Chapter

HSPs under Abiotic Stresses

Noor ul Haq and Samina N. Shakeel

Abstract

Different organisms respond to the altered environmental conditions by different ways. Heat shock proteins' (HSPs) production is one among the different defense mechanisms which defend the photosystem II and thylokoid membrane in plants. There are different types of HSPs based on their size, that is, high molecular weight (60–100 kDa) and low molecular weight heat shock proteins (15–30 kDa). Small HSPs are further classified based on their localization and role in different sub-cellular organelles. Cp-sHSPs are the chloroplast-specific small HSPs that protect the photosystem II and thylokoid membrane. A model to control the Cp-sHSPs in *Chenopodium album* has been put forward in this chapter. According to this model, Cp-sHSPs of *Chenopodium album* are created in cytoplasm and are moved toward chloroplast. The transit peptide is removed on reaching to the target sub-cellular organelle, that is, chloroplast and the premature Cp-sHSPs are converted into mature ones which have multiple roles under different abiotic stress conditions.

Keywords: plants HSPs, abiotic stresses, HSPs model, *Chenopodium album*

1. Introduction

Organisms respond to the changed growth conditions through heat shock proteins' (HSPs) production [1] and that is the way of survival for the cell which responds differentially [2]. Different environmental conditions including abiotic and biotic stress conditions influence the plants' development and production [3]. Different stress conditions like heat, salt, and low water conditions may majorly influence the plants' physiology and production [4–8], but plants response to the changed environmental conditions may vary depending upon duration, intensity, and combination of different environmental growth conditions [9]. Different processes in the plants including biochemistry, development, and physiology may affected by stress conditions and so the expression of different genes may be turned off or on in response to the changed environmental conditions, which may lead to the creation of different proteins and metabolites that protect the cells against such conditions [10].

2. Stress types

Stresses due to living and non-living things can affect the plants' development and production. Different organisms like viruses, bacteria, and fungi may cause stress conditions for the plants [8] which may activate different defense pathways of the plants [9]. There are reports that abiotic stress conditions are responsible to make mostly changes in plant biochemistry and physiology [10]. So plant growth may negatively be affected by abiotic stress conditions also known as non-living factors [6], and any kind of change in environmental conditions may lead the plants toward adaptation under altered growth conditions [11]. Below are the details of different abiotic stress conditions which may affect the plants.

2.1 Types of abiotic stresses and their effects on plants

2.1.1 High temperature or heat stress

Heat stress is the main factor among abiotic stress conditions that affects the plants yield [12] and so different factors in the plants like metabolite concentration, osmolytes, membrane fluidity, proteins structure, and nucleic acids are seriously changed by temperature [13]. Additionally, high-temperature stress affects the chloroplast photochemical activity [14]. Photosystem II is considered as the most sensitive part of thylokoid membrane [15] and heat stress conditions may influence the photosystem II (PS II) reaction center and the light harvesting complexes [16].

Plants adapt their system to the changed growth conditions through complex mechanisms [17]. Thus, different processes at cellular level are reprogrammed under high- and low-temperature growth conditions and many changes in transcription may happen in different parts of the plants, that is, seedlings, roots, pollens, and leaves [18, 19]. Effect on plants may vary with intensity and duration of temperature [20]. One of the plants responses is the reactive oxygen species (ROS) production which is increased by low- and high-temperature stress conditions, while oxidative damage and cell death have also been reported as a result of high-temperature stress conditions [21]. Photosynthesis inhibition has also been reported by researchers under high-temperature conditions [17], additional to the damage of the oxygen evolving complex (OEC) of photosystem II caused by heat stress [22].

Plants adapt to the high-temperature conditions through heat shock proteins (HSPs) production, which are found to be produced in all organisms from prokaryotes to eukaryotes and have role in cell protection under harsh conditions [2]. Establishment of defense mechanism under high-temperature growth conditions is necessary for cells survival which is not specifically occurred only under high temperature but it is also significant under different stress conditions [23].

2.1.2 Low temperature or cold stress

Low temperature represses the plants development without stopping the cell functions and may cause problems to different processes at cellular level [3]. Temperature is the main factor to control the growth changes from vegetative till reproductive level [24]. Low temperature may increase the ROS production additional to the reduction of cellular respiration [25] as well as damages the cell membrane [26].

Low-temperature stress conditions may reduce photosystem I and this effect has been reported to be increased under low light conditions [27]. The same effects have also been observed by different researchers in different plants like winter rye and barley [28, 29].

