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REVIEW

Heavy Metal-Associated Isoprenylated Plant Proteins (HIPPs): Multifunctional Regulators of Metal Homeostasis, Abiotic/Biotic Stress Responses, and Plant Development

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ABSTRACT: Heavy metal-associated isoprenylated plant proteins (HIPPs) are a class of vascular plant specific metallochaperones, characterized by the presence of one or two N-terminal heavy metal-associated (HMA) domains and a C-terminal isoprenylation motif (CaaX). The HMA domain contains a conserved CysXXCys motif that is responsible for binding transition metals such as Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}. The CaaX motif modulates post-translational prenylation that anchors HIPPs to membranes and facilitates protein-protein interactions. This review systematically summarizes the structural features and multifaceted functions of HIPPs in plants. Beyond their well known function in heavy metal detoxification, HIPPs are more recently recognized as key regulators of abiotic stress responses, including drought, cold, and salinity, and biotic stress resistance via interactions with pathogen effectors. Moreover, HIPPs also participate in plant growth and development and in hormone signaling, including the cytokinin and abscisic acid (ABA) pathways. Translating this knowledge holds great promise for developing stress-tolerant crop varieties, breeding low-cadmium crops, and advancing phytoremediation technologies.

KEYWORDS: HIPP; biotic stress; abiotic stress; plant development; heavy metal

1 Introduction

Heavy metal pollution, abiotic stresses (such as cold, drought, and salinity), and pathogen infections are major environmental constraints that severely threaten global crop production and food security [1,2]. Among these, the non-essential heavy metal cadmium (Cd) raises particular concern owing to its high toxicity, persistence in soils, and ease of uptake by crop plants. Excessive Cd not only inhibits plant growth, reduces photosynthesis and biomass accumulation, but also enters the food chain, causing serious health problems, including cancer, renal dysfunction, and osteoporosis in humans [3,4]. Copper (Cu), an essential micronutrient, becomes toxic when present in excess, leading to oxidative stress and impaired growth [5]. Similarly, biotic stresses such as bacterial, fungal, and nematode infections cause devastating yield losses worldwide [6,7]. To cope with these diverse environmental challenges, plants have evolved intricate regulatory networks involving metal chaperones, transporters, and signaling molecules that maintain ion homeostasis and trigger appropriate defence responses [8,9].

Key players in metal homeostasis and stress signaling include HIPPs [10–13]. HIPPs are a plant-specific subfamily of metallochaperones, characterized by the presence of one or two HMA domains at their N-terminus and a C-terminal isoprenylation motif (CaaX box) [14,15]. HIPPs are strictly defined by

the simultaneous presence of at least one HMA domain and a C-terminal CaaX isoprenylation motif. In contrast, HPPs (heavy metal-associated plant proteins) that possess only HMA domains without a CaaX motif are not classified as HIPPs and are discussed only when functionally linked to HIPPs. The HMA domain contains a conserved Cys-XX-Cys motif that directly binds transition metal ions such as Cd^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+}, enabling metal chelation and detoxification [16,17]. The C-terminal isoprenylation motif undergoes post-translational prenylation, which anchors the protein to the plasma membrane or other endomembranes and facilitates protein-protein interactions essential for signal transduction [18,19]. This unique dual-domain architecture distinguishes HIPPs from other metallochaperones that possess only HMA domains. Moreover, HIPP genes are found exclusively in vascular plants, suggesting that they emerged as an evolutionary adaptation to the complex terrestrial environment.

This review critically synthesizes the HIPP literature, distinguishing between mechanistically validated functions and correlative expression data. We prioritize studies that provide functional validation (e.g., mutant or overexpression lines, protein–protein interactions, *in vitro* binding assays) and highlight contradictory findings as well as knowledge gaps. A systematic literature search of PubMed, Web of Science, and Google Scholar was conducted for the period January 2000–March 2026 using the keywords "HIPP", "heavy metal-associated isoprenylated plant protein", "metallochaperone plant", "HMA domain isoprenylation", and "farnesylated plant protein". Compared with earlier reviews (e.g., de Abreu Neto et al., 2013; Barr et al., 2023), this work integrates the rapid expansion of knowledge from over ten crop species published in 2024–2026, provides a mechanistic classification of HIPP in plant metal homeostasis, abiotic/biotic stress responses, and plant development, and offers a translational roadmap for agriculture.

Initial studies on HIPPs focused primarily on their roles in heavy metal detoxification and homeostasis. In *Arabidopsis thaliana* (Arabidopsis), *AtHIPP20*, *AtHIPP22*, *AtHIPP26*, and *AtHIPP27* complement Cd-sensitive yeast mutants, whereas the *athipp20/21/22* triple mutant displays increased Cd sensitivity and reduced Cd accumulation [18,20]. In rice, *OsHIPP29* is upregulated by Cd and Zn; Overexpression of *OsHIPP29* reduces Cd accumulation, thereby improving growth under Cd stress, while knockout lines are more susceptible [21]. *OsHIPP16* and *OsHIPP56* have also been characterized as positive regulators of Cd tolerance and suppressors of Cd accumulation in grains [22,23]. These studies firmly established HIPPs as central regulators of metal stress responses.

In recent years, the functional scope of HIPPs has expanded dramatically. They are now known to participate in abiotic stress responses beyond heavy metals. Recently, a genome-wide identification in tobacco (*Nicotiana tabacum*) and analysis of stress-responsive expression patterns provided a foundation for understanding HIPP functions [24]. A pan-genome analysis and expression profiling of the HIPP gene family in cassava revealed that a total of 59 *MeHIPP* pan-genes were identified and classified into core, softcore, dispensable, and private categories, with uneven chromosomal distribution. The gene family expanded mainly through segmental duplication, with most members under purifying selection. Expression profiles indicated roles in response to *Xanthomonas phaseoli* pv. *Manihotis* (Xpm) infection, cadmium and drought stress, with varying tissue- and cell-specific transcript levels [25]. For example, barley *HvFP1*, a member of the barley HIPP gene family, is induced by drought, cold, and ABA, and overexpression of *HvFP1* delays leaf senescence [26]. In Arabidopsis, AtHIPP26 interacts with the zinc finger homeodomain transcription factor ATHB29 under cold, salt, and drought stress [27], but the exact mode of action of the two regulatory factors HIPP26 and ATHB29 in response of the plants to different stress conditions has to be solved in future experiments. In contrast, grapevine *VvHIPP21* acts as a negative regulator of cold and drought tolerance [28]. *VvHIPP21* expression is suppressed by cold and ABA but induced by drought, and the protein localizes to both the cytoplasm and nucleus. Ectopic expression of *VvHIPP21* in *Arabidopsis* alleviates heavy metal (Cu^{2+},

Cd^{2+}) toxicity and reduces drought tolerance [28]. Regarding biotic stress, HIPP proteins are emerging as common targets of pathogen effectors. The rice blast fungus *Magnaporthe oryzae* effector AVR-Pik directly binds to the HMA domain of rice OsHIPP19 to suppress immunity [29]. The kiwifruit bacterial canker pathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*) effector AvrPto5 targets AcHIPP26 directly [30], but the function of AcHIPP26 in *Psa* resistance is not clear. The movement protein TGB1 of potato mop-top virus interacts with NbHIPP26 to facilitate the virus's long-distance movement [31], and the beet cyst nematode *Heterodera schachtii* relies on AtHIPP27 to complete successful parasitism [32]. These findings indicate that HIPP proteins play an important role in pathogen infection processes. Furthermore, HIPPs also regulate plant development and hormone signaling. HIPP influences the stability of cytokinin oxidase (CKX) by regulating the endoplasmic reticulum-associated degradation (ERAD) pathway in Arabidopsis, thereby finely modulating cytokinin signaling and plant development [33].

Despite these significant advances, several important questions remain unresolved. For example, The molecular mechanisms that how exactly metal ions are transferred from HIPPs to downstream transporters, and how isoprenylation dynamically control HIPP subcellular localization and function are not clear. Additionally, although genome-wide identification of HIPP genes has been performed in several crops, their agricultural potential remains largely unexplored. Notably, the role of HIPPs as pathogen effector hubs has only recently been recognized, whereas the interplay between HIPP-mediated metal homeostasis and plant immunity requires further investigation.

In this review, we aim to introduce the structural features and multifaceted functions of HIPP proteins in heavy metal detoxification, abiotic and biotic stress responses, hormone signaling and plant development. We also discuss the molecular mechanisms underling these related functions. We highlight the potential applications of HIPP genes in stress tolerant and low cadmium crop breeding and outline future research directions to address current knowledge gaps.

2 Structural Characterization of Plant HIPPs

HIPPs are metallochaperones that contain conserved structures, including 1–2 heavy metal-associated domains (HMAs), a C-terminal isoprenylation motif, as well as a flexible glycine-rich and proline-rich region located between these two domains [27]. Here, we generated six representative HIPP proteins (OsHIPP5, OsHIPP9, OsHIPP19, OsHIPP28, OsHIPP42, and OsHIPP56) from rice for protein structure analysis by using AlphaFold 3. We provided UniProt accession numbers and domain boundaries (HMA and CaaX positions) for each protein (Fig. 1), as well as the pLDDT scores, and predicted aligned error (PAE) plots (Fig. 2). Structural analysis of the six predicted structures confirmed that HMA domain cores are conserved while loop regions and C-terminal extensions diverge. For example, OsHIPP56 lacks a CaaX motif at its C-terminus, indicating that it may belong to the HPP gene family, whereas OsHIPP28 contains two conserved HMA domains based on the prediction results. These findings confirm strong conservation of the metal-binding fold between HIPP and HPP proteins. However, differences in the CaaX isoprenylation motif suggest that these HIPP or HPP proteins may interact with distinct protein partners, although direct functional divergence awaits experimental validation (Fig. 1). A genome-wide identification of heavy metal-associated proteins across angiosperms and ancestral plants has revealed evolutionary conservation and functional insights into HIPP proteins [34].

