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Involvement of heat shock proteins in plant tolerance against metal/metalloid toxicity

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Abstract
Plants being sessile organisms are constantly confronted with different environmental stresses, among which toxicity and oxidative damages due to uptake of heavy metals and metalloids from contaminated soil and groundwater has severely threatened the growth and survival of the plants. The matter has been complicated and worsened due to industrial wastes, fertilizer applications, smelting, rampant sewage disposal and such related anthropogenic activities. Cellular accumulation of heavy metals at abnormally high levels causes severe damages in plants due to the accumulation of reactive oxygen species which disrupt the integrity of membrane structure due to lipid peroxidation and also lead to misfolding or unfolding in the structure of several endogenous enzymes and proteins, causing ultimate denaturation and loss in their activity. Therefore, plants are compromised in their vital physiological processes like photosynthesis, respiration, nitrogen assimilation, flowering and seed setting, thereby affecting productivity and yield. As a part of the elaborate and ubiquitous stress response, a set of proteins called heat shock proteins (HSPs) are induced; they constitute a broad family of molecular chaperones that assist the correct folding of stress-accumulated misfolded proteins, and prevent their aggregation often by promoting their proteolytic degradation. Since the HSPs are highly sensitive to even minor assaults, they are regarded as an early warning bio-indicator of cellular hazard. These proteins are regulated by the heat shock factors (HSFs) that bind to the heat shock elements (HSEs) present in the upstream of the *HSP* genes, triggering their expression upon encountering stressors like heavy metals and metalloids. The HSPs are categorized into five classes on the basis of their approximate molecular weight, viz., Hsp100, Hsp90, Hsp70, Hsp60 and small heat-shock proteins (sHsps). One of the first HSPs to be expressed upon increased heavy metal exposure are the HSP70 family proteins. Subsequently, all of them are found to be expressed working in a close orchestrated network to facilitate proper protein folding or proteolytic degradation of the misfolded ones. The sHSP family protein is the most prevalent of all HSPs in plant system and they usually aid the other families of HSPs to work efficiently. This review aims to highlight the involvement of different classes of HSPs in metal and metalloid stress responses in plants.

Keywords: Chaperones, Heat shock proteins, Metal and metalloid toxicity, Plants, Protein denaturation, Stress tolerance

1. Introduction
Pollution due to heavy metals and metalloids is one of the severe environmental hazards in the present era where vast areas of land are subjected to heavy metal contamination due to the excessive use of pesticides, fertilizers, municipal wastes, compost wastes, and also metal wastes released from smelting industries and metalliferous mines. This is a matter of concern since all heavy metals are non-biodegradable, and hence, cannot be purged out naturally from the environment via any possible natural means (Roychoudhury *et al*. 2012) [32]. Although heavy metals such as Cu, Zn and Ni are essential micronutrients and are required in trace amounts for normal physiological attributes of the plants, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms in plant species including low biomass accumulation, disintegration of membrane structure, chlorosis, inhibition of photosynthesis, altered water balance and nutrient assimilation, and oxidative damages due the accumulation of reactive oxygen species (ROS), all of which cumulatively result in the inhibition of vegetative and reproductive development of plants, leading to senescence and ultimately death of the plants (Das and Roychoudhury 2014) [12]. Crop plants that grow in heavy metal-contaminated fields generally accumulate higher amounts of heavy metals, thereby causing contamination of the entire food chain due to consumption by humans and animal species. One of the drastic effects of heavy metal toxicity is the alteration of the native conformation of endogenous cellular proteins together with denaturation of vital enzymes and functional proteins.

