

Heavy Metal Stress and Mitogen Activated Kinase Transcription Factors in Plants: Exploring Heavy Metal-ROS Influences on Plant Signaling Pathways

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Abstract

Due to their stationary nature, plants are exposed to a diverse range of biotic and abiotic stresses, of which heavy metals stress poses as one of the most detrimental abiotic stresses, targeting crucial and vital processes. Heavy metals instigate the over-production of reactive oxygen species (ROS), and in order to mitigate the adverse effects of ROS, plants induce multiple defence mechanisms. Besides the negative implications of overproduction of ROS, these molecules play a multitude of signaling roles in plants, acting as a central player in the complex signaling network of cells. One of the signaling mechanisms it is involved in is the mitogen-activated protein kinase (MAPK) cascade, a signaling pathway used to transduce extracellular stimuli into intracellular responses. Plant MAPKs have been implicated in signaling of stresses, phytohormones and cell cycle cues. However, the influence of various heavy metals on MAPKs activation has not been well documented. In this review, we will attempt to address and summarize several aspects related to various heavy metal-induced ROS signaling, how these signals activate the MAPK cascade and the downstream transcription factors that instigates the plants response to these heavy metals. Moreover, we will highlight a modern research methodology that could characterize the novel genes associated with MAPKs and their roles in heavy metal stress.

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Abstract:

Due to their stationery nature, plants are exposed to a diverse range of biotic and abiotic stresses, of which heavy metals stress poses as one of the most detrimental abiotic stresses, targeting crucial and vital processes. Heavy metals instigate the over-production of reactive oxygen species (ROS), and in order to mitigate the adverse effects of ROS, plants induce multiple defence mechanisms. Besides the negative implications of overproduction of ROS, these molecules play a multitude of signaling roles in plants, acting as a central player in the complex signaling network of cells. One of the signaling mechanisms it is involved in is the mitogen-activated protein kinase (MAPK) cascade, a signaling pathway used to transduce extracellular stimuli into intracellular responses. Plant MAPKs have been implicated in signaling of stresses, phytohormones and cell cycle cues. However, the influence of various heavy metals on MAPKs activation has not been well documented. In this review, we will attempt to address and summarize several aspects related to various heavy metal-induced ROS signaling, how these signals activate the MAPK cascade and the downstream transcription factors that instigates the plants response to these heavy metals. Moreover, we will highlight a modern research methodology that could characterize the novel genes associated with MAPKs and their roles in heavy metal stress.

Keywords: Heavy metal, reactive oxygen species, MAPK, transcription factor, signaling pathway, stress response

Introduction

The emergence of rapid urbanization coupled with various anthropogenic activities such as modern agricultural and farm management practice (phosphate-based fertilizer and metallo-pesticide applications and hard water and wastewater irrigations) is the leading cause of environmental and human health deterioration (Thakur et al., 2022; Rai et al., 2019). This is due to their major contribution of excess metals, especially heavy metals (HM), to soil, water bodies and air environment (Thakur et al., 2022; Rai et al., 2019). Although, heavy metals naturally occur within the Earth's crust (Keyster et al., 2020), the aforementioned activities have been pumping excess amounts of HM into environments and ecosystems, causing them reach threshold levels or to surpass their permissible concentrations (**Table 1**) within various environmental systems (Thakur et al., 2022; Rai et al., 2019). Heavy metals are referred to as such owing to their high densities, atomic weight and/or number (Oladoye et al., 2022). Heavy metals such as cadmium, chromium, lead, arsenic, and mercury are classified among the most toxic metals (Keyster et al., 2020) and therefore they are required to be assessed and controlled within the various environments (**Table 1**). HM are able to cause damaging effects on an extensive variety of ecosystems through food chains and water, mainly because these metal ions are prone to dissolve in water, bioaccumulate in aquatic animals or crops (Oladoye et al., 2022). Hence, they cause notable threats in agricultural fields, living organism, animals, and humans (Xiang et al., 2021; Shao et al., 2022). The toxic heavy metals accumulate in animal and human bones, gastrointestinal tract, liver, lungs, and kidneys, leading to diseases such as cancer, diabetes, emphysema, liver damage, osteoporosis, renal tubular damage, and kidney stone formation (Hossein-Khannazer et al. 2020; Wang et al. 2021).

Within plants, the processes of seed germination, plant growth and development are significantly affected by HM-toxicity, rendering basic plant functions dysfunctional at the physiological (chlorosis, reduction of stomatal density and conductance, inhibition of carbon fixation, reduction in root respiration, transpiration and leaf relative water content, reduction of chlorophyll content and photosynthetic efficiency, decline yield), biochemical (destruction and damage to plant membranes and membranous organelles, altered protein patterning, suppression of antioxidant activity, dysfunctional cellular osmoregulation, and reactive oxygen species (ROS) scavenging system) and molecular level (reduction of crop nutritional value, uptake and transportation of mineral elements, inhibited storage protein catabolism) (Huybrechts et al. 2020; Hussain et al. 2021; Zhou et al. 2021; Wu et al. 2022). However, plants have evolved signaling systems to regulate

responses, in order to adapt or acclimatize to HM-stresses (Keyster et al., 2020), therefore it is important to understand these systems and how they are regulated under HM-stress. However, in plants the relationship between HM-induced ROS production and their regulation of the MAPKs cascade is controversial (Lui et al., 2019). Therefore, the review focuses on the mechanism of HM-uptake into root cells to induce ROS production, and how this induction activates the MAPK cascade. Furthermore, the review wants to unravel which downstream transcription factors are activated in order to instigate a HM-response in plants.

Table 1. Permissible and threshold concentrations of various heavy metals in soil. (Singh et al., 2010; Al-boghdady et al., 2019; Adagunodo et al., 2018)

Heavy metal	Permissible concentration within soils (mg/kg)	Threshold limit (Adagunodo et al., 2018) mg/kg
Cadmium	3-6	1
Chromium	100-180	100
Lead	300	60
Arsenic	50	5
Mercury	1	-
Cu	140-200	100
Zn	200-300	200
Ni	75	50

The (–) value represents that no recorded concentration for that heavy metal found.

