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# Heavy Metal and Mineral Element-Induced Abiotic Stress in Rice Plant

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## Abstract

The adverse effect of nonliving factors on living organisms is described as abiotic stress. It includes drought, excessive watering, extreme temperatures, salinity, and mineral toxicity. Rice is an important cereal crop, grown under diverse ecological and agricultural conditions. Heavy metal contamination of agricultural land causes abiotic stress to the crop plant as well as has a drastic effect on humans. Increased metal concentration in plants leads to the production of reactive oxygen species which results in cell death and thus affects the crop production in plants. In addition, increased heavy metal concentration in the plant has deleterious effects on its consumers. Like other organisms, plants have also designed ways to deal with such stress situations. In this chapter, abiotic stress due to metal toxicity in rice plant, which includes uptake and sequestration mechanisms, biochemical changes taking place in the plant and variation in their gene expression is elucidated. Based on several molecular and biochemical studies in various reviews and research papers, the role of different transporters like zinc-regulated transporter (ZIP), natural resistance-associated macrophage protein (NRAMP), copper transporter (COPT), yellow stripe like (YSL), heavy metal ATPase (HMA), metal tolerance protein (MTP) and other vascular transporters involved in the above processes in rice plant will be discussed in this chapter.

**Keywords:** transporters, reactive oxygen species, vacuolar sequestration capacity, antioxidant system, gene regulation

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## 1. Introduction

Any negative impact of nonliving factors on living organisms in a particular environment can be described as abiotic stress. There are different types of abiotic stress like those due to

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drought, excessive watering, that is, water-logging/flooding, extreme temperatures (cold, frost and heat), salinity and mineral (metal and metalloid) toxicity. These have a negative impact on seed germination, plant growth, development, yield, and seed quality of crops. Changes in environmental conditions affect the biological and physiological response of plants. This chapter deals with abiotic stress due to metal toxicity in rice plants, which includes uptake and sequestration mechanisms and biochemical changes taking place in the plant.

For their overall growth and development, plants require 14 different mineral elements [1]. These elements are present in the soil and are taken up by the roots, translocated to the shoots, and then distributed to different organs and tissues of the plant depending on their needs [1].

Minerals comprise of both metals and metalloids that are toxic to both plants and animals even at a very low concentration. Some of these heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) or selenium (Se), do not perform any known physiological function in plants, and are called nonessential metals. Others, such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn), are essential elements as they are required for the normal growth and metabolism of plants. Essential elements can lead to poisoning when their concentration rises beyond their optimal levels.

Plants growing on metal-contaminated soils are categorized as resistant varieties, which have adapted to this stressed environment. Heavy metal resistance is attained by the plants either by avoidance/tolerance or by both. Plants, which can prevent the entry of metal ions into their cell cytoplasm, are categorized as avoiders, while the plants which can detoxify the metal ions can cross the plasma membrane or organellar membranes are grouped as tolerant varieties. Plants were thus classified into three groups as: metal excluders, indicators and accumulators/hyperaccumulators by Baker and Walker [2] based on the approach used by the plants to grow on metal-contaminated soils. Excluder group of plants restricts the uptake and translocation of metal ions to their shoots thus maintaining low levels of heavy metals in the shoots even when grown over a varied range of metal concentrations in soil. Most of the plants belong to the excluder group. Plants, which are classified as metal indicators, can accumulate metals in their aerial shoot system. The level of metal ions in their aerial biomass generally indicates the concentration of metal in the soil. Plants, which are classified as metal accumulators/hyperaccumulators, can take up, transport and accumulate metals generously in their aerial parts to levels higher than the metal concentration found in the soil [2]. The antioxidant defense system in plants helps to accumulate and tolerate the side effects of high levels of internal metal concentrations. Antioxidant system is activated in order to combat the deleterious effects caused by reactive oxygen species (ROS) generated due to stress [3]. Metals can interfere with mineral nutrition and change the concentration and composition of plant nutrients. Metals can also alter the conformation of proteins, including transporters, or other regulatory proteins [4].

Rice (*Oryza sativa* L.) is one of the most important cereal crops in most part of the world and is cultivated in tropical and temperate regions of the world. Rice is the staple food for half of the world's population majorly for many South East Asian countries. It ranks second next to wheat among the most cultivated cereals in the world to feed the ever-growing population. The genus *Oryza* contains 21 wild and 2 cultivated species (*Oryza sativa* and *O. glaberrima*)

with 10 genome types [5, 6] *O. sativa* is composed of two subspecies: *japonica* and *indica*. It is also a model monocotyledon plant as it has a small genome, which has been sequenced (for both the cultivars *japonica* [7] and *indica* [8]).