Expression of different genes and proteins has been reported to be up- or down-regulated by low-temperature stress conditions [30]. Researchers have reported the up-regulation of the defensive genes under cold stress [24]. For example, almost 300 genes have been reported to get up-regulated under cold stress conditions, while 88 genes (27%) were down-regulated in *Arabidopsis thaliana* [31].

2.1.3 Metal stress

Development of the plants is badly affected by heavy metals [32] and roots are usually damaged by heavy metals which lead to build up different defensive mechanisms for normal growth [33]. Membrane potential and permeability are changed by interactions of heavy metals with membrane components [32]. Plants take up the heavy metals as essential nutrients and are passed to the upper parts of the plants following the pathways of the essential elements transport [34].

Plants respond differentially to the heavy metal toxicity [35] and that is the reason that some plants do not show any phytotoxicity symptoms on heavy metals accumulation [36]. But heavy metals restrict the plants growth and cause cell death due to interruption in different physiological and biochemical pathways [37]. Different essential ions are replaced by heavy metals, for example, Ni replaces Mg ion that results in the changed activity of ribulose-1,5-biphosphate carboxylated oxygenase [38]. Chlorophyll activity is altered [39], while heavy metals break the disulfide bridges of the proteins, which leads to the destabilization of proteins [37]. Besides the formerly mentioned adverse roles in plants, heavy metals interact with the hydroxyl and carboxyl groups of proteins and thus interrupt in the proteins functions [40].

Plants adopt different defense mechanisms while get exposure to heavy metals. These mechanisms include the synthesis of cystein-rich polypeptides phytochelatins and metallothioneins [32]. Researchers have also reported the up-regulation of HSP70 gene and chaperonin 60 family members under different heavy metals, that is, Cd and Ni [41, 42]. Additional to the former HSP families, chloroplast small heat shock proteins (Cp-sHSPs) are also reported to be up-regulated by heavy metals [43].

2.1.4 Salt stress

Based on the response to salt stress, plants may be two types either glycophytes or halophytes. The former kind of plants has no tolerance to the saline environment, while the latter group plants covering are natively grown in saline environment [44]. Halophytes cover almost 1% of the world flora [45]. Salt stress adversely affects the plants growth and productivity by different ways; for example, sodium chloride salt can cause the ionic toxicity and osmotic stress to the plants [46]. Researchers have also reported the adverse effect of salt on growth and photosynthesis of the plants [47] by lowering the intra-cellular CO_2 availability [48] or by changed photosynthetic metabolism [49].

2.1.5 Drought stress

Crops yield and quality are adversely affected by drought conditions. Drought conditions may affect the macro- and micromolecules in a cell including minerals, lipids, proteins, hormones, carbohydrates, or even DNA or RNA [50]. The combination of drought with salt, high- or low-temperature stress conditions becomes more severe for the plants, which affects the plants' growth, development, and signal transduction [51, 52]. Besides the abovementioned macro−/micromolecules, photosynthesis that needs water is adversely affected by environmental stress conditions [53, 54]. Additional to the above, drought conditions may affect the metabolism of the plants because catabolism is enhanced due to hydrolytic enzymatic activity while anabolism is decreased due to lowering synthase activity [52]. In short, drought stress conditions adversely affect the photosynthesis in the chloroplast by decreased nutrient uptake and ion transport [55, 56].

2.2 Effect of stress conditions on gene expression

Stress conditions may activate the defense mechanism of the plants and result the change in different gene expression. The expression of heat shock proteins has been reported to be changed due to heat stress [57]. Heat shock proteins function as chaperones and safeguard the heat sensitive organelles and intra-cellular processes [2].

Proteins other than HSPs have also been reported to get produced and their expression is regulated differentially under heat stress conditions [58]. Besides the HSPs expression under heat stress conditions, these proteins have also been up-regulated under different stress conditions including heavy metal, cold, salt, drought, and oxidative stress conditions [43, 59–62].

3. Heat shock proteins (HSPs)

Heat shock response has been characterized in salivary glands of *Drosophila* [63]. Heat shock proteins have been studied in the result of transcription and translation in chromosomal puffs with active sites [64]. HSPs are produced in all organisms, that is, from bacteria to humans under changed environmental conditions [2] and have chaperone activity that protects the proteins from damage [65].

3.1 Role of heat shock proteins

Genes encoding HSPs respond to abiotic stress factors like high temperature, drought, salt, and low-temperature stress conditions [66]. HSPs having low expression under normal environmental conditions may have different function like chaperone function, prevention of proteins aggregation and folding, as well as to target the miss-folded proteins toward the specific pathways or for degradation [67]. Additional to the HSPs expression under abiotic stress conditions, these proteins have differential expression in different tissues and organelles. Taking all together, HSPs production is to protect the metabolic apparatus for adaptation under different environmental conditions and survival [68].