ROS- Signaling of the MAPK cascade

Over the course of time and due to their stationery nature, plants have developed various acclimatization strategies against heavy metal stresses (Majeed et al., 2023; Lamers et al., 2020). Plants have achieved these acclimatization strategies by means of various molecular systems that entails the perception and transmission of stress signals (reactive oxygen species (ROS), nitric oxide (NOS) and phytohormones) to give rise to a particular response (Majeed et al., 2023; Chen et al., 2014; Jalmi et al., 2015). These perception mechanisms involve the use cellular surface receptors/sensors to detect stress and the subsequent activation of signal transduction pathways, in order to assist the plant to adapt to the HM stress being experienced (Lamers et al., 2020; Jalmi et al., 2015). However, to exhibit a particular response, it is important for the plant to perceive the stimulus and transmit it into the nucleus of the plant cell (Jalmi et al., 2015), and one of the most important changes that occur upon perception of external stimuli is change in redox state (Jalmi et al., 2015). The change in redox state occurs due to the production and accumulation of reactive oxygen species (ROS), which accompanies HM stress (Jalmi et al., 2015). These signals undergo processing and amplification through phosphorylation and dephosphorylation as they are transmitted to plant cells (Hao et al., 2021; Mondal et al., 2022). One of the more popular studied signalling pathways include plant mitogen-activated protein kinases (pMAPKs). This is pathway is highly conserved across species (Kumar et al., 2020) and these proteins belong to the family of protein kinases which play many crucial roles in various cellular process and abiotic stress response (Kumar et al., 2020). Stresses such as heavy metals, in particular, have been shown to have a significant impact on the subsequent activation of MAPK signalling pathways (Jalmi et al., 2018), by initiating a signal perceived by the plasma membrane and subsequently cytoplasm (Majeed et al., 2023). The MAPK cascade is a three-phase system comprised of three protein kinases: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK (Ma et al., 2022). These three kinases are functionally connected via activation by sequential phosphorylation (Kumar et al., 2020). In plants, the activation of MAPK usually consists of a series of reactions carried out by upstream MAP kinase kinases (MAPKKs/MKK), that phosphorylate and activate MAPKs (Sinha et al., 2011). These MAPKs are finally transported to the nucleus where it is involved in controlling the tolerance and the growth and developmental processes in plant through transcriptional programming (Kumar et al., 2020). The transcriptional factors, located downstream of MAPK, are key factors in controlling these programming (Li et al., 2022). Transcription factors possess numerous phosphorylation sites and are pivotal in regulating

responses to heavy metal stress by controlling the expression of downstream genes (Jagodzik et al., 2018). They serve as central components in the regulatory networks responsible for heavy metal detoxification and tolerance (Jagodzik et al., 2018). Currently, extensive research has identified a multitude of transcription factors associated with heavy metal detoxification and tolerance in plants. Among these, transcription factors such as basic leucine zipper (bZIP), heat shock transcription factor (HSF), WRKY, myeloblastosis protein (MYB), and ethylene-responsive transcription factor (ERF) are recognized for their crucial roles in regulating heavy metal detoxification and tolerance in plants (Li et al., 2022; El Rasafi et al., 2022) (**Table 2**).

Several reports have shown that ROS acts upstream of MAPK signaling to regulate the activation and duration activity of MAPKs (Liu et al., 2019). However, as mentioned before, the relationship between particular HM-induced ROS production and MAPK signaling is still poorly understood. Therefore, in this review we focus on elucidating how 5 toxic HM's ROS production activates the MAPK and this is done to gain further insight into early events of HM stress signaling and the involvement of the MAPK cascade in regulating a response to increase plants tolerance to these HM.

The effect of five heavy metal-ROS signaling on MAPK pathways

Cadmium

Cadmium (Cd) is a toxic heavy metal, ranked 7th amongst 20 of the most toxic heavy metals (Kim et al., 2015). It is known as the most carcinogenic heavy metal, and it is introduced into the environment through natural processes such as Cd-containing rocks weathering, forest fires, volcanic eruptions, and anthropogenic systems (Haider et al., 2021; Zulfiqar et al., 2022). Cd is a high metal replacement element that has no biological function. It can replace calcium due to similar charge and ionic properties, inducing adverse effects on biological systems (Lata et al., 2019; El Rasafi et al., 2022). Cd toxicity is gaining serious research attention as it has become global hazard, with agricultural crops being the main Cd entry point into the global food chain (Aprile and De Bellis, 2020; Mikhailenko et al., 2020). Plants grown in Cd-contaminated soil can efficiently absorb Cd through their root system, and it is translocated and accumulates in the edible parts of the plant (Rabêlo et al., 2020). Cadmium occurs in various soil-bound forms; however, a substantial proportion of these forms are not readily accessible for plant uptake (Liu et al., 2018). Cadmium demonstrates a high propensity for absorption and subsequent transportation to the above ground parts of plants, as noted by Shanmugaraj et al. (2019). The extent of Cd absorption, however, exhibits variation across different plant species and genotypes due to morphological traits and physiological characteristics, as well as variations related to plant growth stages and age. (Rizwan et al., 2018). The plant root tissue plays an important role in the uptake of Cd, and it is responsible for approximately 79 – 93% soil Cd accumulation (Li et al., 2021). In general, trace elements are absorbed in their bivalent form (Fontes et al., 2014; Gupta et al., 2016). Cd²⁺ follows a pathway into root cells that involves the same transporters as those for Ca²⁺, Fe²⁺, Mg²⁺, Cu²⁺, and Zn²⁺ (Ismael et al., 2019). The passage of cadmium from the soil solution into plant roots through cell walls can occur via passive transport, primarily through diffusion (Rog Young et al., 2015). Furthermore, it has been observed that active transport mechanisms are employed by Cd to traverse the plasma membrane of root cells, facilitated by non-specific membrane transport proteins, such as zinc transporter (ZIP) and iron regulated transporter (IRT), as well as metal pumping ATPases (Wu et al., 2015; Sebastian & Prasad, 2018). Cd accumulation has been thoroughly investigated, however, the mechanism involved in Cd stress to plants is still not clearly understood. In plant systems, Cd toxicity is a major causative agent of oxidative damage, culminating in reduced plant growth induced by alterations of membrane permeability and the production of reactive oxygen species (ROS) at the organelle level. Hydrogen peroxides (H₂O₂), hydroxyl radical (OH⁻) and superoxide anion (O₂⁻), are among the primary ROS responsible for membranous proteins and lipids oxidation associated with cell death (Jawad Hassan et al., 2020).