Plants are sessile and its roots are exposed to various stresses like deficiency and an excess of mineral elements. Heavy metal contamination and accumulation in water, soil, and air due to various reasons has become a serious environmental problem and has greatly affected rice growth and quality. Heavy metals accumulated in rice are toxic to growth, metabolism, and development of plants. Thus, the transfer of heavy metals from soil to plants of commercial agricultural value, such as rice, is of great concern as it may lead to biomagnification via food chain causing several deleterious effects to the consumer. Heavy metals can enter the human body through the food chain, leading to an increased prevalence of chronic diseases, deformities and cancer. The uptake of heavy metal ions by rice plants poses a threat to the consumer's health. Thus, it is important to understand the uptake and sequestration of heavy metal in the rice plant in order to understand the stress caused by it to the plant.

Availability of metals in soil to plants is controlled by three steps: (1) soil conditions (upland or flooded soil and soil solution pH); (2) mineralization (ionization and complex formation) and (3) uptake and efflux transporters.

In addition to genetic variation, metal uptake is sometimes limited by its bioavailability in the soil. The availability of metal ions like Fe, Zn, and Cd for plant uptake varies mainly depending on soil redox potential. Generally, rice is cultivated under flooded conditions. The practice of flooding paddy field increases Fe availability while decreasing Zn and Cd availability while moderate soil drying improves Zn and Cd uptake and but decreases Fe uptake [9]. In both drained and flooded soils, Zn mainly exists as  $Zn^{2+}$ , while some of it binds to organic substances, and is immobilized as Zn-sulfide (ZnS) in the anaerobic layer of soil [10]. In drained acidic soils, cadmium exists in its ionized state as  $Cd^{2+}$  ion, whereas Cd in alkaline paddy field soils is present in the forms of  $CdCO_3$  and humic acid-bound Cd [11]. Flooding of soil immobilizes Cd as Cd-sulfide (CdS) and colloidal-bound Cd [12]. Drying of soil converts CdS to  $Cd^{2+}$  and increases its availability to plants. In acidic soil, Fe is ionized as  $Fe^{2+}/Fe^{3+}$ , and Fe in aerobic alkaline soils is immobilized as  $Fe(OH)_3$ .

Both Cd and Pb are nonessential elements for plants and are toxic even at a very low concentrations, but are readily transported within the plants. The uptake and sequestration of Cd and Pb by crops is of great concern, due to their accumulation in the edible parts of the plant. The uptake of Cd and Pb, their transport, and accumulation by plants are strongly influenced by soil properties and vary with plant species [13, 14]. Cd is freely taken up by the plants and its uptake increases with increased external concentration. The amount of Cd accumulated after its uptake and its translocation to different organs varies with species and with cultivars within the species. The ability for uptake and sequestration of metals in different parts of the plants varies between different plants. There exists a huge difference in metal uptake and translocation between plant species and even between different cultivars of the same species [15]. Roots of most cereal crop plants are present at a depth of 25 cm from the soil surface from where the heavy metals are absorbed by the plant [16].

Roots are the primary target for the accumulation of metals, and metals like Cd and Pb are accumulated mainly in roots [14]. Plants have developed several strategies to decrease metal ion toxicity, one of which is the cellular transport system. There are several groups of metal transporter proteins identified in plants.

Rice has a distinct root system. Anatomically, rice root is characterized by the two casparian strips present on the exodermis and endodermis. The apoplastic flow of water and movement of mineral elements is prevented in between the cell layers by casparian strips, which act as a barrier [17]. Also, the mature rice root has a well developed aerenchyma, which has well developed vascular bundles for the upstream translocation of metals and other mineral elements from the roots to the shoots. Apoptosis of cortical cells creates the aerenchyma, which has an apoplastic space and no symplastic connections. The apoplastic space is connected by remaining spoke-like connections between the exodermis and the endodermis [18]. Thus to reach the stele, the mineral nutrients in rice roots have to be transported via the symplast of both the exodermis and the endodermis and also through the apoplast of the aerenchyma. The root tips, which lack aerenchyma and casparian strips, can also accumulate mineral elements but in small percentage due to their highly undeveloped vascular system [19]. For efficient translocation of mineral nutrition from the soil solution to the stele in rice, roots require cooperation between both the influx and efflux transporters. Transporters involved in this uptake process have been identified for some mineral elements, but most of them remain unknown. Thus plants take up heavy metals through their roots majorly via various transporters from the soil and accumulate them in their aerial parts.