3.2 Types of HSPs

HSPs are divided into two classes based on their molecular weight, that is, high molecular weight heat shock proteins (HSP100, HSP90, HSP70, HSP60, and HSP40) and low molecular weight heat shock proteins (sHSPs), the weight of which is ranging from 15 to 30 kDa [69].

3.2.1 High molecular weight heat shock proteins

High molecular weight heat shock proteins are further divided into different classes based on molecular weight, that is, HSP100, HSP90, HSP70, and HSP60, the details of which are as below.

3.2.1.1 HSP100

HSP100 (protein family), found in all organisms from prokaryotes to eukaryotes [70], possess two subunits and are reported primarily in prokaryotes, that is, bacteria: (1) large-subunit (ClpA) which is ATP-dependent unfoldase and (2) protease which is a small-subunit ClpP [71]. Nucleotide-binding domain 1 & 2 (NBD1 & NBD2), carboxyl domains, middle domain, and amino and are the five parts of HSP100 proteins family members [72].

HSP100 genes have been reported to be up-regulated under heat stress conditions while the same pattern of expression has not been observed [73] but earlier than these findings, researchers have reported the expression of a member of HSP100 family under abscisic acid (ABA), cold and salt stresses additional to the high-temperature stress conditions [74]. Differential expression of one gene or this family member has been suggested under different abiotic stress conditions [75]. HSP100 family members have been reported with up-regulation under heat stress conditions in different plants like wheat and tobacco [75], rice [74], *Arabidopsis thaliana* [76], soybean [77] and maize [78]. Besides the above, HSP100 family members have also been reported with differential expression at different developmental stages [79] which may be the reason that HSP100 family members have been reported with high concentration in mature seeds of different plants [80].

3.2.1.2 HSP90

All organisms from prokaryotes to eukaryotes have HSP90 [81] and are involved to activate the component proteins involved in proteins transportation, assembling, folding and signal transduction [82]. Seven different isoforms of HSP90 have been identified in *Arabidopsis* and are classified based on sub-cellular localization, that is, three have been reported to be localized in endoplasmic reticulum, chloroplast, and mitochondria while the remaining four are localized in cytosole [83]. Three among the four cytosolic isoforms are expressed constitutively while fourth one is expressed under heat stress conditions [84].

3.2.1.3 HSP70

HSP70 are expressed under normal conditions in plants so these are also named as heat shock cognates [85]. HSP70 are having important role under different environmental conditions including heat stress [86, 87]. This class of proteins may function to stabilize the unstable proteins [82] additional to the proteins transport among sub-cellular compartments and proteins folding [88].

HSP70 family proteins may be classified into four classes based on the sub-cellular localization and thus are localized in four different compartments (cytosol, mitochondria, plastids, and endoplasmic reticulum) of the cell [89].

3.2.1.4 HSP60

HSP60 family members encoded by nuclear DNA [90] are present in prokaryotes to eukaryotes and have function in cells under stress and normal conditions [91]. Bacterial HSP60 plays role in proteins assembling to form complexes (oligomeric) and movement through cell membrane [91] but the same family proteins are involved in organelle (chloroplast and mitochondria)-specific proteins folding [91].

3.2.2 Small heat shock proteins (sHSPs)

Plants' small heat shock proteins having molecular weight from 15 to 30 kDa are encoded by nuclear DNA and are classified into further six classes based on sub-cellular localization [92]. Researchers have classified the abovementioned proteins as per the localization in different cellular organelles, that is, first two are localized in cytosol and the next three classes (III, IV, and V) are localized in endoplasmic reticulum, mitochondria, and plastids, respectively [93]. Additional to the above, class VI has been reported to be localized in endoplasmic reticulum [94].

C-terminal region, N-terminal region, and α-crystallin domain are the three main parts of small heat shock proteins. Small HSPs are characterized by 100 amino acids sequence having α-crystalline domain [95] as well as N-terminal region on one side and C-terminal region on the other side of the formerly mentioned domain [96]. The abovementioned three domains are the conserved regions of small heat shock proteins [97].

Small HSPs expression has been reported in different plants, for example, *Chenopodium album* [43, 62], carrot [98], sugarcane [99], Agave [100], *Arabidopsis* [101], cotton [102], tomato [18], maize [103], tobacco [104], etc. The abovementioned studies of sHSPs in different plants show the importance of this class of HSPs in adaptation under different environmental conditions [92].