The HMA domain is defined by a ferredoxin-like structural fold ($\beta\alpha\beta\beta\alpha\beta$), spans approximately 70 amino acids, and harbors the Cys-XX-Cys metal-binding motif within its first loop, as confirmed by the crystal structure of the OsHIPP19 [8,14,27]. In the yeast Antioxidant Protein 1 (ATX1) protein, the coordination of cysteine residues in the HMA domain with Cu^{2+} is critical for the transfer of Cu^{2+}

between ATX1 and copper transporters [35]. For example, ATX1 can interact with Ca^{2+}-sensitive Cross Complementer (CCC2), a Golgi membrane-localized, Cu-transporting P-type ATPase, at the site where copper is loaded into newly synthesized metalloproteins [8,36]. Isoprenylation (also called prenylation) is a post-translational modification in which a 15-carbon farnesyl group or 20-carbon geranylgeranyl group is covalently attached via a thioether bond to the cysteine residue of a C-terminal CaaX motif, where 'C' represents cysteine, 'a' represents an aliphatic amino acid, and 'X' represents any amino acid [18,37]. Recent studies have found that poplar HIPPs are enriched in plasmodesmata and they may regulate intercellular metal ion or signaling molecule transport through lipid modification [12]. In addition, isoprenylation is also involved in dynamic subcellular localization transitions in response to stress. For example, the rice OsHIPP41 migrates from cytoplasm to perinuclear region under cold stress and activates the expression of stress-resistant genes [10]. More recent research has demonstrated that prenylation is largely required for the localization of AtHIPP7 to plasmodesmata in Arabidopsis [33]. However, the molecular mechanism by which isoprenylation dynamically controls HIPP subcellular localization and function remains unclear. Moreover, prenylated proteins are also important regulators of signal transduction, cytoskeletal organization and intracellular vesicle transport [37].

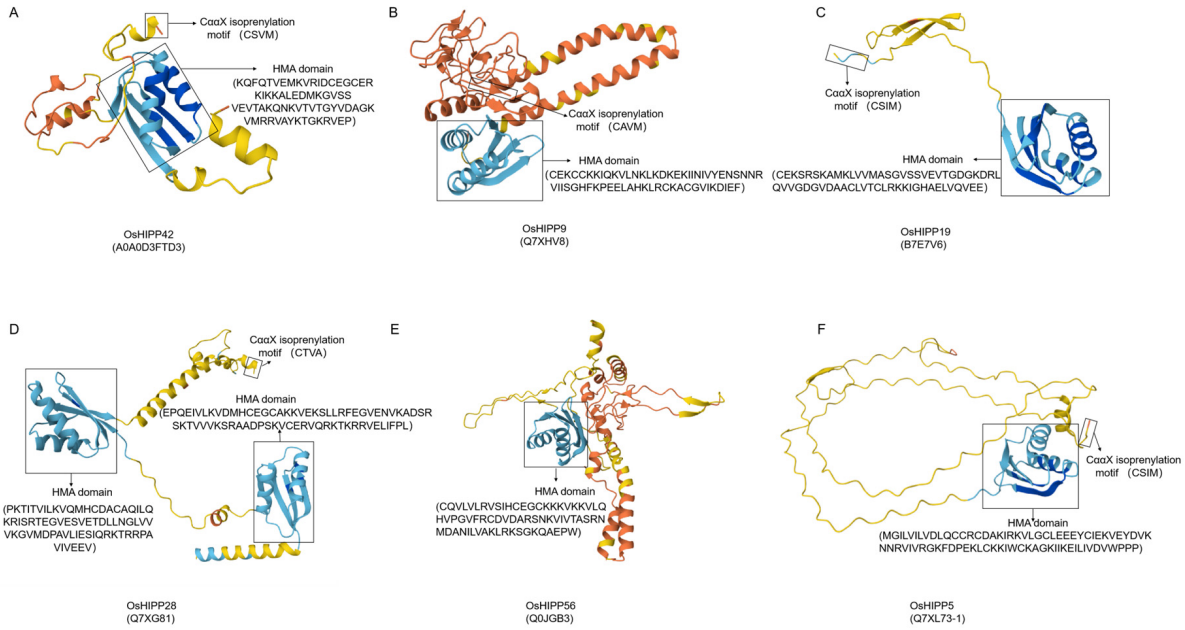

Figure 1: Predictions of HIPP proteins' structure in rice plants using AlphaFold 3. (**A**–**F**) Six representative rice HIPP proteins involved in metal homeostasis and plant immunity were analyzed for protein structure. The HMA domain and the isoprenylation motif positions and sequences in OsHIPP42, OsHIPP9, OsHIPP19, OsHIPP28, OsHIPP56, and OsHIPP5 are highlighted in a black box. UniProt accession numbers are provided in parentheses.

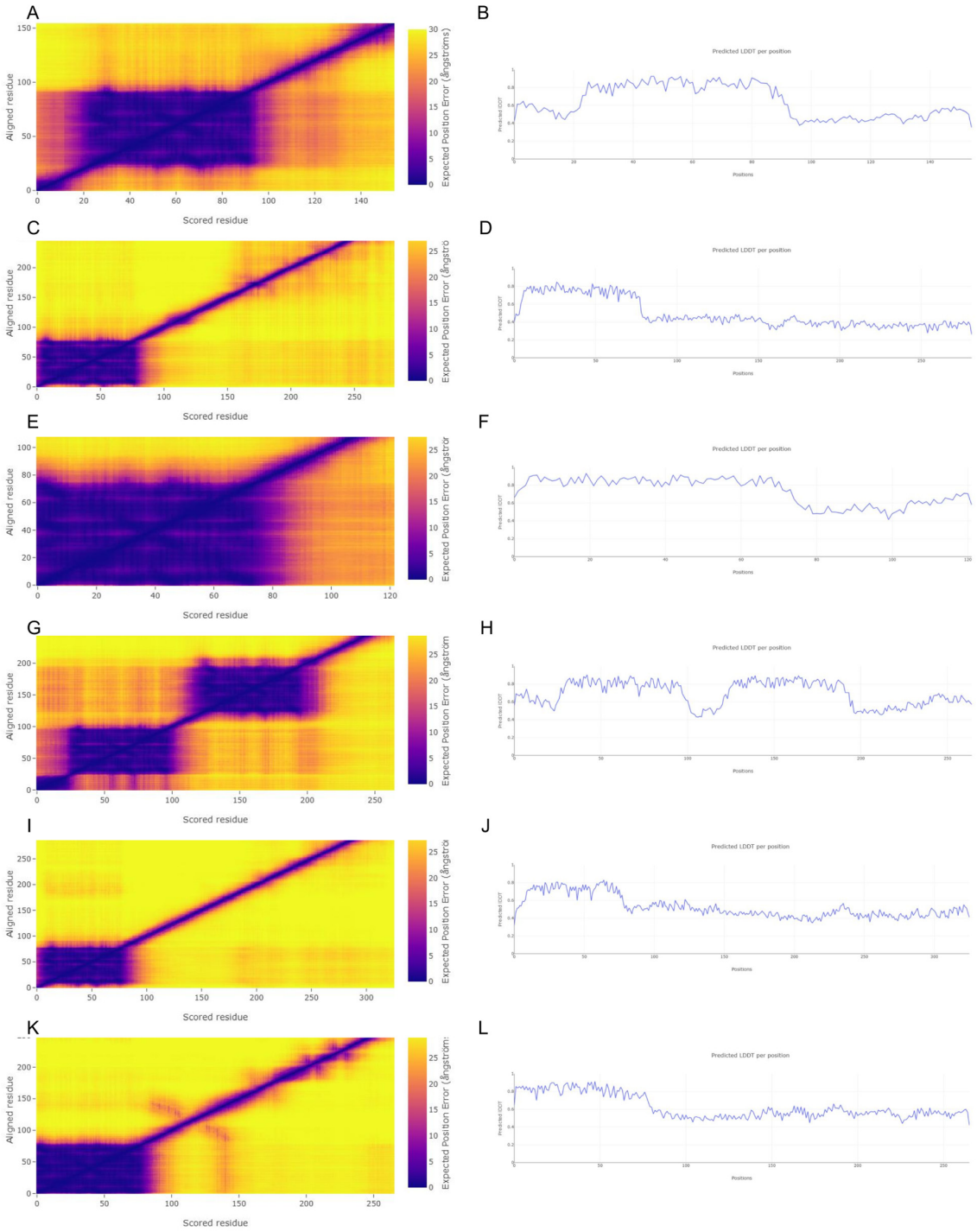

Figure 2: LDDT and PAE of predicted OsHIPPs proteins obtained using AlphaFold 3. LDDT: a per-residue confidence score that ranges from 0 to 1. Higher values (typically >0.9) indicate high confidence in the placement of local atoms, suggesting that the positions of the backbone and side chains are accurate (left panels). PAE: a heatmap that shows the confidence level of the model's prediction for the relative positions of residue pairs. Low PAE values indicate well-defined spatial relationships, while high values suggest uncertainty in the positioning of domains or chains. LDDT and PAE of predicted OsHIPP42 (**A**,**B**); OsHIPP9 (**C**,**D**); OsHIPP19 (**E**,**F**); OsHIPP28 (**G**,**H**); OsHIPP56 (**I**,**J**); and OsHIPP5 (**K**,**L**).

3 HIPPs Participate in Homeostasis and Detoxification of Heavy Metal Ions

3.1 Direct Metal Binding and Biochemical Evidence

Many studies have shown that plant HIPP proteins bind directly to metal ions (Table 1). For example, metal-chelate affinity chromatography experiments showed that recombinant ATFP3 (AtHIPP07) could bind Cu^{2+}, Ni^{2+} and Zn^{2+} while AtFP6 (AtHIPP26) could also bind Cu^{2+} Pb^{2+}, and Cd^{2+} *in vitro* [14,38]; Similarly, AtCdI19 (AtHIPP06) protein was detected by circular dichroism (CD) spectroscopy and shown to bind Cu^{2+} and Hg^{2+} [19]; Furthermore, purified AtHIPP33 protein directly bound with Cd *in vitro* [39]. Using co-incubation of proteins with heavy metal salts, Chen and Xiong [40] demonstrated that OsHIPP24 can chelate Cd^{2+} and Cu^{2+} *in vitro*. These assays establish direct metal-binding capacity, though the metal-binding stoichiometry and affinity constants remain largely undetermined for most HIPPs.