Metal ions are transported from the soil into the root and then distributed throughout the plant, after crossing both cellular and organellar membranes [20]. All the characterized plant transporter proteins, which are responsible for metal homeostasis, are membrane proteins that mediate heavy metal movement through membranes. Transporters in plants either act at the plasma membrane to move metals into the cytoplasm or at the intracellular organellar membrane to re-circulate metals from intracellular compartment into the cytoplasm. They are classified into different families such as natural resistance-associated macrophage protein (NRAMP), zinc-regulated transporter (ZIP), yellow stripe 1-like family (YSL), and Ctr/copper transporter (COPT) family of high-affinity Cu uptake proteins. Plant transporters have been identified in *Arabidopsis thaliana* to be involved in metal efflux from the cytoplasm either across the plasma membrane or into the organelles. They are classified into two families namely  $P_{1B}$ -ATPase family/CPX-ATPase and cation diffusion facilitator (CDF) family/metal tolerance protein1 (MTP1) [21]. Similar metal transporter families are also present in rice.

Various such transporters have been identified in different plant species, which are involved in the metal uptake and sequestration process. This chapter focuses on rice plant transporters that are involved in the uptake of mineral elements in roots and its sequestration into the vacuoles and also the biochemical changes taking place in the rice plant during abiotic stress due to heavy metals.



## 2. ZIP family

ZIP family of transporters is named after the first proteins identified ZRT, iron-regulated transporter (IRT) like protein [20]. They are present in bacteria, fungus, plants, and humans. ZIP proteins can transport divalent cations like  $\text{Fe}^{2+}$ ,  $\text{Zn}^{+2}$ ,  $\text{Mn}^{+2}$  and  $\text{Cd}^{+2}$  [22]. Zn as such cannot be transported across the cell membrane, it requires specific zinc transporters for its transport into the cytoplasm. Generally, ZIP family of transporter proteins transport cations into the cell cytoplasm and play a role in cellular metal ion homeostasis. ZIP proteins generally localized at the plasma membrane and are involved in either moving metals or to remobilize them from intracellular compartments into the cytoplasm [21]. In rice, 14 putative ZIP family of transporter proteins have been identified. It is divided into two subfamilies based on their amino acid sequence similarities. Zinc is taken up by these transporters as a divalent cation and plays role in cellular activities in the form of tetrahedral complexes as it is neither oxidized nor reduced [20].

IRT1 was the first member of ZIP family to be identified. It is a Fe(II) transporter and is involved in the uptake of iron from the soil. Studies have proved that in addition to Fe, it can transport Mn and Zn. IRT1 mediates Cd accumulation in iron-deficient plants. Depending on the plant species, there are two mechanisms for the uptake of Fe by IRT transporter from soil. The first strategy is used by all dicot and non-gramineous monocot plants. In this process, iron uptake by IRT1 transporter happens after ferric Fe is reduced to ferrous Fe by ferric chelate reductase (FRO2) on the plasma membrane [23, 24]. The second strategy is used by graminaceous plants. It is characterized by the secretion of mugineic acid (MA) and forms mugineic acid-ferric complex followed by its uptake by IRT1 transporter [25]. Though the rice root does not have Fe reductase enzyme activity [26], ferrous Fe is abundantly present in paddy fields due to reductive status in soil. Both OsIRT1 and OsIRT2 are involved in Fe uptake in rice plant. They are expressed mostly in roots and induced by Fe deficiency [27]. OsIRT1 can only uptake Fe but not Cu.