3.2.3 Chloroplast small heat shock proteins (Cp-sHSPs) and their role

Cp-sHSPs are produced in cytoplasm followed by its import toward chloroplast [105]. As the name shows, these kinds of proteins are located in chloroplast and have consensus-III or methionine rich region at the N-terminal region additional to the other sHSPs-specific regions [106].

These proteins protect photosynthesis of the plants under heat and oxidative stress conditions [107]. There are different mechanisms to protect photosynthesis, for example, chloroplast membrane stabilization or avoiding everlasting proteins aggregation [108] but the role of Cp-sHSPs is very important in this case [109]. Different researchers have shown the relation of sHSPs with the adaptation of the plants under environmental stress conditions [43, 60–62, 109, 110].

It has been established in vitro by researchers that these chloroplast-specific proteins may protect photosynthetic electron transport under high-temperature stress conditions [59]. Cp-sHSPs associate with photosystem II (PS II) through oxygen-evolving complex (OEC) proteins under high-temperature conditions. It has been confirmed by researchers that these proteins protect PS II from inactivation under heat stress conditions by the protection of oxygen evolution and OEC proteins but have no capability to repair inactivated PS II [107].

4. HSP gene expression and promoters

Promoters regulate gene expression quantitatively and qualitatively [111]. There are three types of promoters that regulate the gene expression, that is, inducible, spatiotemporal, and constitutive promoters. Constitutive promoters promote the gene expression throughout the tissues irrespective to the environmental and developmental conditions, while spatiotemporal promoters direct the target gene expression in specific tissues, but inducible promoters are independent of the endogenous factors but dependent upon the external stimuli and environmental conditions [112]. Almost all kinds of promoters have the same core sequence with TATA-box, initiator, and the TF binding-specific cis-acting motifs specific to the target genes [113].

There are very less reports about the regulation of organelle-localized sHSPs under specific stress conditions or even under combination of stresses though it has been known that these genes are mainly regulated at transcriptional level. Researchers have reported the use of soybean promoter (GmHSP17.3B) to induce the sHSPs expression in *Physcomitrella patens* [114]. Additional to the above, researchers have also reported the rice promoter (Oshsp16.9A) to induce the expression of sHSPs under high-temperature stress conditions [115]. Small heat shock proteins have also been reported to get expressed under different abiotic stress conditions additional to the sHSPs expression at different developmental stages [43, 61, 62, 110, 116].

Heat shock transcription factors (HSFs) and heat shock elements (HSEs) may control the HSPs expression in the result of complex network of interaction [117]. HSFs (more than 20 in number) [118] may control the heat shock response both in vitro and in vivo [119]. Thermotolerance is increased in the result of higher expression of HSPs that is resulted by binding the HSFs to HSEs [120, 121]. Differential expression of HSPs is resulted by the variations in HSEs of HSPs. These HSEs have difference in the location and arrangements of its basic units (nGAAn), for example, AtHsp90–1 gene promoter has heat shock element 1 (HSE1) (tGAAgcTTCtgGAAt), heat shock element 2 (HSE2) (agTCtcGAAacGAAaaGAActTTCtgGAAt), and heat shock element 3 (HSE3) (gGAAgaaTCcaGAAt) [122]. Additional to the above elements, other motifs to regulate HSPs (gap-type 1, gap-type 2, and gap-type 3 with the sequences nTTCnnGAAn[5bp]nGAAn, nTTCn[1bp]nGAAn[5bp]nGAAn and nTTCn[2bp]nGAAn[5bp]nGAAn respectively) have also been reported. Researchers have also reported TTC-rich type regulatory elements with 2–4 units of nTTCn with 0–8 bp gap {e.g., TTC-rich 1 (nTTCn[1bp]nTTCn[6bp]nTTCn) and 3 (nTTCnnTTCn[8bp]nTTCn[1bp]nTTCn)} that have binding capability with HsfA1a of *Arabidopsis*. But some TTC-rich regions are also present with no binding potential with HsfA1a, for example, TTC-rich 2 (nTTCn[5bp]nTTCn[4bp]nTTCn) and TTC-rich 4 (nTTCn[3bp]nTTCn) [119]. Besides the above, other cis-regulatory elements are also present in HSPs promoter to regulate their expression under different growth conditions, for example, stress response elements (STREs), metal response elements (MREs), and CAAT boxes C/EBP [123–125]. Metalothionein gene of animals and plants has also been reported to get activated by heavy metal stress conditions because of the presence of MRE in promoter region of this gene [126–128]. Similarly, another stress-related element, that is, STRE (AGGGG) is also regulated by different abiotic stress conditions in yeast [129].

