	3.2.7.3	Reverse Transcriptase PCR (RT-PCR)	51
	3.2.8	Sequencing of Cp-SHSPs	51
	3.2.8.1	Purification of PCR product	51
	3.2.8.2	Cloning of PCR purified product	52
	3.2.8.3	Plasmid isolation	52
	3.2.9	Relative quantification of Cp-sHSP transcripts in heat and metal treated samples	53
	3.2.10	Cp-sHSP protein expression by Immunoblotting	53
	3.3	Results	54
	3.3.1	Effect of heat/metal stress on physiological parameters	54
	3.3.2	Effect of heat stress and cadmium on thermotolerance of Photosystem II	55
	3.3.3	Full-length Cp-sHSP gene sequencing	55

3.3.4	Heat/metal regulated expression of Cp-sHSP	56
3.4	Discussion	58
Figure 3.1.	Effect of high temperature and metals stress on total chlorophyll contents, peroxidase (POD), superoxide dismutase (SOD) and Cp-sHSP transcript of <i>C. album</i> leaves	62
Figure 3.2.	The effect of heat stress (left panel) and metal stress (right panel) on thermotolerance of Photosystem II efficiency and transcript levels	63
Figure 3.3.	Effect of high temperature (a) and metals stress (b) on Cp-sHSP transcript of <i>C. album</i> leaves	64
Figure 3.4.	Amino acid sequence alignment of CaHSP26.13p from heat & metal transcripts Pakistani ecotype (a) and with four genes of two US ecotypes	65
Figure 3.5:	Amino acid sequence alignment and phylogenetic relationship of CaHSP26.13p with other plants	66
Figure 3.6:	Effect of heat stress and metal stress on Cp-sHSP transcript and protein	67
7	Chapter 04 Molecular characterization of <i>Chenopodium album</i> chloroplast small heat shock protein and its expressions in response to different abiotic stresses	68
	Abstract	68
4.1	Introduction	69
4.2	Materials and Methods	73
4.2.1	Plant materials, growth conditions and heat/metal stresses	73
4.2.2	Physiological parameters	73
4.2.2.1	Chlorophyll contents	73
4.2.2.2	Superoxide dismutase (SOD)	74
4.2.2.3	Peroxidase (POD)	74
4.2.3	Photosystem II efficiency	74
4.2.4	Statistical analysis	74
4.2.5	Identification of Cp-sHSP genes	74

4.2.6 PCR amplification of full length Cp-sHSPs	75
4.2.7 RNA isolation and reverse transcriptase reaction	75
4.2.7.1 Total RNA isolation	75
4.2.7.2 First strand cDNA synthesis	75
4.2.7.3 Reverse Transcriptase PCR (RT-PCR)	76
4.2.8 Sequencing of Cp-SHSPs	76
4.2.8.1 Purification of PCR product	76
4.2.8.2 Cloning and sequencing of transcripts	76
4.2.9 Relative Quantification of Cp-sHSP transcripts in heat and metal treated samples	76
4.2.10 Cp-sHSP protein expression by Immunoblotting	77
4.3 Results	78
4.3.1 Effect of cold/drought/salt stress on physiological parameters	78
4.3.2 Effect of cold/drought/salt stress on thermotolerance of Photosystem II	78
4.3.3 Full-length Cp-sHSP gene sequencing	79
4.3.4 Cold/drought/salt regulated expression of Cp-sHSP	81
4.4 Discussion	84
Figure 4.1: Effect of low temperature, drought and salt stresses on total chlorophyll contents, peroxidase (POD), superoxide dismutase (SOD) and Cp-sHSP transcript of <i>C. album</i> leaves	89
Figure 4.2. The effect of cold, drought and salt stress on thermotolerance of Photosystem II efficiency and transcript levels	90
Figure 4.3: Amino acid sequence alignment of CaHSP26.13p from all five (heat, metal, cold, drought and salt) stresses	91
Figure 4.4: Amino acid sequence alignment and phylogenetic relationship of CaHSP26.13p with other plants	92
Figure 4.5: Effect of cold stress (a), drought stress (b) and salt stress (c) on Cp-sHSP transcript of <i>C. album</i> leaves	93
Figure 4.6: Effect of cold, drought and salt stress on Cp-sHSP transcript and protein	94

8	Conclusion	96
9	Future aspects	97
9	Chapter 5 References	98
10	Papers	
11	Originality Report	