OsZIP1 and OsZIP3 are involved in the transport of Zn but not Fe/Mn. OsZIP4, OsZIP5, and OsZIP8 are functional rice zinc transporters expressed on the plasma membrane [28]. OsZIP1 and OsZIP3 are upregulated in roots and shoots upon Zn deprivation. Zn deficiency upregulates OsZIP2 only in roots [27], whereas Zn deficiency upregulates OsZIP5 and OsZIP7 in rice shoots. In the mature rice plant, OsZIP7a and OsZIP8 are expressed constitutively and weakly in roots, culms, leaves and flowering spikes [29]. Expression of OsZIP9 is also induced by Zn deficiency and it complements the absence of OsZIP5. OsZIP4 is expressed in Zn deficient shoots and roots especially in phloem cells and meristems. Zn deficiency induces the expression of OsZIP8 in both roots and shoots. Expression of OsZIP5 is relatively higher in roots than in shoots and is specific to zinc [27]. OsZIP4 is localized to the plasma membrane. It is a Zn-regulated Zn transporter involved in the transport of Zn. It controls the supply of Zn to developing young leaves and is involved in remobilization of Zn from old to young leaves [28].

In rice Zn transporters OsZIP1, OsZIP3, OsZIP4, and OsZIP5 are induced by Zn deficiency [26, 27, 30]. OsZIP1, OsZIP3, and OsZIP4 are expressed in the vascular bundles in both rice

shoot and root and only in the epidermal cells in rice root [26, 30]. OsIRT1, OsZIP5, and OsZIP4 are upregulated in Zn-deficient roots and OsZIP4, OsZIP5 and OsZIP7 are upregulated in Zn deficient shoots [28].

OsZIP4 is expressed in the vascular bundles in rice root and shoot and also root and shoot meristem during Zn deficiency. Basically, OsZIP1 and OsZIP3 are involved in Zn uptake in roots and Zn homeostasis in shoots [30]. OsZIP4 transports Zn, specifically into vascular bundles and meristem [28]. OsZIP6 is transcriptionally activated in the shoot and root tissues in response to the deficiency in  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$ . Ion transport by OsZIP6 is pH dependent and enhanced transport is observed at acidic pH. OsZIP6 is involved in iron uptake in roots and transport of Fe, Zn, and Mn in the shoots of rice [31]. OsZIP1 and OsZIP3 transport only  $\text{Zn}^{2+}$  and not  $\text{Fe}^{2+}$  or  $\text{Mn}^{2+}$  [30], OsZIP4 transports  $\text{Zn}^{2+}$  and not  $\text{Fe}^{2+}$  [28] and OsIRT1 similarly transports  $\text{Fe}^{2+}$  and not  $\text{Cu}^{2+}$  [32]. OsZIP6 transports at least three transition metal ions, namely,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Cd}^{2+}$ . Substrate affinity for OsZIP6 is in the order  $\text{Co}^{2+} > \text{Cd}^{2+} > \text{Fe}^{2+}$ . ZIP transporters show both high and low affinity transport [31]. In rice, zinc transporter OsZIP1 exhibits enhanced transport at pH 4.7, in contrast to OsZIP3, where maximum activity is observed at pH 6.0 [30]. OsZIP6 is expressed in both roots and shoots at maximum tillering and mid-grain filling stages [33]. OsZIP8 is a plasma membrane zinc transporter in rice that functions in Zn uptake and distribution. During Zn deficiency, it is highly upregulated in shoots and roots [27].

In rice seed, Zn is present in the embryo, endosperm, and the aleurone layer. The Zn content is specifically high in the embryo [34]. During germination, Zn content in the endosperm decreases, while Zn content increases in the radicle and leaf primordium. Zn content increases in the scutellum and its vascular bundle after 24 hrs of sowing [34]. During germination, expression of ZIP family of transporter members decreases [35]. In the embryo meristematic tissues, Zn accumulation is limited. For such a partial localization of Zn, a decrease in OsZIP family transcripts is required. Different rice genotypes vary mainly in their efficiency to utilize Zn and in their grain Zn contents [36]. Thus, the wrong expression of ZIPs could lead to the irregular distribution of the essential micronutrients in the plant.

### 3. Yellow stripe-like (YSL) proteins

Though iron is abundant in the earth's crust, it is mostly unavailable to plants because, at a neutral pH, it forms insoluble ferric oxide complexes in aerobic environment [37]. Graminaceous plants use the chelation-based strategy II. In response to Fe deficiency, these plant cells release phytosiderophores (PSs), which belong to the mugineic acid (MA) family and are derived from the precursor nicotianamine (NA). These molecules bind to Fe(III) and specific plasma membrane transporter proteins to import the Fe(III)-PS complexes [38]. The molecular mechanism controlling Fe(III)-uptake was elucidated by cloning the membrane transporter from the maize yellow stripe 1 (ys1) mutant, which showed characteristic interveinal chlorosis or yellow patches [39]. Because that mutant is deficient in Fe(III)-PS uptake, it has been suggested

that YS1 is the Fe(III)-PS transporter. The YS1 protein is upregulated by Fe deficiencies in roots and shoots, and functions as a proton-coupled symporter to transport Fe(III)-PS [40].