3.2 Protein-Protein Interactions in Metal Transfer

It has been reported that HIPPs may interact with other factors to facilitate the exchange of metal ions, ensuring proper transport and distribution of metals and maintaining metal homeostasis in plants [41]. Take *Saccharomyces cerevisae* ATX1 protein as an example: the MXCXXC motif in ATX1 interacts with the Cu^{2+}-binding domain (MTCXXC) of CCC2 indicating that ATX1 donates Cu^{2+} to CCC2 [42]. In Arabidopsis, the heavy-metal-binding farnesylated protein AtFP6 (AtHIPP26) interacts with acyl-CoA-binding protein ACBP2 to confer tolerance to Cd. Since both AtFP6 and ACBP2 bind Pb^{2+}, Cd^{2+}, and Cu^{2+}, Plasma membrane-localized ACBP2 and AtFP6 likely mediate Pb^{2+}, Cd^{2+} and Cu^{2+} transport in Arabidopsis roots [38]. Moreover, the R2R3-MYB transcription factor MYB49 binds to the promoter of *AtHIPP22* and *AtHIPP44*, resulting in upregulation of their expression and subsequent Cd accumulation in Arabidopsis [43]. In apple (*Malus domestica*), MdWRKY17 acts upstream of MdHIPP1 to enhance its expression and increase Cd tolerance by reducing Cd accumulation [44]. In Arabidopsis, the core autophagy protein ATG8e associates with HIPP33 and recruits it for autophagic degradation in an AIM (ATG8-interacting motif) dependent manner [39]. AtHIPP33 acts as a cargo receptor to modulate the Cd response by facilitating autophagy-mediated vacuolar sequestration of Cd. However, how ATG8 recruits AtHIPP33 remains unknown. Indeed, the exact mechanism by which metal ions are transferred from HIPPs to downstream transporters is still unresolved.

3.3 Transcriptional Responses and Functional Validation in Planta

HIPP genes respond to a variety of heavy metal stresses. The expression of *AtHIPP06* (*AtCdI19*) was induced by Cd^{2+}, Hg^{2+}, Fe^{2+}, and Cu^{2+}, while the expression of *AtHIPP26* (*AtFP6*) was induced by Cd^{2+} and Zn^{2+} in Arabidopsis [19,38]. The expression of *OsHIPP16*, *OsHIPP24*, and *OsHIPP42* was upregulated by Cu^{2+} and Cd^{2+}, whereas the expression of *OsHIPP33* was induced by Zn^{2+} and Fe^{2+} in rice [45,46]. Cd stress led to upregulation of *SlHIPP7*, *SlHIPP21*, *SlHIPP26*, and *SlHIPP32* in tomato roots [47]. The expression of *HIPP26* and *HIPP27* in *Brassica oleracea* var. *Acephala* (kale) was upregulated by $CdCl_2$ [13]. In addition, the expression level of *OsHIPP28* substantially increased in shoots but decreased in roots compared with the control under Mn or Cd treatment, whereas expression of *OsHIPP34* in roots was significantly induced under Mn, Cd, and Cu stress, but in shoots it was significantly reduced only under Mn stress [17]. In maize, Cd treatment confirmed that the expression levels of *ZmHIPP11*, *ZmHIPP30*, and *ZmHIPP48* were generally higher in shoots than roots, while *ZmHIPP02* and *ZmHIPP57* exhibited the opposite pattern [48]. In soybean (*Glycine max*), six genes (*GmHIPP9*, *GmHIPP13*, *GmHIPP29*, *GmHIPP43*, *GmHIPP58*, and *GmHIPP73*) were highly induced by Al [49]. These results indicate that the expression levels of HIPP family members differ across plant tissues under heavy metal stress, suggesting that HIPP family members may play distinct roles

in metal ion homeostasis and detoxification. It is important to note that transcriptional responsiveness (Class III evidence) alone does not demonstrate a functional role. We highlight cases where this has been validated by mutant or overexpression studies below.

Through genetic identification, functional characterization of HIPPs in heavy metal homeostasis has been well studied. Heterologous expression of *AtHIPP20*, *AtHIPP22*, *AtHIPP26*, and *AtHIPP27*, as well as *OsHIPP09* and *OsHIPP16*, in yeast enhanced the tolerance of cadmium-sensitive *ycf1* yeast mutant to cadmium, suggesting that HIPP may have Cd-detoxifying effects [17,18,20]. Moreover, heterologous expression of *OsHIPP17* in copper-sensitive yeast mutants decreased the tolerance to copper [50]. Similarly, heterologous expression of tea plants *CsHIPP22*, *CsHIPP24*, and *CsHIPP36* in Cd-sensitive *ycf1* yeast mutant improved tolerance to cadmium, with *CsHIPP24* conferring much greater cadmium tolerance than *CsHIPP22* and *CsHIPP36* [51]. The HIPP gene family in *Nelumbo nucifera* G. (lotus) plays a crucial role in Cd resistance, as NnHIPP14, NnHIPP21, and NnHIPP33 conferred varying degrees of Cd tolerance when overexpressed in yeast [52]. Functional validation in yeast demonstrated that sorghum SbHIPP40 overexpression enhanced Cd tolerance in the *ycf1* mutant strain [53]. Heterologous expression of *Solanum americanum* SaHIPP41 in yeast enhanced Cd accumulation and sensitivity, a function dependent on its conserved CxxC domain [54]. Overexpression of *TaHIPP1* in the yeast (*Schizosaccharomyces pombe*) significantly increased the cell growth rate under Cu^{2+} stresses [55]. Collectively, these findings suggest that while certain HIPP members are conserved in Cd accumulation, the family as a whole exhibits functional plasticity across different metals and physiological outcomes.

Recent studies have also examined the function of HIPPs in Cd accumulation in relevant transgenic plants. For example, overexpression of *AtHIPP06* and *AtHIPP26* confers tolerance to Cd, while mutant *athipp20/21/22* increases sensitivity to cadmium in Arabidopsis [18,19]. The growth of *OsHIPP16/29/56* overexpression rice plants improved significantly under excessive Cd treatment [21–23]. More recently, overexpression of *IbHIPP7* in sweet potato reduced Cd accumulation and alleviated Cd toxicity, suggesting its potential for phytoremediation or safe crop production [56]. The metallochaperone OsHIPP53 reduces Cd accumulation specifically in rice roots, likely by sequestering Cd and limiting its translocation to shoots [57]. In *Medicago sativa* (alfalfa), overexpression of *MsHIPP12* in Arabidopsis enhances cadmium tolerance by increasing antioxidant enzyme activity and promotes cadmium detoxification by reducing Cd uptake in transgenic lines and mitigating oxidative stress [58]. SbHIPP40 overexpression rice plants accumulated 1.68- to 3.92-fold higher Cd in stems, leaves, and grains compared with wild-type plants, indicating its potential functional role in Cd tolerance in sorghum [53]. The regulatory mechanisms of macroautophagy/autophagy in plant tolerance to Cd remain poorly elucidated. A Recent study showed that overexpression of *SaHIPP41* in the hyperaccumulator *Solanum americanum* enhances Cd accumulation and improves tolerance to Cd stress, offering a valuable gene for phytoremediation [54]. Although HIPPs are involved in Cd accumulation, a striking pattern emerges: some HIPPs reduce Cd accumulation in shoots and grains (e.g., OsHIPP29, OsHIPP53, IbHIPP7), while others promote Cd accumulation, especially in hyperaccumulator species (e.g., SaHIPP41). We propose that HIPPs involved in vacuolar sequestration or root retention reduce shoot/grain Cd, whereas those that enhance xylem loading or leaf storage increase whole-plant Cd accumulation. This distinction is critical for breeding low-Cd crops versus engineering plants for phytoremediation.

In addition to Cd, HIPPs are also involved in other metal metabolism processes in plant cells. For example, *GmHIPP29* overexpression significantly increased Al accumulation in the cell sap of the transgenic soybean hairy root tips, leading to increased Al sensitivity [49]. However, other *GmHIPP* genes, such as *GmHIPP9*, *GmHIPP13*, *GmHIPP43*, *GmHIPP58*, and *GmHIPP73*, are also significantly induced by Al. Whether

these homologous genes have similar or redundant functions requires further investigation. A high-mobility group protein, MdHMGB15, enhances zinc (Zn) tolerance in apple by activating multiple metal homeostasis genes, including *MdHIPP37*, thereby improving metal detoxification and reducing Zn-induced oxidative damage [59]. Notably, research on HIPPs in metal metabolism (other than Cd) is still limited, which is of great interest to the global scientific community.

In addition to binding heavy metal ions or interacting with other proteins to regulate the metal homeostasis in plants, HIPPs can also regulate hormone-dependent signaling pathways to maintain metal homeostasis. Melatonin mediates lead (Pb) accumulation reduction in radish taproot via the RsWRKY26-RsHIPP26-RsASMT2 module, integrating melatonin signaling with HIPP-mediated metal detoxification [60]. In tomato, *SlHIPP4*, *SlHIPP7*, *SlHIPP9*, *SlHIPP21*, *SlHIPP26*, and *SlHIPP32* under cadmium stress caused a decrease in root length and plant height, as well as a decrease in the levels of ABA and salicylic acid (SA), while *SlHIPP7* and *SlHIPP32* led to a decrease in Methyl jasmonate (MeJA) levels [45]. These results suggest that HIPP may respond to metal stress through ABA-, SA-, and MeJA-dependent signaling pathways, and the underlying mechanism should be further investigated.

Table 1: Functions of HIPP family genes in homeostasis and detoxification of heavy metal ions. Based on the levels of evidence in each study, the table classifies evidence strength more clearly into three levels: Class I (Direct metal binding and biochemical evidence), Class II (genetic evidence in planta or yeast complementation), or Class III (correlative expression data).