Heavy metals often bind to the different functional groups in proteins and displace certain essential elements, resulting in the disruption of the overall structure of the proteins, rendering them non-functional (Roychoudhury and Chakraborty 2020) [33]. The gradual accumulation of the aggregates formed by these inactivated proteins might perturb the overall cellular homeostasis and cell death. It is therefore extremely important that the cellular proteins are prevented from inactivation or denaturation by receiving some sort of aids from other cellular machineries or constituents. Therefore, as part of defense mechanism, plants activate molecular chaperones as one of the key components to arrest such protein denaturation. Of the many stress-induced chaperones studied so far, heat shock proteins (HSPs) have been the subject of significant attention. As apparent from the name, HSPs were initially identified as proteins whose concentration increases dramatically under heat stress. However, it has been gradually established that induced synthesis of HSPs is a generalized plant response against any form of environmental stress. HSPs form the main surveillance system that is selectively up regulated under stress for the maintenance of the functional, healthy proteome. Additional groups of proteins or enzymes involved include peptidyl-prolyl cis/trans isomerase, catalysing the isomerization of peptide bonds of proline residues; protein disulphide isomerase (PDI), catalysing disulphide bond formation in the endoplasmic reticulum; and calnexin/calreticulin, assisting the folding of glycosylated proteins in the endoplasmic reticulum. HSPs assist in accurate folding of stress-accumulated misfolded proteins, thereby preventing protein aggregation, as well as promote selective degradation and disposal of misfolded or denatured proteins (Banerjee and Roychoudhury 2018) [6]. The HSPs also promote redox homeostasis by stimulating the antioxidants like ascorbate, glutathione and tocopherol, as well as enzymes which are involved in scavenging of ROS like superoxide dismutase (SOD), catalase (CAT) and peroxidases (PODs). They also function in the stabilization of proteins and membranes, and can assist in protein refolding under stress conditions (Hasan *et al*. 2017) [18]. HSPs are found in cytoplasm and different subcellular compartments like nucleus, mitochondria, chloroplast and endoplasmic reticulum. Members of the HSP60 and HSP70 family take part in correct protein folding and assembly, whereas small HSPs like members of the HSP20 family participate in the formation of ribonucleoprotein complexes involved in storage of mRNAs of housekeeping genes. Despite iterative rounds of such trial via the unfolded protein response (UPR) in the ER, if the native conformation of a protein cannot be reached, there remains no option, but to degrade them via the ubiquitin proteasome process (UPS), also known as ER-associated degradation (ERAD) or through autophagy, to minimize the accumulation of misfolded proteins in cells. This review is primarily aimed to discuss the different classes of HSPs reported in plants with special emphasis on their involvement during metal and metalloid stress response in plants.

2. Regulation of heat shock proteins in plants

The heat shock response is controlled by heat shock factors (HSFs) which bind to highly conserved, cis-acting response elements called heat shock elements (HSEs), found in multiple copies in the upstream regions of heat shock genes. The domain organization in plant HSF proteins is more or less conserved. They contain an N-terminal DNA-binding domain (DBD) which has a central helix-turn-helix motif that is used to specifically bind the HSEs in the promoters of the heat-shock genes. They also contain an oligomerization domain (OD) with a bipartite heptad pattern of hydrophobic amino acids (HR – A/B region). This OD is connected to the DBD by a flexible linker region (Al-Whaibi 2011) [4]. There are three classes of HSFs, viz., HSFA, HSFB and HSFC. HSFA, found in the cytosol as a monomer, is negatively regulated by HSP90 and checked in the form of phospho-proteins under normal conditions. During stress, this repression is reversed due to dissociation of HSP90, so that HSFA homotrimers aggregate and accumulate in the nucleus where they bind to the HSEs, with the consensus sequence, 5'-nGAAnnTTCnnGAAn-3', in the promoter region (Guo *et al*. 2016) [16]. This binding was observed to be promoted by salicylic acid. This induced the transcription of *hsfA2* and *hsfB1* and expression of the HSP70 protein. HSFA2 is only expressed during stressed conditions, when it makes a super activator hetero-oligomer structure with HSFA1 functioning more efficiently than the individual HSFs and they positively regulate the downstream stress-related *HSP* genes. The complex activates the downstream signaling pathway and activates the expression of glutathione S-transferase (GST), glutathione reductase (GR), POD and ascorbate peroxidase (APX). HSP17.8 overexpression led to enhanced SOD activity in Arabidopsis, and HSP16.9 increased the levels of POD, CAT and SOD in tobacco (Wu 1995) [43]. The transcription factor, Dehydration Responsive Element Binding Protein 2 (DREB2) regulated the expression of HSFA during heavy metal-induced stress. Cai et. al. (2017) [9] showed that metal tolerance is positively correlated with melatonin (N-acetyl-5-methoxy tryptamine) synthesis, which in turn is induced by HsfA1a. Post-transcriptional modification such as alternative splicing also regulates the expression and activity of HSFs. HSFA2 binds to its own promoter region and activates its own transcription in a positive auto-regulatory loop (Ul Haq *et al*. 2019) [40].