Similar to maize YS1 gene sequence, 18 such genes have been putatively identified in rice and named as yellow stripe-like (YSL) genes [41]. Though YSL is part of the larger oligopeptide transporter (OPT) family, which is also present in fungi [42], but YSL transporter family members can only be found in plants [43]. YSL family of transporters cannot transport free metals as such but can transport only metals along with nicotianamine (NA) or its derivatives [42]. Nicotianamine is a precursor of phytosiderophores, which are high-affinity Fe ligands exclusively synthesized by Poaceae species and excreted by roots for the chelation and acquisition of Fe [41]. NA is a non-proteogenic amino acid, synthesized from S-adenosyl-methionine by the enzyme NA synthase (NAS) [44]. It is a structural analog of 2'-deoxymugineic acid (DMA), which is formed by NA aminotransferase (NAAT) and DMA synthase (DMAS) [45]. In monocot plants, YSL transporters are associated with metal uptake from the soil, while in both monocots and dicots plants they are involved in long-distance metal translocation [46].

In rice, OsYSL2 transports Fe(II)-NA complex and Mn(II)-NA complex and is mainly expressed in the phloem cells of the vascular bundles, especially in the companion cells of Fe-deficient leaves [47]. It also mediates long-distance transport of manganese, especially to the grain [47, 48]. The expression of OsYSL2 is induced by Fe deficiency in the leaves but not in the roots. OsYSL2 is important for Fe translocation at the early stage of growth [35] also important for long-distance transport during grain filling, particularly for Fe translocation to the endosperm [48].

OsYSL15 is localized to the plasma membrane and mediates the uptake of MA-Fe complex [49]. Fe deficiency induces *OsYSL15* in roots but is unaffected by Zn, Mn or Cu deficiency [49]. Under Fe deficiency, *OsYSL15* is expressed in the epidermis, endodermis, cortex, and vascular bundles of the roots and leaves [49]. The OsYSL15 transporter contribution in paddy soil is little as the secreted MA diffuses out of the rhizosphere. In neutral and alkaline soils, Fe<sup>3+</sup> binds to mineral and organic substances strongly such that ions are hardly available to plants and the iron is solubilized by forming a complex with a phytosiderophore, DMA [50], which is excreted by rice roots by OsTOM1 (rice DMA effluxer) [51]. Fe(III)-DMA complexes in the soil solution is then taken up by OsYSL15 [49]. Under flooded conditions, rice plants may absorb both Fe(III)-DMA and Fe<sup>2+</sup> [26].

OsYSL16 is a Cu-NA transporter which delivers Cu to the developing tissues and seeds through phloem transport. During vegetative growth, OsYSL16 is expressed in the roots, leaves, and unelongated nodes and during the reproductive stage, it is highly expressed in the upper nodes [52].

Among the 18 OsYSL genes in rice, OsYSL15 transports Fe(III)-DMA and Fe(II)-NA [49]. OsYSL15 expression is strongly induced in the roots and shoots by Fe deficiency. OsYSL2, induced by Fe deficiency, is localized to the plasma membrane and transports Fe(II)-NA and Mn(II)-NA, but not Fe(III)-DMA [47]. OsYSL18 is a transporter of Fe(III)-DMA but not of



Fe(II)-NA [53]. Its expression in flowers and the phloem of lamina joints indicates that it is involved in translocating Fe to the reproductive organs and phloem joints.

Expression of OsYSL2, OsYSL9 and OsYSL15 genes increases when Fe is limited. OsYSL9 is induced by Fe deficiency in the shoots but not the roots. On the other hand, OsYSL16 is constitutively expressed in both roots and shoots at levels similar to OsYSL2, OsYSL9 and OsYSL15 genes, but the alteration of Fe concentration has not shown any effect on the expression of OsYSL16 [47].

OsYSL13 is mostly expressed in the shoots, and its expression is reduced under Fe deficient conditions. OsYSL14 is expressed in both roots and shoots irrespective of the external Fe concentration. OsYSL15 is expressed only in roots and Fe deficiency highly induces its expression. OsYSL16 is expressed in both roots and shoots, and Fe deficiency slightly increases its expression in roots.