Species	Gene	Class (I/II/III)	Function/Biological Role	Reference
Rice (*Oryza sativa*)	OsHIPP21/28/41	III	Expression is increased by $CdCl_2$ treatment	[10]
	OsHIPP9	I/II	Binds Cd and Cu *in vitro*; knockout of *OsHIPP9* increased the Cd concentrations of the upper nodes and panicle, but decreased Cd in expanded leaves, and decreased Cu uptake and accumulation in rice	[20]
	OsHIPP16	II/III	upregulated by Cd and Cu stress; overexpression improved plant growth significantly under excessive Cd treatment	[17,22]
	OsHIPP17	II	Negative regulation: enhances copper sensitivity in yeast and Arabidopsis; rice mutants show growth inhibition and Cu accumulation in roots	[50]
	OsHIPP24	II	Heterologous expression in yeast leads to more Cd/Cu accumulation	[40]
	OsHIPP29	II	Detoxifies Cd by reducing its accumulation in *OsHIPP29* overexpression transgenic rice plants	[21]
	OsHIPP33	II/III	upregulated under excessive Zn and Fe stress; accumulation of Zn and Fe in *OsHIPP33* RNAi rice was reduced	[46]
	OsHIPP42	II/III	Expression is induced by excessive Cd, Mn, and Cu; *oshipp42* mutants significantly reduce tolerance to Cd, Mn, Zn, and Cu exposure	[17]
	OsHIPP56	II	Overexpression reduces Cd content in rice	[23]
	OsHIPP34/60	II/III	Induced by excessive Cd, Mn, and Cu, OsHIPP34 and OsHIPP60 were randomly selected to be expressed in yeast (*Saccharomyces cerevisiae*) mutants *pmrl*, *cup2*, *ycf1*, and *zrc1*, exhibiting sensitivity to Mn, Cu, Cd, and Zn toxicity,	[17]
	OsHPP08	II	OsHPP08 plays a role in regulating Cu uptake in rice	[61]
	OsHIPP53	I/II	OsHIPP53 directly binds Cd *in vitro*; *oshipp53* mutant lines exhibited heightened Cd sensitivity, elevated root Cd concentrations, and restricted Cd translocation to the shoots.	[57]

Table 1: *Cont.*

Species	Gene	Class (I/II/III)	Function/Biological Role	Reference
Arabidopsis (*Arabidopsis thaliana*)	AtHIPP20/22/26/27	II	Increase cadmium tolerance in Cd-sensitive yeast; the *athipp20/21/22* triple mutant is more sensitive to Cd and accumulates more Cd	[18]
	AtHIPP44	II	MYB49 binds to the promoter of *AtHIPP44*, resulting in upregulation of its expression and subsequent Cd accumulation	[43]
	AtHIPP26	II/III	Overexpression increases Cd tolerance in Arabidopsis; it binds Cd, Pb, and Cu *in vitro*. Expression is induced by Cd	[27]
	AtFP3	I	Binds Cu, Ni, Zn *in vitro*	[14]
	AtCdI19 (AtHIPP6)	I/II	Binds Cd directly *in vitro*; Overexpression of the CdI19 conferred Cd tolerance in transgenic Arabidopsis	[19]
	HIPP33	II	Mediates selective autophagy-dependent vacuolar Cd sequestration	[39]
Arabis paniculata	ApHIPP26	II	Heterologous overexpression in Arabidopsis enhances Cd tolerance and accumulation.	[62]
Sugar beet (*Beta vulgaris*)	BvHIPP32	II/III	Expression increased by silicon and *BvHIPP32* promotes cadmium accumulation in the root cell wall under silicon treatment	[63]
Grapevine (*Vitis vinifera*)	VvHIPP21	II	Heterologous expression in Arabidopsis alleviates Cd and Cu toxicity.	[28]
Maize (*Zea mays*)	ZmHIPP family	II	ZmHIPP regulates lead tolerance in maize seedlings	[64]
Tomato (*Solanum lycopersicum*)	SlHIPP7/21/26/32	III	Expression was in response to Cd exposure	[47]
Wheat	HIPP1-V	II/III	*HIPP1-V* was upregulated in *H. villosa* after Cd treatment, and transgenic wheat plants overexpressing HIPP1-V showed enhanced Cd tolerance	[65]
Kale (*Brassica oleracea*)	HIPP26/27	III	The expression was induced under Cd stress.	[13]
Sweetpotato (*Ipomoea batatas*)	IbHIPP7	II	Overexpression reduces Cd accumulation and alleviates Cd toxicity	[56]
Soybean (*Glycine max*)	GmHIPP family	II/III	Six genes (GmHIPP9/13/29/43/58/73) were highly induced by Al; *GmHIPP29*-overexpression significantly increased Al accumulation	[49]
Lotus (*Nelumbo nucifera*)	NnHIPP family	II	NnHIPP14, NnHIPP21, and NnHIPP33 conferred varying degrees of Cd tolerance when overexpressed in yeast	[52]
Apple (*Malus domestica*)	MdHIPP1	II	Activated by MdWRKY17 to enhance Cd tolerance	[44]
Medicago sativa	MsHIPP12	II	Overexpression of *MsHIPP12* in Arabidopsis enhances cadmium tolerance	[58]
Sorghum (*Sorghum bicolor*)	SbHIPP40	II	*SbHIPP40* overexpression enhanced Cd tolerance in the *ycf1* mutant strain	[53]
Apple (*Malus domestica*)	MdHMGB15	II	Activates multiple metal homeostasis genes and enhances Zn tolerance	[59]
Solanum americanum	SaHIPP41	II	Heterologous expression of *Solanum americanum* SaHIPP41 in yeast enhances Cd accumulation; Overexpression in the hyperaccumulator *Solanum americanum* enhances Cd accumulation and tolerance	[54]
Grapevine (*Vitis vinifera*)	VvHIPP30	II	Overexpression of VlHIPP30 enhances copper metabolism	[66]
Radish (*Raphanus sativus*)	RsHIPP26	II	Melatonin mediates lead (Pb) accumulation reduction in radish taproot via the RsWRKY26-RsHIPP26-RsASMT2 module	[60]
Tartary buckwheat	HIPPs	III	Genome-wide identification of HIPPs that were responsive to Cd stress	[67]

4 HIPPs Are Involved in the Response to Abiotic Stress in Plants

Besides chelating cytoplasmic metal ions to mediate heavy metal accumulation and detoxification, HIPPs may also function in other abiotic stress responses, as evidenced by the responsiveness of several rice HIPP genes to cold, drought, and salt stress [10] (Table 2). We distinguish between transcriptional responsiveness (Class III) and functionally validated roles (Class II) below. For example, *OsHIPP23* and *OsHIPP15* are downregulated under drought and salt stress, respectively. *OsHIPP41* exhibits upregulation under both drought and cold conditions. In contrast, *OsHIPP11* and *OsHIPP45* show downregulation exclusively in response to cold stress. Although expression profiling suggests involvement, functional proof via mutant analysis is lacking for *OsHIPP11* and *OsHIPP45* in cold stress. Genome-wide identification of HIPP genes and subsequent functional analysis of *GsHIPP79* in wild soybean demonstrated that *GsHIPP79* is involved in the alkaline stress response, expanding the knowledge of HIPPs in response to abiotic stress beyond heavy metals [68].

4.1 Protein–Protein Interaction-Mediated Stress Regulation

HIPPs regulate abiotic stress tolerance by interacting with other proteins. In Arabidopsis, *AtHIPP26* is induced during drought, cold, and salt stress. ATHB29, a zinc-finger homeodomain transcription factor involved in drought stress response, was shown to interact strongly with AtHIPP26 through the two central cysteines of the AtHIPP26 heavy metal-associated domain [27]. However, the interaction between HIPP26 and ATHB29 in response to different stress conditions requires further investigation. In *Chenopodium quinoa*, CqHIPP34 can interact with a drought-responsive zinc finger homologous domain protein CqZF-HD14. Transient overexpression of *ZF-HD14* or *CqHIPP34* alone enhances drought tolerance, while transient co-overexpression of *CqHIPP34* and *CqZF-HD14* results in a significant increase in drought tolerance. This result suggests that they may cooperate to withstand drought stress [69]. In sweet cherry, a basic helix-loop-helix protein, PavHLH106, interacts with PavHIPP16 to positively regulate the cold tolerance of tobacco [70,71]. Interestingly, in grape, *VvHIPP21* negatively regulates the cold tolerance by interacting with the E3 ubiquitin ligase High Expression of Osmotically Responsive Gene 1, VvHOS1, to co-regulate cold stress [28]. These opposite functions may arise from species-specific evolutionary adaptations, differences in interacting partners, or distinct downstream signaling pathways. Therefore, the role of HIPP proteins in cold response is not inherently conserved but rather depends on the broader regulatory context within each species. Further comparative studies are needed to elucidate the molecular mechanisms underlying such functional diversification. The HIPP26L-Nuclear Factor Y subunit C9 (NF-YC9)-Salt Responsive MYB Transcription Factor (SRMT) module regulates drought response, demonstrating a signaling pathway where an HIPP protein participates in abiotic stress adaptation in poplar [72]. It is noteworthy that the experiments were restricted to poplar seedling saplings in a greenhouse. Because poplar is a perennial species, future studies should extend the growth period and incorporate field experiments to achieve a more comprehensive perspective.

4.2 Hormone Pathway Associations

In addition to protein interactions, HIPP genes also play a role in the phytohormone signaling networks that operate under abiotic stress. Although *HvFP1* is induced by drought, senescence, and ABA treatment, its overexpression represses ABA biosynthesis-related genes, suggesting that *HvFP1* participates in a negative feedback loop within the ABA signaling pathway [26]. In tomato, SlHIPP4, SlHIPP7, SlHIPP9, SlHIPP21, SlHIPP26, and SlHIPP32 physically interact with hormone-responsive transcription factors in yeast two-hybrid assays, and their co-expression with ABA- and JA-signaling components suggests they

form stress-responsive protein complexes [47]. However, observed changes in hormone levels should be viewed as possible signaling associations rather than confirmed causal mechanisms unless supported by genetic epistasis or inhibitor studies.

Table 2: HIPP family genes in response to abiotic stress in plants. Class II (genetic evidence in planta or yeast complementation), Class III (correlative expression data).