Multiple studies have been conducted attempting to elucidate the role of Cd-induced ROS production upstream of the MAPK cascade. A study by Liu et al., 2019, by means of in-gel kinase assays, identified two ERK-like MAPKs in response to cadmium treatment (CdCl₂) in *Zea mays* roots, ZmMPK3-1 (43 kDa) and ZmMPK6-1 (45 kDa). In addition, the authors also noted via CMH2CFDA fluorescence, there was

Cd-induced ROS production in *Z. mays* roots (Liu et al., 2019). However, to validate the involvement of Cd-induced ROS production in the activation of these two ERK-like MAPKs, the authors used a two-pronged approach in order to assess 1. The influence of Cd-ROS on the activation of the MAPKs and 2. whether MAPK signaling influenced Cd-induction of ROS production. ROS inhibitors (DMTU and DPI) were used prior to the activation of the MAPKs and noted that inhibition of ROS reduced Cd-activation of both ZmMPK3-1 and ZmMPK6-1, whereas inhibition of MAPK signaling (by use of UO126) did not disturb Cd-induced ROS production (Lui et al., 2019). These two approaches indicated that Cd-treatments activated the two ERK-like MAPKs via ROS production (Lui et al., 2019). Two previous studies also validated the Cd-induced ROS activation of the MAPK cascade elucidated by Lui et al., 2019, however the two studies implemented two other approaches (using a ROS stimulus and ROS scavenger) to assess the relationship between Cd-ROS and MAPK activation. Wang et al., 2010 and Liu et al., 2010 used hydrogen peroxide stimulus (H₂O₂) and glutathione (ROS scavenger), respectively to elucidate the relationship between Cd-ROS and MAPK activation. Both studies identified MPK3, whereas Liu et al., 2010 also identified MPK6 in their respective plant species (*Zea mays* and *Arabidopsis thaliana*, respectively). The three studies above attempted to elucidate the relationship between Cd-ROS and MAPK activation, using different approaches (ROS inhibitor, ROS stimulus and ROS scavenger), and all concluded that the accumulation of Cd-ROS is necessary for the activation of MPK3 and MPK6 in both *Z. mays* and *A. thaliana*, validating the role of Cd-ROS accumulation in the activation of the MAPK cascade. However, neither studies elaborated on what downstream effects these MAPK's posed in response to Cd treatments. Zhao et al., 2021 clarified that the downstream effects of activating ZmMPK6 led to it phosphorylating ZmWRKY104 transcription factor, in *Z. mays* plants under drought stress. This transcription factor was further revealed via functional analysis, to be involved in abscisic acid (ABA)-induced antioxidant defence and that its activity depends on ZmMPK6 (Zhao et al., 2021). Hence, it could have been proposed that activation of the ZmMPK6 under Cd treatment could have likewise activated ZmWRKY104 in order to increase *Z. mays* and *A. thaliana*'s tolerance to Cd-stress. Various other studies has indicated that under Cd-induced stress condition, MAPKs govern the activity of other transcription factors like bZIP, MYB, MYC, and WRKY (Ghori et al., 2019; Sharma et al., 2021) (**Table 2**). Furthermore, a crosstalk has been described between ABA, auxin, and MAPK signaling pathways, which contributes to Cd-stress tolerance in rice (Zhao et al., 2014). In soybean seedlings, the upregulation of MAPK2 has been observed in response to Cd-stress (Chmielowska-Bąk et al., 2013).

Chromium

Recently research investigating the impact of heavy metals such as chromium (Cr) namely chromium trivalent and chromium hexavalent (Cr (VI)) in plants has gained significant attention. Studies have shown that plants grown in the presence of Cr(VI) often display a reduction in growth and development resulting in subsequent loss of quality and yield (Sharma et al., 2020). Among the various (HMs), chromium hexavalent (Cr (VI) or Cr⁺⁶), stands out due to its structural similarity to phosphate (PO₄³⁻) and sulphate (SO₄²⁻) ions. This similarity is notable as Cr(VI) happens to be one of the more stable forms of chromium found in soils (Liu et al., 2021). Subsequently as a result of these similarities, Cr(VI) is taken up by plant roots potentially through the use of phosphate and sulphate transporters (Cervantes et al., 2001). A study by Xu et al., 2021 reported Cr (VI) as having a high affinity for the sulphate transporter Sultr1;2. The authors confirmed that Sultr 1;2 significantly influence Cr (VI) uptake in as indicated by the increase in shoot Cr(VI) in Sultr1;2 double knockdown mutant and increased Cr(VI) uptake in *Arabidopsis* plants overexpressing Sultr1;2 (Xu et al., 2021). Additionally, López-Bucio et al., 2014 reported that Cr(VI) influenced the expression of low phosphorus inducible reporter genes, namely, AtPT1 and AtPT2 in *Arabidopsis*. Furthermore, in their study the authors also observed decreased primary root development as a symptom of Cr(VI) stress which was alleviated by the addition of phosphorus (López-Bucio et al., 2014). Their study highlighted the importance of phosphorus in Cr(VI) stress responses and to a greater degree the potential of improving plant tolerance to chromate via phosphate deficiency mediated responses.

In higher plants MAPK cascades have been shown to influence the regulation of the WRKY gene family, one of the largest families of transcription factors (TFs). Studies have demonstrated that, in response to biotic and