OsYSL2 is localized in the plasma membrane. It is not a Fe(III)-phytosiderophore transporter which is involved in the uptake of Fe from the soil [47]. In rice plant, OsYSL2 is expressed in the root companion cells and leaves phloem. OsYSL2 can also transport manganese-NA complex [47].

OsYSL6 is required for detoxification of excess Mn in rice thus helps in Mn tolerance. Irrespective of the Fe status, OsYSL6 is constitutively expressed in both roots and leaves. While OsYSL6 expression is slightly reduced under Fe-deficiency condition. The expression level increases with leaf age. This pattern is similar for Mn concentration in the different leaves [48].

YSL16 is a plasma membrane-localized transporter and is directly involved in distribution and remobilization of Cu as Cu-NA complex in the developing tissues and rice seed. It loads Cu-NA complex into the phloem, which is required for remobilization of Cu from older leaves to developing tissues like young leaves and seeds [52]. OsYSL16 is expressed in many cell types and is more preferentially expressed in the vascular tissues of roots and leaves. It has been studied that enhanced tolerance to a low-Fe environment can be achieved through over expression of OsYSL16.

OsYSL18 is a Fe(III)-DMA transporter which is involved in Fe distribution mediated by DMA in the reproductive organs, lamina joints, and phloem cells at the leaf sheath base. It is localized in the plasma membrane [54]. The other remaining putative OsYSL transporters in rice need to be functionally characterized in future.

#### **4. Ctr/COPT family: copper transporter**

This family of transporters is found only in eukaryotes. In plants, it is known as COPT transporters [55] and in animals and fungi as Ctr [56]. COPT family of proteins are important for copper uptake from soil and its transport to pollen in plants [57]. COPT proteins are localized on the plasma membrane and are involved in the transport of Cu from extracellular spaces

into cytosol or vacuoles or are localized on the lysosomal membrane and are involved in the supply of Cu from vacuoles or lysosomes to the cytosol [58].

The COPT family of transporters in rice consists of seven members: COPT1–COPT7. In rice plant, COPT proteins are specifically involved in Cu transport. It can transport only Cu(I) but not other bivalent ions such as Mn, Zn or Fe. In rice plant, COPT1–COPT7 are plasma membrane-localized proteins. As these transporters can form symmetrical homotrimer or heterotrimer structure with a diameter that is only suitable for Cu(I) transport and not other divalent ions [59] or heterocomplex with themselves or each other [60] or heterocomplex with other proteins which are involved in Cu transport [61].

COPT1 and COPT5 can exist as homodimers or a heterodimer. Only COPT1 and COPT5 bind to rice XA13 protein, a protein which is susceptible to pathogenic bacterium *Xanthomonas oryzae pv. Oryzae* (Xoo) [61] and mediate Cu transport in rice plant. In rice plant, all the COPTs except COPT1 and COPT5, function independently or together and mediate Cu transport in different tissues. COPT6 acts as a cofactor and aids the efficient localization of COPT2, COPT3 or COPT4 to the plasma membrane for mediating Cu transport. COPT1 and COPT5 show similar tissue and also develop-specific expression patterns. When compared to the sheath, stem, and panicle expression levels of these two genes, they have a higher level of expression in root and leaf tissues. COPT4 has higher expression level in root in comparison to other tissues. COPT1, COPT4, and COPT5 have higher expression level in young leaves than in old leaves, particularly COPT1 and COPT4. COPT2, COPT3, and COPT7 show higher expression levels in old leaves when compared with young leaves. COPT6 is not expressed in root and is highly expressed in leaf than in other tissues. COPT6 is constitutively expressed in different-aged leaves but has a low level of expression in shoot tissue at seedling stage. The expression of rice COPT1 and COPT5 are induced by Cu deficiency and suppressed by excess Cu in both shoot and root tissues. COPT1 and COPT5 together mediate Cu transport in rice plant [61]. The expression of rice COPT2, COPT3, COPT4, COPT6, and COPT7, is also affected by the variation in Cu levels. In both root and shoot tissues, COPT7 shows a similar response as COPT1 and COPT5 to Cu deficiency and overdose. In different-aged leaves of a mature plant, COPT6 is constitutively expressed while in the seedling stage, the shoot tissue has low expression levels. In shoot, COPT6 is induced under Cu deficiency state and suppressed in Cu overdose but no COPT6 expression is detected in root either with or without Cu deficiency. Cu overdose suppresses the expression of COPT2, COPT3, and COPT4 in both root and shoot tissues but their expression is not influenced by Cu deficiency. Expression of COPTs is also influenced by other bivalent cations. Mn deficiency induces expression of COPT1 in root and COPT3 and COPT7 in shoot and slightly suppresses the expression of COPT2 and COPT4 in root. The expression of COPT1, COPT5, and COPT7 is induced by Zn deficiency and COPT4 expression is slightly suppressed in the root. Zn deficiency also induces the expression of COPT5, COPT6, and COPT7 in the shoot. In root, Fe deficiency moderately induces COPT1 and suppresses COPT2 and COPT5 while in the shoot, it induces COPT2, COPT5, COPT6, and COPT7. In root, no COPT6 expression has been found either in the presence or absence of Mn, Zn, or Fe deficiency. COPT family of transporter proteins functions uniquely in different tissues, during various developmental stages, and in different environmental conditions. In rice plant, COPT2, COPT3, COPT4, COPT6 and COPT7 mediate Cu transport either