Species	Gene	Class (II/III)	Function/Biological Role	Reference
Rice (*Oryza sativa*)	OsHIPP11/45	III	Transcriptionally responsive to cold stress	[10]
	OsHIPP15	III	Transcriptionally responsive to salt stress	
	OsHIPP23/40	III	Transcriptionally responsive to drought stress	
	OsHIPP41	III	Transcriptionally responsive to drought and cold stress	
Arabidopsis (*Arabidopsis thaliana*)	AtHIPP26	III	Interacts with transcription factor ATHB29 and responds to drought stress	[27]
Quinoa (*Chenopodium quinoa*)	CqHIPP34	II	Interacts with CqZF-HD14 to enhance drought tolerance in quinoa	[69]
Barley (*Hordeum vulgare*)	HvFP1	III	Transcriptionally responsive to drought, salt, cold stress, and leaf senescence	[73,74]
Wheat (*Triticum aestivum*)	TaHIPP1	III	Responsive to drought, cold, high light, ABA, and leaf senescence	[75]
Grapevine (*Vitis vinifera*)	VvHIPP21	II	Negatively regulates cold and drought stress; heterologous expression in Arabidopsis increases cold sensitivity	[28]
Nicotiana benthamiana	HIPP26	III	Upregulated under drought tolerance	[31]
Sweet cherry (*Prunus avium*)	PavHIPP16	II	*PavHIPP16* OE Arabidopsis thaliana exhibited enhanced adaptability compared to WT plants under cold conditions	[76]
Tomato (*Solanum lycopersicum*)	SlHIPP4/7/9/21/26/32	III	Transcriptional response to osmotic and salt stresses	[47]
Tobacco (*Nicotiana tabacum*)	NtHIPP family	III	Transcriptional response to cold and drought stresses	[24]
Glycine soja	GsHIPP79	II/III	Upregulated by alkaline stress; Overexpression of GsHIPP79 in transgenic soybean hairy roots conferred enhanced alkaline stress tolerance	[68]
Cassava (*Manihot esculenta*)	HIPP family	III	Transcriptional response to drought stress	[25]
Poplar (*Populus*)	HIPP26L-NF-YC9-SRMT	II/III	Upregulated by drought stress in poplar; Overexpression enhances drought tolerance of poplar	[72]
Sugar beet	BvHIPP32	II/III	Si induced BvHIPP32 expression	[63]

5 HIPPs Are Involved in Plant-Pathogen Interactions

Plants can recognize effector proteins secreted by microbial plant pathogens by plant intracellular nucleotide-binding leucine-rich repeat (NLR) receptors, which contain an HMA domain or other proteins like some HIPPs to induce an immune response [45,77] (Table 3). HIPP roles in immunity can be classified as effector targets, susceptibility/resistance factors, or components of ubiquitination/receptor-associated immune pathways.

5.1 HIPPs as Effector Targets

AVR-Pik is an effector protein secreted by *Magnaporthe oryzae* during infection of rice leaves, and it exists in multiple variants. To date, six AVR-Pik (A-F) variants have been identified in rice [29]. The study demonstrates that rice OsHIPP19 is a genuine virulence target of the *Magnaporthe oryzae* effector AVR-Pik. The effector binds the HMA domain of OsHIPP19 with high affinity, including stealthy variants such as

AVR-PikC and AVR-PikF that escape nucleotide-binding and leucine-rich repeat (NLR) domain-containing immune receptor Pik-1. In contrast, the integrated OsHIPP19-HMA domain of the NLR receptor Pik-1 acts as a decoy that structurally mimics the HIPP target and directly binds AVR-Pik to trigger immunity. This highlights the pathogen's evolutionary strategy to bypass host immunity while retaining its ability to interact with host targets critical for virulence [77]. Notably, OsHIPP19 is not a helper component, nor is it physically integrated into an NLR receptor; instead, it is a host susceptibility factor that the pathogen manipulates, and scientists have independently co-opted its HMA domain into an NLR as a surveillance module. The *Magnaporthe oryzae* effector Pwl2 alters HIPP43 localization in host cells to suppress plant immunity, demonstrating a pathogen strategy to manipulate host metal-associated proteins [78]. Moreover, a recent study found that a novel effector, MgMO289, interacts with a new rice copper metallochaperone OsHPP04. Overexpressing *OsHPP04* or *MgMO289* exhibited an increased susceptibility to *Meloidogyne graminicola* [79]. *OsHIPP28* contains two HMA domains and is preferentially expressed in leaf blades and sheaths, implicating it in defense mechanisms associated with photosynthetic tissues. The interaction between OsHIPP28 and the fungal effector protein RsMf8HN has been experimentally validated. This interaction likely facilitates pathogen infection by interfering with host metal metabolism or immune signaling pathways, thereby attenuating plant defense responses [80]; however, this mechanism requires further investigation.

5.2 HIPPs as Susceptibility or Resistance Factors and Components of Ubiquitination/Receptor-Associated Immune Pathways

Isopreneylated motifs and proline-rich regions in HIPPs also play an important role in pathogen resistance [75]. OsHIPP5, also known as Pi21, is a negative regulator in rice blast disease. Pi21 may help optimize defense mechanisms by slowing the initiation of plant defense responses, but it weakens resistance to the pathogen *Magnaporthe oryzae* in both rice and Arabidopsis [81,82]. The *pi21* mutant is missing a proline-rich region. pi21-mediated resistance does not rely on hypersensitive response (HR), but is achieved by delaying the expansion of pathogenic hyphae between cells [81]. These findings suggest that the integrity of the proline-rich motif in the OsHIPP5 protein is closely associated with susceptibility to rice blast disease. Additionally, the role of ubiquitination in HIPP-mediated immunity is also of great interest. HIPP1-V from *Haynaldia villosa* positively regulates resistance to powdery mildew (Pm) caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*). Overexpression of *HIPP1-V* significantly inhibited haustorium formation, reduced the infection index, and provided broad-spectrum resistance against 10 Pm isolates. Importantly, isoprenylation of HIPP1-V was essential for its plasma membrane (PM) localization, interaction with the E3 ligase CMPG1-V, and Pm resistance function [75]. The prenylation modification ensures that HIPP1-V localizes to the plasma membrane and recruits CMPG1-V to the PM to form a defense complex. Inhibition of isoprenylation, either by mutating the CaaX motif or using the inhibitor tipifarnib, results in the shedding of HIPP1-V from the PM and significantly reduces disease resistance [75]. OsFBX388, an E3 ligase, orchestrates the ubiquitination of OsHIPP56 and blast fungus MAX effectors in a coordinated manner, a process that regulates rice immunity [83]. Notably, the question remains unresolved whether the "dual ubiquitination" strategy, whereby OsFBX388 ubiquitinates both host HIPP proteins and pathogen effectors, commonly exists in other plant-pathogen interaction systems. How is this dynamic balance regulated, and what are the mechanisms underlying the conservation and specificity of ubiquitination-mediated regulation among different HIPP proteins?

5.3 The Metal–Immunity Interface

Moreover, HIPP can also be involved in the response of pathogenic microorganisms through hormone-dependent pathways. In Arabidopsis, the zinc-binding nuclear protein HIPP3 has been identified as a dual-function regulator that operates upstream of both the SA-dependent immune pathway and flowering time regulation. Zschiesche et al. [84]. demonstrated through experimental inoculation with the pathogenic bacterium *Pseudomonas syringae* pv. Tomato, that *AtHIPP3* expression undergoes rapid and significant induction following pathogen infection in Arabidopsis leaves. Mechanistic studies further revealed that HIPP3 acts as a negative regulator of the SA signaling pathway. This regulatory function may be mediated through its zinc-binding capacity, potentially modulating the activity of key immune proteins such as Nonexpressor of pathogenesis-related genes 1, NPR1, thereby suppressing the activation of SA-dependent immune responses [84]. Chitin elicitor receptor kinase 1, OsCERK1, physically interacts with and phosphorylates OsHPP08, regulating copper uptake and conferring blast resistance in rice, linking copper homeostasis with disease resistance [61], which deepens our understanding of the intricate interplay between biotic and abiotic signals in rice. The VlMYB149-VlHIPP30 regulatory module confers enhanced resistance to *Botrytis cinerea* in grapevine by upregulating the antioxidant system and copper metabolism [66]. The emerging picture is that pathogens target HIPPs to perturb host metal pools, suppress reactive oxygen species (ROS) bursts, and subvert hormone signaling. Conversely, plants may have co-opted these metallochaperones as surveillance components. However, the exact molecular consequences for immunity remain unresolved for several interactions, and definitive evidence of immune signaling function requires further genetic dissection.

Table 3: HIPP family genes involved in plant immunity and development. Class II (genetic evidence in planta or yeast complementation), Class III (correlative expression data).

Species	Gene	Class (II/III)	Function/Biological Role	Reference
Rice (*Oryza sativa*)	OsHIPP28	III	Interacts with the Rhizoctonia solani secreted protein RsMf8HN, and expression is in response to fungal infection	[80]
	OsHIPP5 (Pi21)	II	Contributes to a particularly durable resistance to a fungal rice blast disease	[81]
	OsHPP04	II	Overexpression increased plant susceptibility to M. graminicola	[79]
	OsHIPP19	II	Its HMA domain interacts with the blast fungus effector AVR-Pik, activating plant immunity	[29]
	OsHIPP56	II	OsHIPP56 overexpression enhances resistance to rice blast and bacterial blight	[83]
	OsHIPP16	II	OsHIPP16 mediates ovule development	[85]
	OsHIPP20	II	OsHIPP20 causes enhanced disease resistance towards the blast pathogen	[86]
Arabidopsis (*Arabidopsis thaliana*)	AtHIPP1	II	Triggers degradation of cytokinin oxidase/dehydrogenase CKX1	[33]
	AtHIPP3/6	II/III	Overexpression causes delayed flowering; regulates the salicylic acid-dependent pathogen defense pathway; is responsive to bacterial infection	[84]
	AtHIPP7	II	Interacts with CKX1; altered development of overexpressing plants is causally linked to enhanced cytokinin activity	[33]
	AtHIPP40 (AtHMAD1)	II	Knockout confers resistance to virulent Pseudomonas (DC3000)	[87]
Barley	HIPP43	II	HIPP43 overexpression increased disease susceptibility	[78]

Table 3: *Cont.*

Species	Gene	Class (II/III)	Function/Biological Role	Reference
Wheat (*Triticum aestivum*)	TaHIPP1	II	Knock-down plants show improved *stripe rust* resistance	[75]
Nicotiana benthamiana	HIPP26	II/III	Responds to Potato mop-top virus (PMTV) infection; interacts with the viral movement protein TGB1, involved in long-distance movement of the virus	[31]
Kiwifruit (*Actinidia*)	AcHIPP26	II	May serve as a potential target of AvrPto5 during *Pseudomonas syringae* pv. *actinidiae* infection	[30,83]
Grapevine (*Vitis vinifera*)	VlHIPP30	II	Enhances the antioxidant system and copper metabolism for Botrytis resistance	[66]

6 HIPP Is Involved in Plant Growth and Development

HIPP may also be involved in the regulation of plant growth and development (Table 3). For example, overexpression of *PavHIPP16* in tobacco promotes germination and root elongation [76]; however, the underlying mechanism remains unknown. Additionally, overexpression of *AtHIPP3* resulted in delayed flowering in Arabidopsis; however, the exact mode of action of *AtHIPP3* in flower development has not been clarified [84]. Moreover, long-term field trials demonstrated that the mutation of *OsHIPP33* resulted in shortened internodes, decreased tillering, stunted panicle development, and significantly reduced grain fertility and yield [46], but the underlying mechanism by which *OsHIPP33* regulates agronomic traits requires further investigation.