abiotic stress, WRKY-associated TFs play crucial roles in various plant processes (Jiang et al., 2017). A study by Shen *et al.* (2021) observed WRKY33 as an important TF involved in the remodelling of root architecture during phosphate-deficiency (Pi deficiency) response. The authors reported that the disruption of WRKY 33 resulted in accumulation of Fe^{3+} at the root tips, inhibiting primary root growth and promotion of root hairs. Their study demonstrated signalling crosstalk between WRKY33 and the modulation of an aluminium mediated malate transporter 1 (ALMT1) in regulating Pi-deficiency response, through the modulation of root structural architecture (Shen et al., 2021). Furthermore, Zhang *et al.* (2023) showed that WRKY33 is directly involved in the direct activation of ATL31, a ubiquitin ligase, during cadmium (Cd) stress. In their study, the subsequent activation of ATL31 positively regulated Cd tolerance in *Arabidopsis* through controlling heavy metal uptake by regulating the activity of iron-regulated transporter 1 (IRT1) (Zhang et al., 2023). Although the above-mentioned studies highlight the importance of WRKY33 in Pi-deficiency and heavy metal stress responses. Research investigating the potential functional roles of WRKY33 during plant Cr(VI) responses remains to be limited. Given that Cr(VI) uptake utilizes mechanisms involved in phosphorus uptake, investigating the activity of WRKY33 during Cr(VI) stress responses could provide insights into the function of this TF in the regulatory mechanisms of Cr(VI) tolerance. Moreover, research has shown that in plants, WRKY33 is one of the WRKY TF that gets regulated by the MAPKs (Adachi et al., 2015). Other WRKY-TFs such as WRKY23 and WRKY47 have been shown to regulate processes such as auxin mediated root development and cell wall modification such as binding capacity respectively, all of which regulate nutrient and subsequent heavy metal up take (Grunewald et al., 2012, Trinh et al., 2014). Furthermore, a recent study by Ali and authors (2023) also reported WRKY and Ap2/ERF as important TFs in Cr(VI) signalling cascades during plant heavy metal defence responses (Ali et al., 2023). In plants, basic region/leucine zipper motif (bZIP) has also been shown to be regulated by MAPKs. These TFs play a role in many essential processes such as seed maturation, pathogen defences as well as stress responses to various abiotic stresses (Yu et al., 2020). Furthermore, studies have shown that bZIPs can be phosphorylated during plant HM stress responses to various heavy metals (i.e Cd, Zn and Pb etc.) (Li et al., 2022). Additionally, Dubey and colleagues reported that bZIP Tfs influenced shielding responses against various heavy metals like Cr(VI), As, Cd, and Pb in *Oryza sativa* (Dubey et al., 2014). Furthermore, in a study by Fang et al. (2017), the authors observed increased activity in the bZIP (TGA3) in response to Cr(VI) stress. This triggered hydrogen sulfide (H_2S) mediated defence responses as a result the subsequent increase in the transcription of L-cysteine desulfhydrase (LCD) by TGA3. (Fang et al., 2017). A recent study by Chai and authors (2022), described GmbZIP152, a soybean bZIP as an important TF involved in multiple stress responses (i.e., abiotic and biotic) (**Table 2**). In their study, the overexpression of GmbZIP152 improved *S. sclerotiorum* -disease resistance as well as tolerance to salinity, drought and heavy metals in soybean. The overexpression of GmbZIP152 improved the expression levels of antioxidant genes, namely superoxide dismutase, peroxidases, and catalase, resulting in the subsequent improved biotic/abiotic stress resistance/tolerance (Chai et al., 2022). In a study by Fan *et al.*, (2019) the authors observed the downregulation of RsbZIP010 in response to Cr(VI) in radish, (Fan et al., 2019). Furthermore, earlier studies have shown bZIP10 to be involved in plant oxidative stress responses and cell death though its interaction with lysine-specific demethylase 1 (LSD1) (Kaminaka et al., 2006). Myeloblastosis viral oncogene homolog (MYB) transcription factors (MYB) form one of the largest families of TFs involved in the biosynthesis of secondary metabolites in plants. Transcription factors in this family have been shown to be involved in a plethora of essential biological processes such as cellular morphogenesis, growth/development as well environmental stress responses (Cao et al., 2020) (**Table 2**). In a study by Baek et al., (2013), the authors reported AtMYB2 as an important regulator of P-deficiency response through its activation of miR399f transcript which regulated the root system architecture (Baek et al., 2013). Given that Cr (VI) uptake occurs via P and S transporters. The mechanism of regulating Cr in plants could also be influenced by the activity TFs such as MYBs. Studies investigating the functional roles of MYB in plant heavy metal stress have been done. A study by Wang et al. (2017) reported on the function of OsARM1, an arsenite-responsive MYB1 transcription factor, and its subsequent binding to key promoters involved in As transporters (Wang et al., 2017). Additionally, Hu and authors also observed increased sensitivity to Cd in OsMYB45 mutants, attributed to an increase in ROS accumulation and decreased antioxidant activity compared to the wild type (Hu et al., 2017). Although

these studies highlight the importance of MYB in plant responses to HM-stress via adaptive root remodelling and redox regulation, research on the functional roles of MYB with relation to Cr (VI) stress responses and MYB2 in plant Cr(VI) sensitivity is still limited. Furthermore, many studies have alluded to the notion that MYB TFs form part of the downstream targets of MAPKs as indicated by the dual alteration in gene expression of both MAPKs and MYBs in plants during heavy metal stress (Jalmi et al., 2018). Therefore, investigating the role of MAPK-mediated MYB activity in response to Cr(VI)-stress could provide insights to the functional contribution of MYB to chromate sensitivity versus tolerance (**Table 2**).

Table 2: Summary of the MAPK activated transcription factors involved in regulating heavy metal stress in plants. The four major MAPK-activated transcription factors are shown. Furthermore, the genes activated, and their roles involved in various heavy metals are shown.

TF	Genes	Response to/ Regulation	Processes involved in	Reference
WRKY	<i>AtWRKY12</i>	Up regulated under Cd	Represses GSH1 expression to negatively regulates cadmium tolerance in Arabidopsis	Han et al, 2019
	<i>AtWRKY13</i>	Up regulated under Cd	Activates PDR8 expression to positively regulate cadmium tolerance in Arabidopsis	Sheng et al., 2019
	<i>GmWRKY142</i>	Up regulated under Cd	Confers cadmium resistance by upregulating the cadmium tolerance 1-like genes	Li et al, 2022
	<i>WRKY33</i> <i>Ubiquitin ligase-ATL31</i>	Upregulation under HM-stress (i.e., Cd)	Regulating HM uptake via iron-regulated transporter 1 regulation	Zhang et al., 2023
	<i>RsWRKY</i>	Up regulated under Cd and Pb	unknown	Karanja et al., 2017
bZIP	<i>ZmbZIP1</i>	Differential regulation in response to various stresses (i.e Cd)	Enhances antioxidant enzyme activity	Wang et al., 2010

TF	Genes	Response to/ Regulation	Processes involved in	Reference
MYB	<i>GubZIP</i>	Expressed specifically in different tissues under Cd stress	Affect protein–protein interaction networks and plays an important role in plant growth and stress responses	Han et al., 2021
	<i>OsBZIP23</i>	Cultivar dependant	Related to the tolerance of rice to Cd stress and affected Cd accumulation in rice grains	Pan et al., 2021
	<i>BnbZIP2/3</i>	Up regulated under drought and heavy metal Cd stress.	Involved in plant growth, development, and stress signalling	Huang et al., 2016
	<i>AB15</i>	Up regulated under Cd	Interacts with MYB49 and prevented its binding to the downstream genes, resulting in inactivation of IRT1 and reduced Cd uptake	Zhang et al., 2019
	<i>TGA3L</i>	Upregulation under Chromate (Cr(VI))	Generation of H ₂ S, triggering HM-defence, reducing Cr(VI) accumulation	Fang et al., 2017
	<i>GmbZIP152A</i>	Upregulation under multiple stresses including Cr(VI)	Regulation of antioxidant enzyme activity	Chai et al., 2022
	<i>RSbZIP10</i>	Downregulation under Cr(VI)	Oxidative stress responses	Fan et al., 2019
	<i>OsMYB45</i>	Downregulation increases Cd sensitivity	Maintaining redox homeostasis via antioxidant activity	Hu et al., 2017
	<i>SbMYB15</i>	Up regulated under Cd Ni	Cadmium and nickel tolerance in transgenic tobacco	Sapara et al., 2019

TF	Genes	Response to/ Regulation	Processes involved in	Reference
HSF	<i>AtMYB4</i>	Up regulated under Cd	Improves antioxidant defence systems and enhances expression of PCS1 and MT1C under Cd-stress in Arabidopsis	Agarwal et al., 2020
	<i>JrMYB2</i>	Up regulated under Cd	Improve CdCl2 stress tolerance	Xu et al., 2018
	<i>OsARM1</i>	Upregulation reduces HM (i.e As) tolerance, Downregulation improves tolerance	Regulation of shoot to root HM uptake	Wang et al., 2017
	<i>SaHSfA4c</i>	Up regulated under Cd	Enhances Cd tolerance by ROS -scavenger activities and shock proteins expression	Chen et al., 2020
	<i>TaHsfA4a</i>	Up regulated under Cd	Upregulates MT gene expression for Cd tolerance	Shim et al., 2009.
	<i>PvBip1</i>	Up regulated under Cd	Participates in the reconstruction of proteins and improves intracellular homeostasis to increase cadmium tolerance.	Song et al., 2018
	<i>HSF1A</i>	Up regulated under Cd	Upregulates melatonin biosynthesis to confer cadmium tolerance in tomato plants	Cai et al., 2017

Abbreviations are as follows: transcription factor (TF), basic leucine zipper (bZIP), Myeloblastosis viral oncogene homolog (MYB), and heat shock factor (HSF).