solely or cooperatively with each other. In different tissues of rice plant COPT2, COPT3, or COPT4 function along with COPT6 for Cu transport except in root. Expression of COPT2, COPT3, COPT4, and COPT6 has been observed in stem, sheath, leaf, and panicle tissues. Root shows relatively high expression levels of COPT3 and COPT4 but no expression of COPT6. In leaves, the expression of COPT2, COPT3, and COPT4 is developmentally regulated but not that of COPT6. In rice shoot, Cu deficiency strongly induces the expression of COPT6 but not COPT2, COPT3, and COPT4. COPT7 mediates Cu transport in rice all by itself. Based on its expression pattern, it has been suggested that COPT7 functions in different tissues and is unaffected by Cu deficiency [62].

## 5. NRAMP family

Natural resistance-associated macrophage protein (NRAMP) family of transporters are found in the three domains of life [63]. NRAMP transporters have a wide range of metal substrates, typically transport  $\text{Fe}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Co}^{+2}$ , and  $\text{Zn}^{+2}$  [63].

The first plant NRAMP genes cloned were from rice [64]. In rice, there are seven Nramp transporters, OsNRAMP1-OsNRAMP7. Though, not all have been functionally characterized [63]. Many of the NRAMP family proteins function as Fe transporters. *OsNRAMP1* is highly upregulated by Fe deficiency. *OsNRAMP1* is a plasma membrane-localized transporter and is involved in the transport of Cd and Fe. *OsNRAMP1* expression is observed mainly in roots at the vegetative state and is involved in cellular uptake of Cd and is responsible for high Cd accumulation in rice [65]. The differences observed in Cd accumulation among different rice cultivars are because of differences in *OsNRAMP1* expression levels in roots [65]. *OsNRAMP1* expression is higher during the reproductive stage in leaf blade and stem.

OsNRAMP3 is localized to the plasma membrane and is specifically expressed in vascular bundles, particularly in companion cells of phloem. *OsNRAMP3* is constitutively expressed in the rice node [66]. *OsNRAMP3* is a Mn-influx transporter involved in Mn distribution and redistribution to young leaves from old leaf via phloem cells. With leaf aging, the expression of *OsNRAMP3* in leaves increases slightly in rice plants. *OsNRAMP3* transports Mn from the enlarged vascular bundles to the younger tissues and panicles during Mn deficiency in order to meet its minimal growth requirement. On the other hand, when Mn is in excess, *OsNRAMP3* is internalized in vesicles and rapidly degraded. Then, Mn is preferentially loaded into the older leaves, which are directly connected to the enlarged vascular bundles, thereby protecting the developing tissues from Mn toxicity. This indicates the role of post-translational regulation of *OsNRAMP3* in response to environmental nutrient availability. Rice plant utilizes *OsNRAMP3* to respond to environmental changes to Mn availability. *OsNRAMP3* is involved in Mn translocation but not Mn uptake [67].

OsNRAMP4 is also known as Nramp aluminum transporter1 (Nrat1) is the first transporter in this family to be identified as the trivalent Al ion transporter [68]. In contrast to other rice NRAMP members, *OsNRAMP4* does not show transport activity for other divalent metal ions, like Zn, Mn, and Fe. It also shares relatively low similarity with the other *OsNRAMP*

members [68]. NRAT1 plays an important role in rice Al tolerance by reducing the level of toxic Al in the root cell wall and transporting Al into the root cell vacuole for sequestration. Rice is the most Al tolerant of all the cereal crops and OsNRAMP4 plays an important role in this [69].