6.1 Cytokinin Signaling and ERAD

The plant hormone cytokinin mediates diverse developmental processes, including differentiation in meristems and cell proliferation and developing organs, the onset of senescence, and responses to environmental signals [33]. HIPP affects the stability of cytokinin oxidase (CKX) by regulating the endoplasmic reticulum-associated degradation (ERAD) pathway in Arabidopsis, thereby finely regulating cytokinin signaling and plant development [33]. Specifically, AtHIPP7 interacts with CKX1 and promotes its degradation via the ERAD pathway, leading to elevated cytokinin levels. This results in altered root meristem size, delayed leaf senescence, and modified shoot architecture. The interaction is mediated by the isoprenylation motif of HIPPs, revealing a prenylation-dependent regulatory mechanism that directly impacts developmental programs. Nevertheless, the mechanism by which HIPPs execute their function remains unclear. It remains to be determined, for example, whether HIPPs act solely through interaction with ERAD substrates or whether they also target components of the ERAD machinery.

6.2 Direct vs. Indirect Developmental Effects

Notably, although the HIPP proteins have been demonstrated to be involved in the development of many species, the underlying molecular mechanism remains largely unknown. It is critical to distinguish whether developmental phenotypes are direct consequences of HIPP activity or secondary effects of altered metal homeostasis and hormone signaling. For CKX-regulating HIPPs, the effect on cytokinin signaling is likely direct [33]. For OsHIPP33, the dwarf and fertility phenotypes may arise from both Zn/Fe deficiency-induced metabolic stress and altered hormonal balance [46]. Future studies should uncouple these possibilities using tissue-specific complementation and metal supplementation experiments.

7 Conclusions and Future Perspectives

Plant HIPPs are a unique family of metallochaperones that integrate heavy metal homeostasis, abiotic stress tolerance, and pathogen resistance through their HMA domains and isoprenylation motifs (Fig. 3). Recent genome-wide studies across multiple crop species have greatly expanded our understanding of HIPP family diversity and stress-responsive expression patterns. Functional analyses have revealed both positive and negative regulators of metal tolerance, as well as novel mechanisms such as autophagy-mediated Cd sequestration and ubiquitination-based immune regulation. A conceptual model emerges in which HIPPs serve as molecular hubs that coordinate environmental sensing, metal buffering, and signaling across multiple stress and developmental pathways.

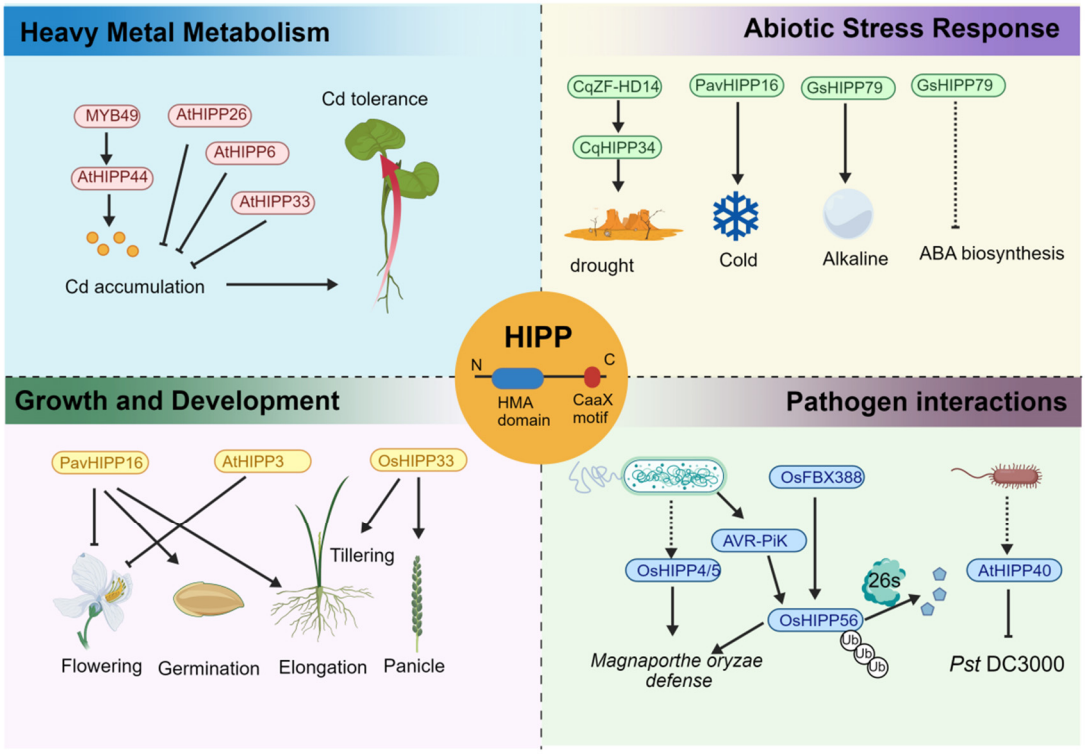

Figure 3: Schematic representation of the multifunctional roles of HIPP proteins in plants. The central structure shows the conserved N-terminal HMA domain (with the Cys-XX-Cys metal-binding motif) and the C-terminal CaaX isoprenylation motif, which anchors HIPPs to membranes. Four surrounding quadrants summarize the major biological functions: (1) heavy metal detoxification (e.g., Cd^{2+}), leading to reduced metal accumulation and enhanced tolerance; (2) abiotic stress responses (drought, cold, salt, alkaline) through interactions with transcription factors and involvement in hormone signaling (e.g., ABA); (3) regulation of plant growth and development, including flowering, germination, spike development, and root elongation; and (4) plant–pathogen interactions, where HIPPs are targeted by pathogen effectors or act as resistance factors against bacterial pathogens. The schematic was constructed using BioGDP (www.BioGDP.com). Solid represents direct binding, and dotted represents hypothesized connections.

Future research should focus on a set of unresolved problems and potential applications of HIPPs in agriculture. For example, elucidating metal transfer mechanisms will require structural biology approaches such as cryo-EM, together with *in vitro* reconstitution assays, to determine how HIPPs deliver metal ions to downstream transporters. The dynamic regulation of isoprenylation can be investigated in parallel using chemical biology tools, including photoactivatable prenyl probes, combined with live-cell imaging to

track HIPP relocalization in real time after exposure to stress. In order to overcome functional redundancy, which has long masked single-mutant phenotypes, the CRISPR-based multiplex editing of HIPP genes, as well as single-cell transcriptomics can help exploring their core functions. It is important to engineer HIPP specificity via structure-guided mutagenesis which offers the possibility of altering metal selectivity, for example, enhancing Cd binding for phytoremediation or designing HIPP decoys that interacting with pathogen effectors without disrupting endogenous metal homeostasis. On the other hand, low-cadmium crop varieties could be developed by overexpression or gain-of-function of HIPP genes under root-specific promoters. It has been proven that grafting can be an effective strategy for reducing heavy metal accumulation in the edible parts of plants while maintaining crop productivity [88]. This example supports our argument that both genetic and agronomic approaches can be leveraged to mitigate heavy metal stress in crop production systems. Additionally, overexpressing HIPPs in non-food plants may enable rhizofiltration, while integrating HIPP-mediated stress priming (or eustress/hormesis) into crop management could exploit controlled low-dose stress to pre-activate HIPP-dependent protective pathways [89]. Finally, the interplay between HIPP-mediated metal homeostasis and plant immunity should be dissected using dual-transcriptomic analyses performed during infection, in conjunction with metal-flux biosensors.

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References

1. Zhao FJ, Ma Y, Zhu YG, Tang Z, McGrath SP. Soil contamination in China: Current status and mitigation strategies. Environ Sci Technol. 2015;49(2):750–9. [CrossRef].
2. Clemens S, Ma JF. Toxic heavy metal and metalloid accumulation in crop plants and foods. Annu Rev Plant Biol. 2016;67:489–512. [CrossRef].
3. Rizwan M, Ali S, Adrees M, Rizvi H, Zia-Ur-Rehman M, Hannan F, et al. Cadmium stress in rice: Toxic effects, tolerance mechanisms, and management: A critical review. Environ Sci Pollut Res Int. 2016;23(18):17859–79. [CrossRef].
4. Song Y, Wang Y, Mao W, Sui H, Yong L, Yang D, et al. Dietary cadmium exposure assessment among the Chinese population. PLoS One. 2017;12(5):e0177978. [CrossRef].
5. Burkhead JL, Gogolin Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon M. Copper homeostasis. New Phytol. 2009;182(4):799–816. [CrossRef].
6. Jones JD, Dangl JL. The plant immune system. Nature. 2006;444(7117):323–9. [CrossRef].
7. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, di Pietro A, Spanu PD, et al. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012;13(4):414–30. [CrossRef].
8. Robinson NJ, Winge DR. Copper metallochaperones. Annu Rev Biochem. 2010;79:537–62. [CrossRef].