Lead

Lead, like arsenic, cadmium and coronium may enter the environment and harm plants through a variety of means, including mining (Dong et al., 2009), the burning of fossil fuels, and the use of synthetic agricultural pesticides. Out of a total of 53 naturally occurring heavy metals (HMs), 17 are biologically available and play

a crucial role in the ecosystem (Alamri et al., 2018). However, for plants, animals and humans, lead (Pb) is considered a nonessential element that is categorized as the second most toxic heavy metal after Arsenic (As) (Duffus, 2002; Arias et al., 2010). When the rate of intake of heavy metals (HMs) by biological systems exceeds the rate of excretion, we refer to this as bioaccumulation of HMs in biological systems (Wang and Fisher, 1999; Ali et al., 2019). This phenomenon is especially concerning in the case of lead (Pb), which has the potential to account for approximately 10% of the total pollution caused by HMs (Collin et al., 2022). Furthermore, Pb can persist in the soil for over 2000 years, induces a wide array of toxic effects on plants, encompassed by physiological, morphological, and biochemical changes. (Kumar et al., 2020). The molecular mechanism of Pb uptake in roots has not been elucidated yet (Kumar et al., 2020). It is suggested that numerous pathways can facilitate the uptake of Pb, such as ionic channels (Kumar et al., 2020), however, Pb is a non-selective phenomenon and is independent of the H⁺/ATPase pump (Kumar et al., 2020). Transgenic plant studies have indicated that Pb can also penetrate into roots through other alternative non-selective pathways such as cyclic nucleotide-gated ion channels and low-affinity cation transporters. According to research on the association between lead (Pb) toxicity and an increase in ROS molecules, ROS might possibly produce phytotoxicity by causing damage to tissue ultrastructure, cellular components, and macromolecules, eventually leading to programmed cell death in plants. ROS molecules are ideal for initiating stress-signalling transduction pathways, since several processes are involved in their formation or scavenging (Hancock et al., 2001). Among these ROS processes, ionic mechanism of action believed to be sensed by calcium binding protein has been linked with affecting mineral uptake in *Zea mays* (Seregin et al.2004), *O.sativa* (Chatterjee et al.2004), *Brassica oleracea* (Sinha et al.2006), and *Medicago sativa* (Lopez et al.2007). A study by Wang et al., 2013 used transcriptomic analysis of *R. sativus*(radish) roots and revealed upregulation of four MAPKs (MAPKKK7, MAPK6, MAPK18 and MAPK20) in response to Pb treatments. Furthermore, looking back a study by Huang and Huang, 2008 indicated that Pb induced ROS production and activated two MAPKs (40- and 42 kDa) in *O. sativa* roots. By means of ROS scavenger, glutathione, and diphenylene iodonium (DPI) the authors further elucidated the relationship between Pb-induced root cell death and MAPK activation, which was that MAPK activation was dependent on Pb²⁺-cell death. The authors suggested that ROS may function in Pb²⁺-triggered cell death and MAP Kinase signaling pathway in rice roots (Huang and Huang, 2008). A study by Karanja et al., 2017 discovered 20 RsWRKY transcripts were significantly increased in *Raphanus sativus* L. (radish), in response to Pb treatments. RsWRKY31 and RsWRKY114 had the highest expression levels under all the abiotic stresses in their study, including Pb stress (Karanja et al., 2017). They concluded that WRKY genes may have multifunctional roles under various abiotic stresses, and could contribute to controlling signaling processes linked to transcriptional adjustments in plants under harsh conditions such as Pb stress (Karanja et al., 2017). In summary, this review gives insight on the complex effects of lead poisoning in plants, addressing environmental, molecular, and cellular issues. Understanding these consequences is critical for developing methods to reduce lead's negative effects on plant health and the larger environment.

Arsenic

Arsenic may enter the environment and therefore affect plants through mining, the burning of fossil fuels and the use of synthetic agricultural chemicals. Arsenic has been noted to cause epigenetic changes in plants, such as reduced methylation at concentrations as low as 100 mg. kg⁻¹ (Beniwal, Yadav and Ramakrishna, 2023). Furthermore, plants exposed to As also had their nitric oxide metabolism, hormone production and nutrient acquisition reduced (Ahmad *et al.* , 2020; Bhat, Ahmad and Corpas, 2021; Beniwal, Yadav and Ramakrishna, 2023). It is well known that heavy metals such as arsenic have the ability to induce ROS. These ROS molecules are able to initiate stress-singling transduction pathways (Mittler *et al.* , 2022). Within these pathways, calcium-dependant proteins kinases (CDPKs) as well as mitogen-activated protein kinases (MAPKs) play a key role. The mechanism governing arsenic uptake and detoxification are fundamentally alike across plants, yeast, and bacteria (Navarro et al., 2022). It has been stated that arsenic can mimic natural substrates such as phosphate (Pi), glucose, and glycerol well enough to share their uptake systems, suggesting that uptake of various forms of arsenic, from the soil to the root cells, can occur via numerous kinds of transporters (Garbinski et al., 2019) such as phosphate transporters, silicon transporters and aquaporins