3. HSPs suppress endoplasmic reticulum stress

Continuous exposure to metal stress leads to the accumulation of misfolded proteins in the endoplasmic reticulum (ER), leading to ER stress. To alleviate the ER stress, the Unfolded Protein Response (UPR) is initiated through transcriptional and translational events. UPR aims to restore the normal functioning of the cell by suppressing the production of secreted and membrane proteins, removing the misfolded proteins through ER-associated degradation (ERAD) systems, and activating the signaling pathways to up regulate the production of molecular chaperones which are involved in protein folding. If the stress exposure increases to a level which is beyond the limit of UPR to restore cell homeostasis, the UPR after a certain time span, activates the programmed cell death. To overcome the ER stress, the misfolded or unfolded proteins are tagged with the HSP70 chaperone, binding immunoglobulin protein (BiP, also known as heat shock 70 kDa protein 5, HSPA5), in the ER, and induces the Unfolded Protein Response (UPR). The activation of downstream signaling pathway by BiP is further regulated by stress sensors embedded in the ER membrane like Inositol-requiring enzyme 1 (IRE1) (Srivastava *et al* 2018) [39]. IRE1 is an ER stress sensor, localized in the ER membrane. In absence of stress, IRE1 interacts with BiP, keeping it inactivated. Accumulation of unfolded proteins

leads to the onset of ER stress, causing BiP disassociation from IRE1. Plant IRE1, now free, undergoes oligomerization, followed by autophosphorylation of the cytosolic kinase domains. This activates the IRE1 ribonuclease domain which causes the unconventional splicing of basic leucine zipper 60 (bZIP60) mRNA. In addition, there is bulk degradation of selected mRNAs through Regulated IRE1-Dependent Decay (RIDD). Spliced and now mature bZIP60 mRNA is translated into an active transcription factor, which migrates to the nucleus and leads to transcriptional activation of genes involved in UPR, including the activation of heat shock proteins (Srivastava *et al* 2018) [39].

4. HSPs aid in proteasomal degradation

Misfolded proteins are also degraded by ubiquitin proteosome pathway. Under heavy metal stress, the expression of *polyubiquitin* genes is one of the important indications that the Ubiquitin Proteasome System is involved in the regulation of plant heavy metal stress tolerance. Ubiquitin is encoded by multiple *polyubiquitin* genes, *UBQ3*, *UBQ4*, *UBQIO*, *UBQ11*, and *UBQ14* which are unregulated at the onset of stress. It has been found in *Arabidopsis thaliana* that carboxyl terminus of Hsc70-Interacting Protein (AtCHIP) is a E3 ligase and is named for its sequence similarity to mammalian co-chaperone, CHIP which targets the damaged proteins for degradation by the 26S proteasome. CHIP inhibits hydrolysis of ATP and, therefore, attenuates substrate binding to HSP70. This results in the inhibition of the HSP70-DnaJ protein folding-refolding pathway. CHIP is a chaperone-associated E3 ligase that catalyzes the transfer of the ubiquitin from the E2 enzyme to a lysine residue on the target protein, thus targeting it for proteasomal degradation (Lee *et al*. 2009) [25].