9. Rono JK, Sun D, Yang ZM. Metallochaperones: A critical regulator of metal homeostasis and beyond. Gene. 2022;822:146352. [CrossRef].
10. de Abreu-Neto JB, Turchetto-Zolet AC, de Oliveira LF, Zanettini MH, Margis-Pinheiro M. Heavy metal-associated isoprenylated plant protein (HIPP): Characterization of a family of proteins exclusive to plants. FEBS J. 2013;280(7):1604–16. [CrossRef].
11. Tan M, Cheng D, Yang Y, Zhang G, Qin M, Chen J, et al. Co-expression network analysis of the transcriptomes of rice roots exposed to various cadmium stresses reveals universal cadmium-responsive genes. BMC Plant Biol. 2017;17(1):194. [CrossRef].
12. Barr ZK, Werner T, Tilsner J. Heavy metal-associated isoprenylated plant proteins (HIPPs) at plasmodesmata: Exploring the link between localization and function. Plants. 2023;12(16):3015. [CrossRef].
13. Šutković J, van Wieren A, Peljto E, Yildirim A. Phytoremediation potential of *Brassica oleracea* varieties through cadmium tolerance gene expression analysis. J Genet Eng Biotechnol. 2024;22(2):100381. [CrossRef].
14. Dykema PE, Sipes PR, Marie A, Biermann BJ, Crowell DN, Randall SK. A new class of proteins capable of binding transition metals. Plant Mol Biol. 1999;41(1):139–50. [CrossRef].
15. Freisinger E. Structural features specific to plant metallothioneins. J Biol Inorg Chem. 2011;16(7):1035–45. [CrossRef].
16. Crowell DN, Huizinga DH. Protein isoprenylation: The fat of the matter. Trends Plant Sci. 2009;14(3):163–70. [CrossRef].
17. Khan IU, Rono JK, Zhang BQ, Liu XS, Wang MQ, Wang LL, et al. Identification of novel rice (*Oryza sativa*) *HPP* and HIPP genes tolerant to heavy metal toxicity. Ecotoxicol Environ Saf. 2019;175:8–18. [CrossRef].
18. Tehseen M, Cairns N, Sherson S, Cobbett CS. Metallochaperone-like genes in *Arabidopsis thaliana*. Metallomics. 2010;2(8):556–64. [CrossRef].
19. Suzuki N, Yamaguchi Y, Koizumi N, Sano H. Functional characterization of a heavy metal binding protein CdI19 from *Arabidopsis*. Plant J. 2002;32(2):165–73. [CrossRef].
20. Xiong S, Kong X, Chen G, Tian L, Qian D, Zhu Z, et al. Metallochaperone OsHIPP9 is involved in the retention of cadmium and copper in rice. Plant Cell Environ. 2023;46(6):1946–61. [CrossRef].
21. Zhang BQ, Liu XS, Feng SJ, Zhao YN, Wang LL, Rono JK, et al. Developing a cadmium resistant rice genotype with OsHIPP29 locus for limiting cadmium accumulation in the paddy crop. Chemosphere. 2020;247:125958. [CrossRef].
22. Cao HW, Zhao YN, Liu XS, Rono JK, Yang ZM. A metal chaperone OsHIPP16 detoxifies cadmium by repressing its accumulation in rice crops. Environ Pollut. 2022;311:120058. [CrossRef].
23. Zhao YN, Wang MQ, Li C, Cao HW, Rono JK, Yang ZM. The metallochaperone OsHIPP56 gene is required for cadmium detoxification in rice crops. Environ Exp Bot. 2022;193:104680. [CrossRef].
24. Wang H, Du J, Niu C, Yang X, Wu D, Sun H, et al. Genome-wide identification and stress response expression analysis of the heavy metal-associated isoprenylated plant protein gene family in tobacco (*Nicotiana tabacum* L.). BMC Plant Biol. 2025;25(1):1506. [CrossRef].
25. Xia Z, Zhao J, Wang C, Wu S, Zang Y, Wang D, et al. Pan-genome analysis and expression profiling of *HIPP* gene family in cassava. Genes. 2026;17(2):136. [CrossRef].
26. Parasyri A. Barley HIPP protein HvFP1 functions in a crosstalk between abiotic stress responses and developmental signaling pathways, including leaf senescence [dissertation]. Sachsen-Anhalt, Germany: Martin-Luther-University Halle-Wittenberg; 2023. 116 p.
27. Barth O, Vogt S, Uhlemann R, Zschiesche W, Humbeck K. Stress induced and nuclear localized HIPP26 from *Arabidopsis thaliana* interacts via its heavy metal associated domain with the drought stress related zinc finger transcription factor ATHB29. Plant Mol Biol. 2009;69(1–2):213–26. [CrossRef].
28. Zheng Q, Yu Q, Wu N, Yao W, Li J, Lv K, et al. A grape VvHOS1-interacting HIPP protein (VvHIPP21) negatively regulates cold and drought stress. Environ Exp Bot. 2023;207:105203. [CrossRef].
29. Maidment JHR, Franceschetti M, Maqbool A, Saitoh H, Jantasuriyarat C, Kamoun S, et al. Multiple variants of the fungal effector AVR-Pik bind the HMA domain of the rice protein OsHIPP19, providing a foundation to engineer plant defense. J Biol Chem. 2021;296:100371. [CrossRef].

30. Dharmaraj K, Cui W, Rikkerink EHA, Templeton MD. Construction of a kiwifruit yeast two-hybrid cDNA library to identify host targets of the *Pseudomonas syringae* pv. actinidiae effector AvrPto5. BMC Res Notes. 2019;12(1):63. [CrossRef].
31. Cowan GH, Roberts AG, Jones S, Kumar P, Kalyandurg PB, Gil JF, et al. Potato mop-top virus co-opts the stress sensor HIPP26 for long-distance movement. Plant Physiol. 2018;176(3):2052–70. [CrossRef].
32. Radakovic ZS, Anjam MS, Escobar E, Chopra D, Cabrera J, Silva AC, et al. Arabidopsis *HIPP27* is a host susceptibility gene for the beet cyst nematode *Heterodera schachtii*. Mol Plant Pathol. 2018;19(8):1917–28. [CrossRef].
33. Guo T, Weber H, Niemann MCE, Theisl L, Leonte G, Novák O, et al. Arabidopsis HIPP proteins regulate endoplasmic reticulum-associated degradation of CKX proteins and cytokinin responses. Mol Plant. 2021;14(11):1918–34. [CrossRef].
34. Sánchez-Pérez CR, Castaño E, Rodríguez-López CE, Urrea-López R, Pereira-Santana A. Heavy metal-associated proteins in plants: Genome-wide identification and functional insights from angiosperms and ancestral plants. J Mol Evol. 2025;93(4):527–42. [CrossRef].
35. Zhang Y, Chen K, Zhao FJ, Sun C, Jin C, Shi Y, et al. OsATX1 interacts with heavy metal P1B-type ATPases and affects copper transport and distribution. Plant Physiol. 2018;178(1):329–44. [CrossRef].
36. Huffman DL, O'Halloran TV. Function, structure, and mechanism of intracellular copper trafficking proteins. Annu Rev Biochem. 2001;70:677–701. [CrossRef].
37. Crowell DN. Functional implications of protein isoprenylation in plants. Prog Lipid Res. 2000;39(5):393–408. [CrossRef].
38. Gao W, Xiao S, Li HY, Tsao SW, Chye ML. *Arabidopsis thaliana* acyl-CoA-binding protein ACBP2 interacts with heavy-metal-binding farnesylated protein AtFP6. New Phytol. 2009;181(1):89–102. [CrossRef].
39. Wu W, Zhao T, Zheng Y, Liu T, Zhou S, Chen W, et al. HIPP33 contributes to selective autophagy-mediated vacuolar sequestration of cadmium in *Arabidopsis*. Plant Cell Environ. 2025;48(9):7072–88. [CrossRef].
40. Chen G, Xiong S. OsHIPP24 is a copper metallochaperone which affects rice growth. J Plant Biol. 2021;64(2):145–53. [CrossRef].
41. Rong F, Zhang Y, Ni F, Zhang L, Yu M, Hong Z, et al. miR408-5p and miR408-3p cooperatively reduce cadmium uptake and accumulation in rice. PLoS Biol. 2026;24(6):e3003811. [CrossRef].
42. Pufahl RA, Singer CP, Peariso KL, Lin SJ, Schmidt PJ, Fahrni CJ, et al. Metal ion chaperone function of the soluble Cu(I) receptor Atx1. Science. 1997;278(5339):853–6. [CrossRef].
43. Zhang P, Wang R, Ju Q, Li W, Tran LP, Xu J. The R2R3-MYB transcription factor MYB49 regulates cadmium accumulation. Plant Physiol. 2019;180(1):529–42. [CrossRef].
44. Wang C, Shan D, Bai Y, Hu Z, Yan T, Kong J, et al. MdWRKY17 enhances cadmium tolerance in *Malus domestica* via the transcriptional activation of the gene *MdHIPP1* encoding heavy-metal-associated isoprenylated plant protein. J Agric Food Chem. 2025;73(48):30802–13. [CrossRef].
45. Lin CY, Trinh NN, Fu SF, Hsiung YC, Chia LC, Lin CW, et al. Comparison of early transcriptome responses to copper and cadmium in rice roots. Plant Mol Biol. 2013;81(4–5):507–22. [CrossRef].
46. Cao HW, Li C, Zhang BQ, Rono JK, Yang ZM. A metallochaperone HIPP33 is required for rice zinc and iron homeostasis and productivity. Agronomy. 2022;12(2):488. [CrossRef].
47. Xu J, Cui J, He Q, Liu Y, Lu X, Qi J, et al. Genome-wide identification of HIPP and mechanism of Sl-HIPP4/7/9/21/26/32 mediated phytohormones response to Cd, osmotic, and salt stresses in tomato. Plant Physiol Biochem. 2024;217:109220. [CrossRef].
48. Gao C, Zhang Z, Zhu Y, Tian J, Yu K, Hou J, et al. Genome-wide analysis of *HIPP* gene family in maize reveals its role in the cadmium stress response. Genes. 2025;16(7):770. [CrossRef].
49. Li J, Tian J, Liang C, Wang T, Lu X. Characterization of the soybean (*Glycine max*) heavy-metal-associated isoprenylated plant protein (HIPP) gene family in response to aluminum. Plants. 2025;14(23):3582. [CrossRef].
50. Shi Y, Jiang N, Wang M, Du Z, Chen J, Huang Y, et al. *OsHIPP17* is involved in regulating the tolerance of rice to copper stress. Front Plant Sci. 2023;14:1183445. [CrossRef].
51. Wei Y, Peng X, Wang X, Wang C. The heavy metal-associated isoprenylated plant protein (HIPP) gene family plays a crucial role in cadmium resistance and accumulation in the tea plant (*Camellia sinensis* L.). Ecotoxicol Environ Saf. 2023;260:115077. [CrossRef].