(Khan et al., 2021). As(V) are mainly facilitated by Pi transporters (Abedi and Mojiri., 2020). Whereas shown in *Oryza sativa* (rice) plants, arsenite (As(III)) is transported into root cells via silicon transporters (Khan et al., 2021). It has been shown that MAPKs are activated in response to As(III), suggesting their implication in arsenic signaling (Navarro et al., 2022) and it has been documented that arsenic lead to production of ROS and NO in arsenic treated *O. sativa* seedling roots (Rao et al., 2011), however, whether this MAPKs response was instigated by ROS production was to be validated. Rao et al., 2011 observed that in *O. sativa* roots a 42- and 44-kDa MPK was activated in response to arsenite treatment. Transcript analysis of the MAPK family revealed them to be *OsMPK3* and *OsMPK4* (Rao et al., 2011). Gupta et al., 2009 compared two varieties of *Brassica juncea* with varying arsenite tolerance (Pusa bold (more tolerant) and Varuna) and observed induction of ROS under As(III) treatment in both varieties. In-gel kinetic assays revealed activation of a 46 kDa MAPK in both varieties in response to As(III) treatment (Gupta et al., 2009), with the activation more prominent in the Pusa bold variety, indicating that MAPK might be playing important role in transducing As(III) signal for an appropriate cellular response (Gupta et al., 2009). A study by (Huang *et al.* , 2012) performed on rice exposed to arsenic highlighted that one MAPK (*OsMPK6*) belonging to the GMGC group and seven MAPKKK belonging to the STE group were upregulated in response to the stress. The same study also showed that transcription factors belonging to the HsfA and HsfB were upregulated during the experiment (Huang *et al.* , 2012). Other transcription factors which have garnered recent attention for their potential ability to improve arsenic tolerance include the WRKY transcription factors (Mirza, Haque and Gupta, 2022). These transcription factors were found to be the substrates of MAPK, which is able to phosphorylate them. A study by (Zeeshan *et al.* , 2022) observed that the transcription factors under the WRKY family, *GmWRKY6* (mediating arsenate transport), *GmWRKY46* (ABA signalling and auxin homeostasis) (Lal *et al.* , 2023), *GmWRKY56* and *GMWRKY106* had only a slightly higher expression when grown under arsenic stress when compared to the negative control. However, the same study showed that the application of zinc oxide nanoparticles led to a significant increase in the expression of these transcription factors, improved soybean development, and lowered arsenate uptake by reducing the phosphate and arsenate transport genes (Zeeshan *et al.* , 2022). The findings of the aforementioned transcription factors show the importance of their activation and the need to understand their role in improving arsenic tolerance. Another transcription factor that is activated by MAPK is MYB40. In a study by (Chen, Wang and Chen, 2021) the transcriptional factor was induced by arsenate; however, when this transcriptional factor was overexpressed, it increased the plant's resistance to arsenate by reducing the uptake of the metal. The reduction in arsenate was obtained through the expression of PCS1 (phytochelatin synthase) in *Arabidopsis* which was positively regulated by MYB40 (Chen, Wang and Chen, 2021). The findings of these studies show the importance between ROS stimulation of the Mitogen-activated Protein Kinases and the activation of certain transcription factors under arsenic toxicity.

Mercury

Mercury (Hg) is greatly distributed in nature (Bhattacharya, 2018), owing to its application in fertilizers, lime, sludges, and manures (Azevedo and Rodriguez, 2012). Mercury's lengthy half-life and extremely detrimental effects under low concentrations, has granted it as the heavy metal of significant toxicity (Chen et al, 2014; Shao et al, 2022). Entry of mercury into plants is mainly theorized to occur through ionic channels, where this heavy metal competes with other metals such as zinc, copper, iron, and cadmium (Azevedo and Rodriguez, 2012). Once in plants, this HM has no nutritional function (Shao et al, 2022) and tends to accumulate within the roots of several plant species (Chen et al, 2014), and here its accumulation is heavily phytotoxic causing disruptions to numerous cellular-level functions and inhibits plant growth and development (Chen et al, 2014). Mercury has been shown to be connected to an excess production of reactive oxygen species (ROS), which may cause lipid peroxidation, enzyme inactivation, inhibition of photosynthesis, transpiration and nutrient transport in plants as well as causing DNA and membrane damage (Chen et al, 2014; Shao et al, 2022). A study by (Chen et al., 2014) validated this as the authors noted that mercury stress led to an induction of ROS production (superoxide and hydrogen peroxide), in *Oryza sativa* (rice) roots. Through whole-genome array on rice roots, Chen et al., 2014 examined the transcriptome responses to short- and long-term mercury stress (Chen et al., 2014). Through in-gel kinase activity assays the authors

discovered two MAPKs of roughly 40- and 42-kDa that was dose-dependently activated in rice roots exposed to mercury (Chen et al., 2014). The authors though did not elucidate on 1. The identity of the two MAPKs and 2. The relationship between mercury-induced ROS production and these two MAPKs activation.

However, a study by Wang et al., 2012 elucidated these via comparative proteomic approach, using wild-type *O. sativa* and mercury (Hg^{2+})-tolerant mutant roots. Using Mass spectrometry (MS) in the signal transduction category, a MAP kinase 2 (MAPK2) was upregulated in the mutant and wild-type rice roots during Hg treatment (Wang et al., 2012), while it was weakly expressed in the untreated rice roots. The authors previous study indicated that the mercury induced ROS accumulation, and that OsMPK3 is a ROS-induced MAPK (Yeh et al., 2003; Wang et al., 2009). Therefore, in view of Hg^{2+} induced up-regulation of MAP kinase 2 and protein kinase domain containing protein, we suggest that the MAPK signaling pathways are required during plant response to Hg^{2+} stress (Wang et al., 2012). Yeh et al., 2003 indicated that H_2O_2 was involved in the induction of a 42-kDa MAPK-like kinase by $CuCl_2$ in rice roots, by using ROS scavenger, glutathione. The author indicated that pre-treatment of rice roots with glutathione, greatly decreased the level of copper-induced MAPK-like activation (Yeh et al., 2003). Suggesting that $CuCl_2$ treatments result in ROS production and activation of MAPK-like kinase, at least in part (Yeh et al., 2003). The authors discovered that the 42-kDa MBP was in good accordance with those of OsMAPK2 (Yeh et al., 2003). In plants, higher amount of copper, cadmium and mercury ions lead to activation of a novel MAPK gene *OsMSRMK2* from japonica-type rice (cv. Nipponbare). (Sinha et al., 2011). *OsMSRMK2* was shown to be a multiple stress responsive MAP kinase gene. It was indicated that this gene accumulated rapidly suggesting the role of *OsMRMK2* in transmitting signals produced in response to environmental cues (Agrawal et al., 2002). Chen et al., 2014 noted that of their multiple differently expressed genes in response to Hg-treatment, WRKY and heat shock factor (HSF) transcription factors were highly upregulated in rice roots. Numerous studies have previously reported that WRKY and HSF serves essential roles in plant resistance to biotic and abiotic stresses (Chen et al., 2014). In the authors array data, they discovered 5 group I WRKY transcription factors (*OsWRKY24, 30, 38, 53* and *70*) that were upregulated under mercury stress, hence, leading to the authors to assume that group I *OsWRKY* genes may serve particular roles in the mercury-stress response by activating MAPKs (Chen et al., 2014).

Regarding all the heavy metals discussed above it can be noted that among the HM-induced ROS production and MAPK activation that MPK3 and MPK6 were two of the MAPK's that were in response to most of the heavy metals indicating their roles in HM-ROS-MAPK response pathway in various plants. Hence, these MPK should be further investigated to elucidate further how they confer a degree of tolerance to various metals and further to assess the downstream transcriptional programming that confers a degree of tolerance to HM-stress.