5. Model to express the Cp-sHSPs under different environmental conditions

There is no model put forward by researchers to control the expression of chloroplast-specific small heat shock proteins (Cp-sHSPs), but a model (**Figure 1**) to control the formerly mentioned genes has been proposed by Haq et al. [62]. According to this model, the presence of different cis-regulatory elements in Cp-sHSPs promoter shows the role of Cp-sHSPs under different abiotic stress

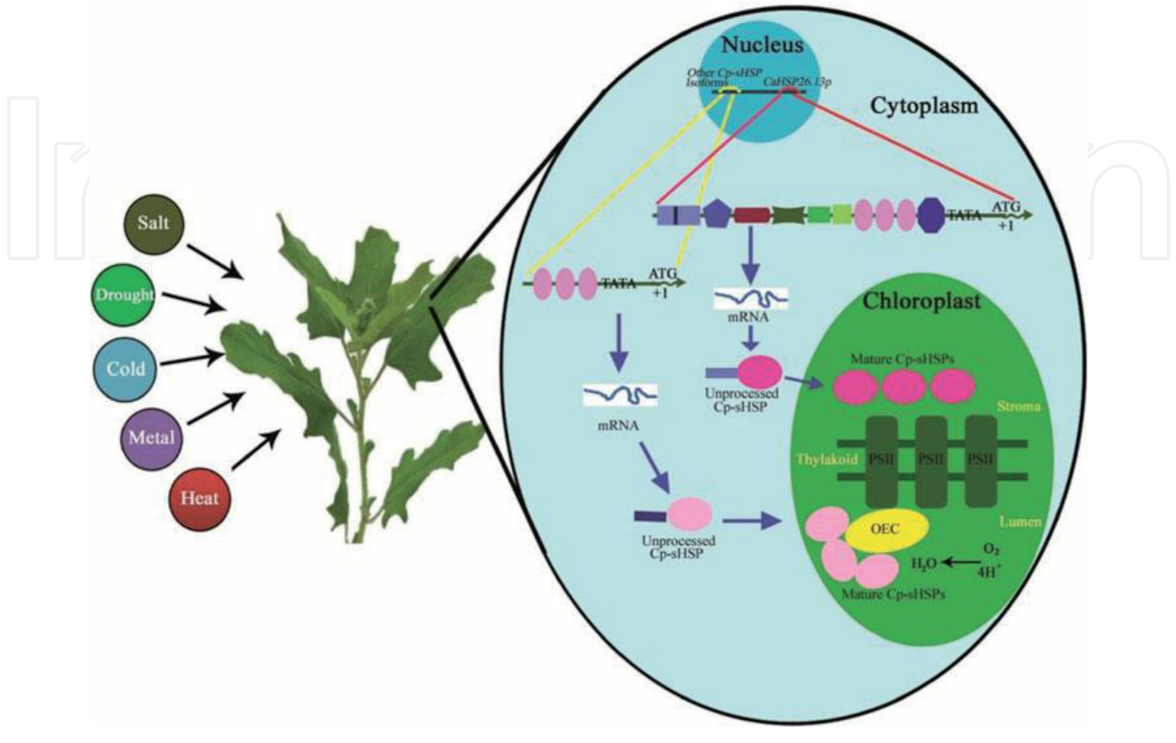

Figure 1.
Proposed model of expression and role of Cp-sHSPs [65].

conditions, that is, salt, drought, cold, metal, and high-temperature stress conditions. Cp-sHSPs in *Chenopodium album* have been shown to protect thylakoid membranes and photosystem II under different abiotic stress conditions. Different abiotic stress conditions, that is, heat, cold, heavy metal, drought, and salt stress conditions may regulate the single Cp-sHSP transcript in *C. album* which produces the precursor proteins that have transit peptide which directs that toward chloroplast. The transit peptide is detached from the proteins while reaching toward chloroplast in the result of which these proteins are matured that have the function in chloroplast. As per this proposed model, differential regulation of the same Cp-sHSP family member in *C. album* makes it able to play multiple roles under different abiotic stress conditions, that is, salt, drought, heavy metal, cold, and heat stress conditions [62].

Author details

Noor ul Haq[1]* and Samina N. Shakeel[2]

1 Department of Computer Science and Bioinformatics, Khushal Khan Khattak University, Karak, Khyber-Pakhtunkhwa, Pakistan

2 Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan

*Address all correspondence to: noorqu@gmail.com; noorulhaq@kkkuk.edu.pk

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