5. Different classes of HSPs and their functions

5.1. Hsp70

Hsp70 chaperones, along with their co-chaperones (e.g., DnaJ/Hsp40 and GrpE), carry out a broad range of protein folding processes in most of the cellular compartments. Structurally, Hsp70 consists of a highly conserved 44 kDa N-terminal ATPase domain and a 25 kDa C-terminal peptide-binding domain. Successive cycles of substrate binding and release are coupled to the intrinsic ATPase activity of Hsp70 (Wang *et al*. 2004) [42]. Hsp70 has vital functions in preventing aggregation, and in assisting refolding of non-native proteins under both normal and stressful conditions (Hartl 1996; Frydman 2001) [17, 13]. They are also involved in protein import and translocation processes, and in aiding the proteolytic degradation of unstable proteins by targeting the proteins to lysosomes or proteasomes. Some family members of Hsp70 which are constitutively expressed are known as Hsc70 (70-kDa heat-shock cognate). The members of Hsc70 family are frequently involved in assisting the folding of de novo synthesized polypeptides and the import/translocation of precursor proteins. During precursor protein transportation, HSC70 is required for cell-to-cell transport in conjunction with the plasmodesmatal translocation pathway. Other family members are expressed only during unfavorable environmental conditions. Consequently, they are more involved in facilitating refolding and proteolytic degradation of non-native proteins (Miernyk 1997) [27]. Many Hsp70 proteins have been identified in different plant species (Boston *et al*. 1996; Vierling 1991) [7, 41]. Two-fold higher accumulation of HSP70 was observed in cadmium-accumulating genotype (Enrei) of soybean, while in lower Cd-accumulating genotype (Harosoy), there was lower HSP70 expression (Hossain *et al*. 2012) [21]. A study conducted by Neumann *et al*. (1994) [29] showed that when cell cultures of *Lycopersicon peruvianum* L. are exposed to 10^{-3} M $CdSO_4$, changes occur in the ultrastructure, starting with the plasmalemma and then extending to the ER and the mitochondrial envelope. A portion of the membrane material is extruded, with the development of osmiophilic droplets that increase in size and number during the stress period. Almost 20% of the cells die after 4 hours of application of the stress. However, when a short heat stress is applied prior to the heavy metal stress, tolerance is induced by preventing membrane damage. The cells exhibit normal ultrastructure except for the fact that cytoplasmic heat-shock granules are formed. When flax was cultured on heavy metal-treated media, many heavy metal-binding proteins, including HSP70, were accumulated, while HSP83 showed down regulation. HSP70 has been shown to alleviate Cd toxicity in tomato, *Lemma minor*, *Populus* sp., etc., arsenic toxicity in rice, and Hg toxicity in *Suaeda salsa*. Heat-stress granules in the cytoplasm contains mostly HSP17, whereas HSP70 is localized in the nucleus, cytoplasm and most interestingly in the plasmalemma. This discovery that a significant amount of HSP70 is bound to membranes, particularly to the plasmalemma, post cadmium stress is probably related to the formation of complexes between heavy metals and proteins which cause subsequent denaturation of proteins. HSP70 has a high affinity for misfolded proteins and assists their refolding, which is possibly connected with reintegration into the proper complex of membrane proteins. It was also seen that this protection of membranes against cadmium damage which is offered by heat-stress-induced proteins can be abolished by the application of cycloheximide. This is because cycloheximide prevents HSP synthesis and so the protective effect against cadmium-induced membrane damage was not observed. HSP70 induction not only reduces the proteotoxic effects of metal ions, but also aids in their sequestration and detoxification by metallothioneins.