Pipeline

In the forthcoming years, heavy metals will harshly threaten crops due to climate change and continuous heavy industrialization. Therefore, there is an urgent need to validate the functional roles of MAPK genes and downstream transcriptional factor activation, in order to develop heavy metal resistant crops. This review highlights a pipeline that can be implemented across a variety of plant species to determine and elucidate the relationship between HM-induced ROS production and the activation of MAPKs, under various HM-treatments (Figure). In which the first step would be to conduct the analysis on fully sequenced genomes (Arabidopsis and rice) or on emerging sequence information (Populus, Medicago, lotus, tomato, maize, and chickpea). This is an essential aspect for validating or discovering new players within this signaling pathway and will assist with efficiently determining the function of multiple genes simultaneously (Sinha et al., 2011). The via three approaches (ROS stimulus, ROS inhibitors, or ROS scavengers) one can assess the relationship between HM-ROS production and MAPK activation. Thirdly, to identify MAPKs that are differentially regulated under HM-treatments, in-gel kinase assays can be conducted, and MAPK can be identified based on their molecular weight. In order to confirm the activity of MBP phosphorylating kinase is due to a MAP kinase, immunokinase assays can be performed (Figure). These steps above all assist in validating the MAPKs activation via HM-ROS production, however, various other approaches can be incorporated or

explored to further elucidate more players within the MAPK pathway. A pipeline elucidating downstream MAPK transcription factors has not yet been properly developed. In order to identify more MAPK gene families in plants related to HM-stress and to identify their functional analysis multiple approaches such as CRISPR/Cas technology, DNA/RNA sequencing, transcriptomics, proteomics, and metabolomics can be explored to allow the analysis and clarification of a regulation network that controls HM-stress response.

To gain more insight into the cellular responses of plants to heavy metals, we could perform a large-scale analysis of the plant's transcriptome during these heavy metal stresses (Chen et al, 2014). Identifying and further characterization of the acquired heavy metal- responsive TF and genes may be helpful for better understanding the mechanisms of heavy metals in plants (Chen et al, 2014). Once these key TF and genes have been identified, mutational studies (gene knock-ins and knock-outs) can be conducted to observe functional roles of these key players in heavy metal responses. Proteomics is another approach which could be used to address the biological function of proteins in response to the various HM-stresses, in order to assist with understanding the injury mechanisms induced under the HM-stresses as well as understanding the adaptation processes of the plants to these conditions (Wang et al., 2012). The study of plant stress responses at the proteome level has provided deeper insights into the functions of protein/protein-networks during plant adaptative stress responses. Proteins play important functional roles in the maintenance of cellular homeostasis and the regulation of adaptive stress response mechanisms such as regulating phenotypic plasticity (Zhu et al., 2023, Mackenzie and Kundariya, 2020).

In the post-genomics era, the use of mass spectrometry (MS) based proteomics has significantly improved the ability of researchers to understand how plants respond to varying conditions or stresses (Liu et al., 2019). By coupling enrichment techniques with MS-based proteomics, researchers gained crucial information about the about the function and subcellular localization of proteins as well as their post translational modification (PTM) (Komatsu and Hashiguchi, 2018, Zhao and Jensen, 2009). Of the numerous PTMS, protein phosphorylation continues to be one of most extensively researched. During this process kinases mediate the addition/removal phosphate groups present on serine, threonine and tyrosine residues which alter the function of a protein (Ramazi and Zahiri, 2021). Hence, the use of phosphoproteomics has become a powerful in understanding mechanism phosphorylation-dependant signalling response, such as MAPK in plants (Wang et al., 2020)

In the context of plant stress responses, MAPKs have been shown to be involved protein regulation via phosphorylation mediated PTMs. In a study by Forzani *et al* .,(2011) study and authors reported that serine/threonine protein kinase Pto-interacting 1-4 (PTI1-4) as one of the targets of MAPK (MPK6) for signalling during oxidative stress responses in *Arabidopsis*(Forzani et al., 2011). Additionally in separate study several MAPK's, namely SIMK, MMK2, MMK3, and SAMK, were shown to be induced during plant heavy metal stress response in alfalfa roots (Jonak et al., 2004). In a study by Zhong et al., (2017), using phosphoproteomics the authors observed a significant increase the phosphorylation of proteins in Cd-stressed rice. In their study many of the proteins were involved in ABA mediated abiotic stress responses, some of which included the MAPK signalling protein DSM1, that has been shown to be involved in the regulation of ROS scavenging (Zhong et al., 2017).

In this review we discussed the various processes MAPKs are involved in, such as plant development and stress responses. The activation of proteins by MAPK-phosphorylation plays an essential role in plant heavy metal stress responses, affecting processes such as metal mobility, managing ion toxicity, and minimizing oxidative damage. The use of modification-specific proteomics tools such as phosphoproteomics could assist with deepening our understanding of the contribution of how phosphorylation-dependant, MAPK-mediated changes influences plant HM responses. This holds great potential for the future progression of plant heavy metal stress tolerance, benefiting both agricultural and environmental conservation.

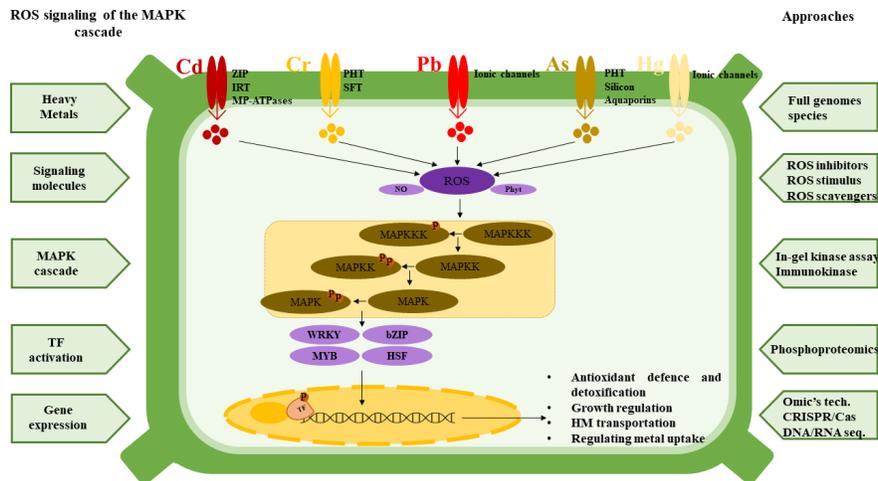


Figure 1: Schematic diagram showing the heavy metal’s ROS signaling of the MAPKs cascade and a possible pipeline to identifying and elucidating more key players in this pathway.