52. Chunyan G, Yuxuan Z, Hualei X, Xinyue P, Kaili Y, Xi G, et al. The heavy metal-associated isoprenylated plant protein (HIPP) gene family plays a crucial role in cadmium resistance in lotus (*Nelumbo nucifera* G.). Horticulturae. 2025;11(9):1136. [CrossRef].
53. Zhang H, Zhai G, Ni X, Liu Z, Song T, Han Y, et al. Genome-wide identification of HIPP genes family in sorghum reveals the novel role of SbHIPP40 in accumulation of cadmium. J Hazard Mater. 2025;494:138478. [CrossRef].
54. Zhu J, Wu M, Lai Y, Wang L, Xiao P, Chen J, et al. SaHIPP41 overexpression enhances cadmium accumulation and tolerance in the hyperaccumulator Solanum americanum. SSRN. 2026. [CrossRef].
55. Zhang X, Feng H, Feng C, Xu H, Huang X, Wang Q, et al. Isolation and characterisation of cDNA encoding a wheat heavy metal-associated isoprenylated protein involved in stress responses. Plant Biol. 2015;17(6):1176–86. [CrossRef].
56. Dong P, Yin Y, Zhang S, Fan Y, Zhang X, Zhang M, et al. Overexpression of the heavy metal-associated isoprenylated plant protein gene *IbHIPP7* reduces cadmium accumulation and alleviates cadmium toxicity in sweetpotato. Hortic Res. 2026;13(3):uhaf323. [CrossRef].
57. Wang L, Chen C, Huang J, Shen R, Zhu X. Metallochaperone protein OsHIPP53 reduces cadmium accumulation in rice (*Oryza sativa* L.) roots. Plant Cell Rep. 2026;45(3):63. [CrossRef].
58. Xia H, Jing X, He H, Peng J, Liu Y, Sun W, et al. Genome-wide identification of the HIPPs gene family and functional validation of MsHIPP12 in enhancing cadmium tolerance in *Medicago sativa*. J Hazard Mater. 2025;491:137894. [CrossRef].
59. Liu RX, Wang MK, Wang YN, Qin XH, Lv YX, Li SS, et al. MdHMGB15 enhances zinc tolerance by activating multiple metal homeostasis genes in apple. J Hazard Mater. 2026;506:141677. [CrossRef].
60. Zhang X, Tang M, Chu W, Liu M, Song Q, Li B, et al. Melatonin mediates lead accumulation reduction via the RsWRKY26-RsHIPP26-RsASMT2 module in radish taproot. J Hazard Mater. 2026;501:140556. [CrossRef].
61. Chen Y, Liu Z, Yang L, Wu F, Cao Z, Shi H, et al. OsCERK1 interacts with OsHPP08 to regulate copper uptake and blast resistance in rice. Rice Sci. 2025;32(2):203–16. [CrossRef].
62. Zhou L, Ye L, Pang B, Hou Y, Yu J, Du X, et al. Overexpression of ApHIPP26 from the hyperaccumulator Arabis paniculata confers enhanced cadmium tolerance and accumulation to Arabidopsis thaliana. Int J Mol Sci. 2023;24(20):15052. [CrossRef].
63. Kabir AH, Das U, Rahman MA, Lee KW. Silicon induces metallochaperone-driven cadmium binding to the cell wall and restores redox status through elevated glutathione in Cd-stressed sugar beet. Physiol Plant. 2021;173(1):352–68. [CrossRef].
64. Ma L, An R, Jiang L, Zhang C, Li Z, Zou C, et al. Effects of ZmHIPP on lead tolerance in maize seedlings: Novel ideas for soil bioremediation. J Hazard Mater. 2022;430:128457. [CrossRef].
65. Zhang H, Zhang X, Liu J, Niu Y, Chen Y, Hao Y, et al. Characterization of the heavy-metal-associated isoprenylated plant protein (*HIPP*) gene family from *Triticeae* species. Int J Mol Sci. 2020;21(17):6191. [CrossRef].
66. Zhang X, Zhou X, Wu G, Du Y, Sun F, Zhou S, et al. The VlMYB149-VlHIPP30 regulatory module enhances grapevine resistance to *Botrytis cinerea* by activating the antioxidant system and copper metabolism. Mol Plant Pathol. 2026;27(1):e70197. [CrossRef].
67. Ye X, Liu C, Yan H, Wan Y, Wu Q, Wu X, et al. Genome-wide identification and transcriptome analysis of the heavy metal-associated (HMA) gene family in Tartary buckwheat and their regulatory roles under cadmium stress. Gene. 2022;847:146884. [CrossRef].
68. Zhang C, Wei Z, Ding D, Nisa ZU, Jin X, Chen C. Genome-wide analysis of *HIPP* genes and functional analysis of *GsHIPP79* in response to alkaline stress in *Glycine soja*. Plants. 2026;15(6):850. [CrossRef].
69. Sun W, Wei J, Wu G, Xu H, Chen Y, Yao M, et al. CqZF-HD14 enhances drought tolerance in quinoa seedlings through interaction with CqHIPP34 and CqNAC79. Plant Sci. 2022;323:111406. [CrossRef].
70. Hou Q, Shen T, Yu R, Deng H, Wen X, Qiao G. Functional analysis of sweet cherry PavbHLH106 in the regulation of cold stress. Plant Cell Rep. 2023;43(1):7. [CrossRef].
71. Yu R, Hou Q, Deng H, Xiao L, Liu K, Wu Y, et al. Molecular identification and expression patterns of sweet cherry HIPPs and functional analysis of PavHIPP16 in cold stress. Planta. 2024;260(6):134. [CrossRef].
72. Chen K, Tong S, Huang H, Li J, Shi W, Li Y, et al. HIPP26L-NF-YC9-SRMT module regulates drought response in poplar. Cell Rep. 2025;44(6):115770. [CrossRef].

73. Barth O, Zschiesche W, Siersleben S, Humbeck K. Isolation of a novel barley cDNA encoding a nuclear protein involved in stress response and leaf senescence. Physiol Plant. 2004;121(2):282–93. [CrossRef].
74. Parasyri A, Barth O, Zschiesche W, Humbeck K. The barley heavy metal associated isoprenylated plant protein HvFP1 is involved in a crosstalk between the leaf development and abscisic acid-related drought stress responses. Plants. 2022;11(21):2851. [CrossRef].
75. Wang Z, Zhang H, Li Y, Chen Y, Tang X, Zhao J, et al. Isoprenylation modification is required for HIPP1-mediated powdery mildew resistance in wheat. Plant Cell Environ. 2023;46(1):288–305. [CrossRef].
76. Yu R, Hou Q, Deng H, Xiao L, Cai X, Shang C, et al. Overexpression of *PavHIPP16* from *Prunus avium* enhances cold stress tolerance in transgenic tobacco. BMC Plant Biol. 2024;24(1):536. [CrossRef].
77. Maidment JHR, Shimizu M, Bentham AR, Vera S, Franceschetti M, Longya A, et al. Effector target-guided engineering of an integrated domain expands the disease resistance profile of a rice NLR immune receptor. Elife. 2023;12:e81123. [CrossRef].
78. Were VM, Yan X, Foster AJ, Sklenar J, Langner T, Gentle A, et al. The *Magnaporthe oryzae* effector Pwl2 alters HIPP43 localization to suppress host immunity. Plant Cell. 2025;37(6):koaf116. [CrossRef].
79. Song H, Lin B, Huang Q, Sun L, Chen J, Hu L, et al. The *Meloidogyne graminicola* effector MgMO289 targets a novel copper metallochaperone to suppress immunity in rice. J Exp Bot. 2021;72(15):5638–55. [CrossRef].
80. Niu X, Yamamoto N, Yang G, Lin H, Jiang L, Liu Y, et al. A small secreted protein, RsMf8HN, in *Rhizoctonia solani* triggers plant immune response, which interacts with rice OsHIPP28. Microbiol Res. 2023;266:127219. [CrossRef].
81. Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, et al. Loss of function of a proline-containing protein confers durable disease resistance in rice. Science. 2009;325(5943):998–1001. [CrossRef].
82. Nakao M, Nakamura R, Kita K, Inukai R, Ishikawa A. Non-host resistance to penetration and hyphal growth of *Magnaporthe oryzae* in *Arabidopsis*. Sci Rep. 2011;1:171. [CrossRef].
83. Qiu T, Cheng Y, Fang K, Feng Y, Bai Q, Bhadauria V, et al. Coordinated ubiquitination of OsHIPP56 and blast fungus MAX effectors by OsFBX388 regulates rice immunity. Agric Commun. 2026;4(1):100123. [CrossRef].
84. Zschiesche W, Barth O, Daniel K, Böhme S, Rausche J, Humbeck K. The zinc-binding nuclear protein HIPP3 acts as an upstream regulator of the salicylate-dependent plant immunity pathway and of flowering time in *Arabidopsis thaliana*. New Phytol. 2015;207(4):1084–96. [CrossRef].
85. Huang Y. Molecular mechanisms of the rice HAM domain gene *OsHIPP16* in ovule development. Biosci Evid. 2024;14(5):218–26. [CrossRef].
86. Oikawa K, Fujisaki K, Shimizu M, Takeda T, Nemoto K, Saitoh H, et al. The blast pathogen effector AVR-Pik binds and stabilizes rice heavy metal-associated (HMA) proteins to co-opt their function in immunity. PLoS Pathog. 2024;20(11):e1012647. [CrossRef].
87. Imran QM, Falak N, Hussain A, Mun BG, Sharma A, Lee SU, et al. Nitric oxide responsive heavy metal-associated gene *AtHMAD1* contributes to development and disease resistance in *Arabidopsis thaliana*. Front Plant Sci. 2016;7:1712. [CrossRef].
88. Coşkun ÖF, Aydın A, Başak H, Mavi K, Yetişir H, Toprak S. Perspectives on morphology, physiology, genetic polymorphism and machine learning in cucumber grafting under zinc toxicity. BMC Plant Biol. 2025;25(1):1647. [CrossRef].
89. Coşkun ÖF. Eustress and hormesis improve quality resilience and resource efficiency in horticultural crops through controlled preharvest stress management. Discov Plants. 2026;3(1):112. [CrossRef].