5.2. Chaperonin (Hsp60) family

The term chaperonin was first coined to describe a class of molecular chaperones which are evolutionarily homologous to *E. coli* GroEL (Hemmingsen *et al*. 1988) [20]. Chaperonins are found in prokaryotes and in the mitochondria and plastids of eukaryotes (Boston *et al*. 1996) [7]. The main examples of this class of molecular chaperones include the prokaryotic GroEL and the eukaryotic equivalent Hsp60. Chaperonins play a vital role by aiding a variety of newly synthesised and newly translocated proteins to achieve their native forms (Bukau and Horwich 1998; Frydman 2001) [8, 13]. Studies have shown that HSP60 is essential for cellular functions at normal as well as stressful environments including metal stress. A study was conducted by Sarry *et al* (2006) [35] in which *Arabidopsis thaliana* cells were exposed to different concentrations of cadmium (Cd). Following a 24 h treatment, soluble proteins extracted from untreated and treated cells were separated by 2-D-polyacrylamide gel electrophoresis and proteins up- and down-regulated in response to Cd were identified by Mass Spectrometry (MS). A significant increase in expression levels of several HSPs was seen including HSP70, chaperonin and mitochondrial HSP60. The induction of HSPs was found to be associated with the induction of PDI which also plays a crucial role in protein folding. Such molecular chaperones which are induced during Cd stress possibly prevent the irreversible protein denaturation which occurs due to the oxidative stress

linked to Cd exposure. Likewise, molecular responses of hydroponically cultivated tomato plants to As(V) or Cr(VI) were also assessed through analysis of accumulation of transcripts of genes coding for products potentially involved in heavy metal tolerance. Tomato plants were treated for 24 h with As (V) or Cr(VI) at concentrations, ranging from 80 to 640 mM to mimic the potential effect of these heavy metals in polluted soil and to assess the response of the plants to this metalloid at the molecular level. Following this, RNA was isolated from tomato roots or shoots and quantitative real-time PCR was done with primers specific for the genes encoding Hsp90-1, MT2- and GR1-like proteins. It was observed that both As(V) and Cr(VI) treatments induced *Hsp90-1* transcript accumulation in tomato plants. However, As(V) induction was marked in roots, while Cr(VI) induction in shoots (Goupil *et al*. 2009) [15].

5.3. Hsp90 family

Apart from its role in assisting in protein folding, Hsp90 also plays a crucial role in signal-transduction networks, cell-cycle control, protein degradation and protein trafficking (Richter and Buchner 2001) [30]. HSP90 functions in association with other proteins such as actin, calmodulin, tubulin and other signaling kinases. Although Hsp90 chaperones are constitutively expressed in most organisms, their expression increases in response to stress in both prokaryotes and eukaryotes. Expression of Hsp90 in Arabidopsis is developmentally regulated and responds to heat, cold, salinity, heavy metals, phytohormones, and light and dark transitions (Krishna and Gloor 2001) [23]. HSP90.3 helped in the enhancement of stress tolerance to Cd by lowering the germination rate, mediated by the antioxidants in *Arabidopsis*.

5.4. Hsp100/Clp family

The Hsp100/Clp family chaperones are members of the large AAA ATPase superfamily, having a broad spectrum of diverse functional properties (Agarwal *et al*. 2001) [1]. Hsp100/Clp proteins have been reported in many plant species like Arabidopsis, soybean, tobacco, rice, wheat, maize, and Lima bean (*Phaseolus lunatus*). Hsp100/Clp family chaperones are often constitutively expressed in plants, but their expression is developmentally regulated and is induced during various abiotic stresses like heat, cold, dehydration, high salt and dark-induced etiolation (Wang *et al*. 2004) [42]. Hsp100 acts in tandem with Hsp70 to prevent protein aggregation. Expression of rice Hsp101 cDNA in *hsp104* deficient yeast (tolerance to arsenite is governed by *hsp104* in yeast) has shown to cause recovery in tolerance against arsenite stress. However, there is little substantial evidence implicating the role of HSp100/Clp proteins in heavy metal tolerance in plants (Agarwal *et al*. 2003) [2].