Conclusion

Heavy metals do not decompose in the soil, and therefore poses a major threat to plant growth and development (Rashid et al., 2023). HM toxicity becomes more evident when their concentrations exceed threshold levels (Table 1) within the soil (Rashid et al., 2023). ROS production and alteration in plant’s redox state is a shared response among heavy metal stresses (Singh et al., 2019). Overaccumulation of ROS has been shown to be detrimental to plant development; however, plants have adapted signaling pathways to which the perceived stress signal is sent to the nucleus for an appropriate response (Singh et al., 2019). While there has been much research conducted on HMs and plants adaptive mechanisms, there has been a knowledge gap between signaling pathways and how individual HM-ROS signals interact and activate a particular signaling pathway, the MAPK pathway and further how activation of MAPKs triggers a downstream activation of transcription factors to impart resistance against HM-stress.

In animals MAPKs regulatory network has been extensively studied under heavy metal stress, more so than in plants, therefore, it will be greatly beneficial to investigate the importance of MAPKs crosstalk in heavy metal stress in plants. Limited investigations report on the regulatory network of MAPKs with transcription factors, therefore indicates towards the need for more in depth experiments in response to heavy metals in plants. In this review, the influence of HM-ROS on the activation of MAPKs along with transcription factors are reported. Numerous studies across various scientific groups assisted to elucidate that various HM do indeed instigate the production of ROS which interplay with MAPK to induce a stress response. Furthermore, this review delved into the downstream transcription factors (WRKY, bZIP, MYB and HSF) that are activated to highlight particular stress responses (detoxification, HM-uptake, HM-transport, and growth and development) under various HM-stresses. The pipeline proposed in Section 4 and Figure 1 indicates an approach to assist in bridging the gap between HM-ROS induced MAPK and the downstream transcription factors activated. Hence, the purpose is to confirm the functional roles of MAPK genes, and the transcription factors and their regulation by ROS signaling to develop heavy metal resistance plants.

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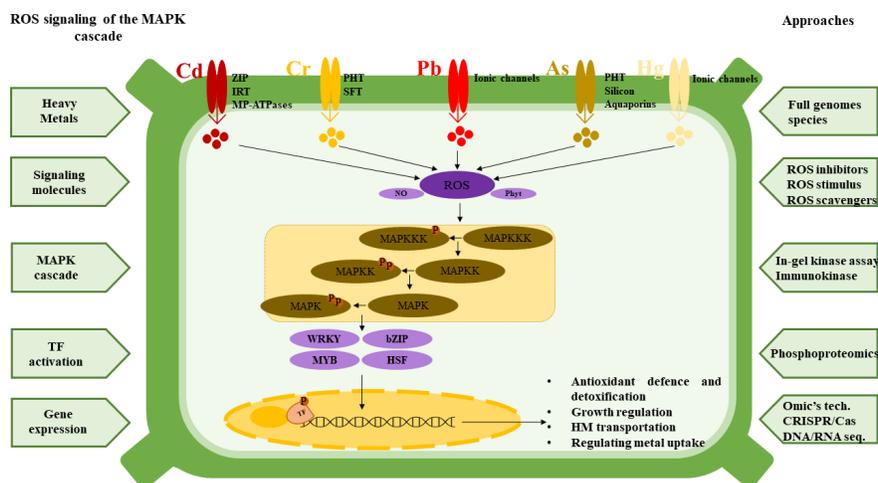
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TF	Gene	Response to Regulation	Processes involved in	Reference
WRKY	<i>AtWRKY12</i>	Up regulated under Cd	Represses GHR1 expression to negatively regulate cadmium tolerance in Arabidopsis	Han et al., 2019
	<i>AtWRKY13</i>	Up regulated under Cd	Activates PDR8 expression to positively regulate cadmium tolerance in Arabidopsis	Bong et al., 2019
	<i>GmWRKY142</i>	Up regulated under Cd	Confers cadmium resistance by upregulating the cadmium tolerance 1 like genes	Li et al., 2022
	<i>WRKY33 Chiquito (Yucca-CTL3)</i>	Upregulation under HMs stress (i.e., Cd)	Regulating HM uptake via iron-regulated transporter 1 regulation	Zhang et al., 2023
<i>AtWRKY7</i>	Up regulated under Cd and Pb	unknown		Karapınar et al., 2017
ZIP	<i>ZmZIP1</i>	Differential regulation in response to various stresses (i.e. Cd)	Enhance antioxidant enzyme activity	Wang et al., 2010
	<i>GmZIP</i>	Expressed specifically in different tissues under Cd stress	Affect protein-protein interactions networks and plays an important role in plant growth and stress responses	Han et al., 2021
	<i>OaZIP3</i>	Cultivar dependent	Related to the tolerance of rice to Cd stress and affected Cd accumulation in rice grains	Pan et al., 2021
	<i>BnaZIP29</i>	Up regulated under drought and heavy metal Cd stress	Involved in plant growth, development, and stress signaling	Huang et al., 2016
	<i>AtZIP</i>	Up regulated under Cd	Interacts with MYD49 and prevented its binding to the downstream gene, resulting in inactivation of IRT1 and reduced Cd uptake	Zhang et al., 2019
	<i>ZmZIP</i>	Upregulation under Chromium (Cr(VI))	Generation of H ₂ O ₂ , triggering HM-defence, reducing Cr(VI) accumulation	Fang et al., 2017
	<i>GmZIP154</i>	Upregulation under multiple stresses including Cr(VI)	Regulation of antioxidant enzyme activity	Chai et al., 2022
	<i>ZmZIP10</i>	Downregulation under Cr(VI)	Oxidative stress responses	Fan et al., 2019
MYB	<i>GmMYB45</i>	Downregulation increase Cd sensitivity	Maintaining redox homeostasis via antioxidant activity	Hu et al., 2017
	<i>SaMYB15</i>	Up regulated under Cd ²⁺	Cadmium and nickel tolerance transgenic tobacco	Saparov et al., 2019
	<i>AtMYB4</i>	Up regulated under Cd	Improves antioxidant defence systems and enhances expression of PCS1 and MT1C under Cd-stress in Arabidopsis	Agarwal et al., 2020
	<i>AtMYB2</i>	Up regulated under Cd	Improve Cd ²⁺ stress tolerance	Xu et al., 2018
	<i>GmMYB4</i>	Upregulation reduces HM (i.e. As) tolerance, Downregulation improve tolerance	Regulation of shoot to root HM uptake	Wang et al., 2017
HSF	<i>SaHSF46</i>	Up regulated under Cd	Enhance Cd tolerance by ROS-scavenger activities and shock proteins expression	Chen et al., 2020
	<i>TaHsf44a</i>	Up regulated under Cd	Upregulates MT genes expression for Cd tolerance	Shen et al., 2009
	<i>PtHsf1</i>	Up regulated under Cd	Participates in the reconstruction of proteins and improve intracellular homeostasis to increase cadmium tolerance	Song et al., 2018
	<i>HsHf14</i>	Up regulated under Cd	Upregulates melanin biosynthesis to confer cadmium tolerance in tomato plants	Cui et al., 2017