5.5. Small heat shock protein (sHsp) family

The sHsps have lower molecular weight ranging from 12–40 kDa. Out of the five conserved families of Hsps (Hsp70, Hsp60, Hsp90, Hsp100 and sHsp), sHsps are the most ubiquitous in plants (Banerjee and Roychoudhury 2018) [6]. The high diversification of plant sHsps probably suggests a molecular adaptation to stress conditions that are unique to plants. Unlike other classes of chaperone like DnaK or Clp/DnaK, this group of protein does not require ATP for their function. sHSPs basically maintain denatured proteins in a folding-competent state and allow subsequent ATP-dependent disaggregation through the HSP70/90 chaperone system, thereby facilitating their subsequent refolding. BAG3 protein has been shown to act as a modulator, by bringing the proteins together in a chaperone complex and co-ordinating the potential function of Hsp22 and Hsp70 to refold the misfolded proteins. Several studies have suggested a strong correlation between sHSP accumulation and plant tolerance, particularly to heavy metal stress. Certain chaperones like Cp-sHSPs or HSP26.13p performs dual role of protecting the plant from heat stress as well as metals like Cd, Ni and Zn stress. A study was conducted to understand the role of a small, low-molecular weight heat shock protein gene called *Oryza sativa multiple stress-responsive3* (*OsMSR3*) under Cu stress in *Arabidopsis thaliana*. During Cu stress, transgenic *A. thaliana* overexpressing *OsMSR3* gene showed higher tolerance to Cu. The transgenic plants had longer roots, higher survival rates, biomass, and relative water content than the wild type, with more accumulation of abscisic acid (ABA), chlorophyll, carotenoid, SOD and POD than the wild type plants. Additionally, expression of *OsMSR3* gene in *A. thaliana* increased the expression of antioxidant-related and ABA-responsive genes, thereby regulating Cu tolerance (Cui *et al*. 2019) [10]. Safeguarding photosynthesis against excess metal toxicity with the aid of sHSPs was demonstrated in near-isogenic genotypes of *Agrostis stolonifera* (Heckathorn *et al*. 2004) [19].

6. HSPs and metal/metalloid tolerance

The involvement of HSPs from different plant species in response against metal and metalloid stress (Table 1) is represented in the literature, though to a comparatively lesser extent as compared to other environmental stresses. Upon exposure of rice seedlings to Cd stress, 21 proteins, identified by MALDI-TOF MS, were up regulated. These proteins included DnaK Bip proteins, a subfamily of HSP70, which could prevent proteins from unfolding, mainly in the ER, and maintain cellular homeostasis during heavy metal toxicity in the soil. Similarly, up regulation of HSP80 and HSP17.9 were also detected by microarray technique in rice seeds (Ashan *et al*. 2007) [3]. Cd toxicity was overcome in *Arabidopsis thaliana* via significant increase in the expression of HSP70, chaperonins and mitochondrial HSP60. Jorge Rodríguez-Celma *et al*. (2003) [31] showed that in tomato, seven proteins related to protein metabolism were up regulated in plants subjected to 10 μM Cd, of which three were HSPs, viz., HSP68, HSP60 and chaperonin 60 β-subunit. This induction was correlated with that of PDIs, which are also involved in proper protein folding. These molecular chaperones could prevent irreversible denaturation of proteins or brought about proteolytic degradation of the denatured proteins. However, constitutive expression of HSP90.3 in *Arabidopsis thaliana* impaired plant tolerance to Cd toxicity by lowering germination potential and root and shoot growth, reducing the contents of phytochelatins (PCs) and reduced glutathione (GSH), inhibiting the activities of SOD, CAT and PODs, and increasing malondialdehyde (MDA) level (Sarry *et al*. 2006; Song *et al*. 2012) [35, 38]. The differential expression pattern of HSP was noted using a comparative proteome analysis of poplar (*Populus yunnanensis*) leaves under Cd toxicity. During earlier stages when Cd^{2+} ions were initially increasing, production of defense-response molecules, photosynthesis- and energy-associated proteins, antioxidant enzymes and HSPs were transiently induced. Upon their accumulation, protein stability was enhanced and subsequently a new cellular homeostasis was established. During second stage (prolonged Cd^{2+} ion treatment), the concentration levels of ribulose-1,5-bisphosphate

carboxylase (RuBisCO) and HSPs were markedly reduced, leading to imbalance of photosynthetic system (Yang *et al*. 2015) [44]. Lomaglio *et al*. (2015) [26] studied the effect of short-term cadmium (50 µM $CdSO_4$) stress in detached leaves of *Populus nigra* L. where HSP70 protein was up regulated 13.8 fold, as compared to the control set. Common duckweed (*Lemna minor*) and toothed wrack (*Fucus serratus*), exposed to osmotic and cadmium stress for 24 hours displayed elevated concentrations of HSP70. For both the stresses concerned, HSP70 levels reached a peak and then started to decline as the stressor levels increased further. It was suggested that HSP70 could be potentially applied for stress detection in aquatic species as tested by indirect competitive ELISA (Ireland *et al*. 2004) [22].When tomato plants were exposed to Cd stress, melatonin biosynthesis was induced by HsfA1a. Silencing of *HsfA1a* gene decreased Cd tolerance, whereas overexpression led to enhanced tolerance. Under Cd stress, *HsfA1a*-silenced plants exhibited reduced level of melatonin. *HsfA1a*-overexpressed plants showed stimulated melatonin accumulation along with induced expression of *caffeic acid O-methyltransferase 1* (*COMT1*), the gene responsible for melatonin biosynthesis. Moreover, in *HsfA1a*-overexpressing plants, Cd stress induced the expression of HSPs robustly, while in *HsfA1a*-silenced plants, there was decrease in *HSP* expression under Cd stress. If the *COMT1* gene was silenced in *HsfA1a*-overexpressing plants, melatonin accumulation decreased, resulting in the suppression of Cd tolerance (Cai *et al*. 2017) [9]. Melatonin has already been shown to participate in stress tolerance of plant species against Cd, aluminium, boron and lanthanum toxicity (Samanta *et al*. 2021) [34]. Similarly, increase in the expression of HSP26-A was observed in soybean (*Glycine max*) under Cd^{2+} and/or As(V) toxicity.

The transcriptome profiling in *Arabidopsis* identified 200 and 69 genes encoding transcription factors that were expressed differentially with exposure to arsenic in arsenic-tolerant and arsenic-sensitive varieties, respectively. The tolerant variety showed few more genes which included heat-shock transcription factors, HSFB2A, HSF2A and heat-shock-protein 20-like chaperone superfamily protein (AT1G52560). In *Lemna minor*, the response to As(V) involved cellular protein machineries including ubiquitin-proteasome pathway and HSP synthesis (Fu *et al*. 2014) [14].

In plants, Al^{3+} ions strongly bind proteins, nucleic acids and phospholipids, thus inhibiting cell division, cell extension and transport at the cellular level. At the molecular level, high Al^{3+} ion concentration results in oxidative damages to cellular components due to high accumulation of ROS like superoxide radicals, hydroxyl radicals, etc. along with MDA. When *Lotus corniculatus* was exposed to high Al concentration, a rapid membrane depolarization in root cells was observed, thus suggesting the role of Al toxicity in the inhibition of root cell elongation. However, an increase in the expression of HSP90 was observed in response to this toxicity. HSP90 further appeared to elicit the activity of antioxidative enzymes such as CAT, SOD and glutathione peroxidase, at very high levels of Al^{3+}ions (Navascues *et al*. 2012) [28].

High levels of Pb^{2+} can replace essential ions within the plants, thus disrupting redox balance and inhibiting mineral acquisition and photosynthesis. High levels of As(V) negatively regulate plant metabolism, photosynthesis, growth, and root and shoot proliferation. An increase in expression of HSP17.7, a cytosolic sHSP, in carrot (*Daucus carota*) was reported under high levels of Pb^{2+} and/or As(V). However, in non-stressed leaf tissues of carrot where such toxic ion levels were low, this protein was present at a lower level (Lee and Ahn 2013) [24].

Small HSPs like HSP17 was expressed in roots of *Armeria maritima* plants grown on Cu-rich soils (Neumann *et al*. 1994) [29] and the same HSP17 was also shown to increase in cell cultures of *Silene vulgaris* and *Lycopersicon peruvianum* in response to a range of heavy metal treatments. Increase in HSP70 translation was also seen in the seaweed, *Enteromorpha intestinalis* after exposing it to a variety of metal stresses such as copper.

Table 1: Different metal/metalloid stress against which HSPs function in plant species (Hasan *et al*. 2017) [18]

Metal/ metalloid	Members of HSP	Plant species
Cadmium	HSP70 HSP21, HSP60, HSP70, HSP81.4, 88.1 and 89.1 HSP60, HSP68 BiP, HSP81-1 HSC70 HSP17 HSP20, 23p	*Glycine max, Populus tremula* *Arabidopsis thaliana* *Solanum lycopersicum* *Oryza sativa* *Phytolacca americana* *Lycopersicon peruvianum* *Kandelia candel*
Arsenic	HSP17.4, 17.5 HSP90-1, ClpB-C HSP70, HSP82, HSP101	*Tamarix hispida* *Arabidopsis thaliana* *Oryza sativa*
Lead	HSP70 HSP90	*Elodea canadensis, Lemna minor* *Arabidopsis thaliana*
Iron	HSP17.4	*Oryza sativa*
Zinc	HSP24 HSP17.4	*Capsicum annuum* *Oryza sativa*
Chromium	HSP23 HSP20, 21, 22, 70-2 HSP90-1	*Glycine max* *Raphanus sativus* *Solanum lycopersicum*
Mercury	HSP70 HSP60	*Suaeda salsa* *Oryza sativa*
Copper	HSP18.3, HSP81-2, ClpB-C HSP90-1 HSP17	*Oryza sativa* *Lemna gibba* *Armeria maritima*
Aluminium	HSP23.9	*Oryza sativa*

7. Conclusion and future perspectives

Heat shock proteins show remarkable variation in their expression level under several abiotic stress conditions in plants. The molecular characterization and regulation

pattern of different *hsp* genes have thrown light on their broad range of functional capabilities, primarily targeted against the aggregation of misfolded and unfolded proteins through their molecular chaperone activity and ultimately allowing the cell to restore normal homeostasis. HSPs as chaperones also play a role in membrane stability, using ROS as a signal molecule and scavenging them by positively regulating the antioxidant enzymes, along with maintaining plant growth and development under normal conditions. Hsp70 and HSP60 have been proposed as biomarkers of exposure levels and toxicity. In addition to its direct role, HSP also inhibits translation of housekeeping proteins under stress condition, while helping in the restoration of the normal level of expression during recovery period. Although the role of HSPs in conferring tolerance to metal and metalloid stress in plants is less explored, prominent cellular protection by HSPs has been observed against cadmium, lead, zinc, aluminium, arsenic, etc. By virtue of their chaperone activity, HSPs play a crucial role in alleviating metal toxicity in plants. This review ponders upon the possibility of engineering plants, tolerant to heavy metal stress, using this aspect of the stress-response signaling. However, we are still far from proper understanding of the mechanism by which HSPs/chaperones, as regulatory molecules, participate in stress sensing, signal transduction and transcription activation of stress genes. Deeper insights are required into the regulatory cross-talk events, occurring among the different heat shock proteins and other chaperones upon encountering any form of stress, including that arising from heavy metal contamination. Future research should be extended to explore other regulatory mechanisms such as alternative splicing, microRNAs and their interaction and cross-talk with complex HSFs, HSPs, phytohormones and protective enzymes in regulating plant growth, development and metabolism, under normal and stress conditions. This will provide avenues for the development of stress-tolerant crops by exploiting HSPs through biotechnological approaches and molecular breeding.

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