OsNRAMP5 is a plasma membrane protein involved in Mn and Fe transport [70]. OsNRAMP5 gene expression increases slightly in the roots when plants are under Fe or Zn deficiency but varying levels of Mn in the surrounding does not affect it [71]. It is expressed in the mature root zone at the PM of the exodermal and endodermal layers [70, 71]. OsNRAMP5 in rice plant is essential for the uptake of Mn from the soil. In rice plant, OsNRAMP5 is constitutively involved in Fe and Mn uptake, it also plays a role in Fe and Mn transport during flowering and seed development [70].

OsNRAMP5 is highly expressed in hulls. It is also expressed in leaves but the expression level decreases with leaf age. In rice plant, OsNRAMP5 transporter is present in the vascular bundles of roots and shoots particularly the parenchyma cells surrounding the xylem. *OsNRAMP5* is also highly expressed in stele cells especially in the xylem region, thus plays an important role in the xylem-mediated root-to-shoot transport. Thus OsNRAMP5 plays an important role in the uptake, translocation, and distribution of Mn in rice plants. *OsNRAMP5* is highly expressed in stele cells especially in the xylem region, thus plays an important role in the xylem-mediated root-to-shoot transport [72]. OsNRAMP5 is a major transporter for Cd uptake in rice [71].

Recently, OsNRAMP6 has been identified to be involved in uptake of Fe and Mn. It is a plasma membrane-localized protein. It negatively regulates the rice plant immunity as loss of its function results in increased resistance against *M. oryzae* [73].

## 6. Heavy metal ATPases (HMAs)

The P<sub>1B</sub>-type ATPases, known as heavy metal ATPases (HMAs) in plants, play an important role in metal transport. HMAs vary in their tissue distribution, subcellular localization, and metal specificity. HMA transporters can be divided into two subgroups based on their metal-substrate specificity, they are Cu/Ag group and Zn/Co/Cd/Pb group. Rice plant has nine such *HMA* genes. OsHMA1–OsHMA3 are members of the Zn/Co/Cd/Pb subgroup in rice. Unlike dicots, only a few reports on HMAs from monocots are available. OsHMA2 plays an important role in root-to-shoot translocation of Zn and Cd and participates in their transport to developing seeds in rice. OsHMA9 phylogenetically belongs to the Cu/Ag subgroup but also plays a role in Zn, Cd, and Pb transport [74]. OsHMA3 transports only Cd and in root cells is involved in the sequestration of Cd into vacuoles [75, 76]. *OsHMA3* has been identified as a responsive gene for quantitative trait loci of Cd concentration in the rice cultivars Anjana Dhan and Cho-kokoku, and loss of function of this protein leads to high Cd accumulation in the shoots [75–77]. There is little information available on the role of OsHMA1 and is thought to be involved in Zn transport. *OsHMA1* expression in shoot tissue is highly upregulated by Zn deficiency [78]. OsHMA1 is suggested to play a role in Zn transport in the



plant throughout its growth and developmental stages [74]. Enhanced activity of OsHMA3 is related to increased storage of Cd in roots and its decreased transport to the shoot and the final accumulation in rice grains [74]. OsHMA2 is localized at the root pericycle and plays a major role in transport of Zn and Cd during xylem loading [74, 75].

*OsHMA3* gene selectively sequesters Cd into the vacuoles thus limits the root-to-shoot translocation of Cd [75, 76]. In rice plant, *OsHMA2* gene has also been shown to be involved in the translocation of Cd through xylem from root to shoot [79, 80].

In root cells, OsHMA4 is a vacuolar membrane-localized transporter and is involved in sequestering Cu into the vacuoles. OsHMA4 specifically transports Cu. Increased Cu accumulation in rice grain due to increased root-to-shoot translocation of Cu has been observed when OsHMA4 function is lost. In rice OsHMA4–OsHMA9 are members of the Cu/Ag subgroup of HMAs. OsHMA5 is a Cu transporter, localized to the plasma membrane [81]. In rice, OsHMA5 is involved in transferring Cu into the xylem for its root-to-shoot translocation and/or Cu detoxification in roots [81]. OsHMA4 is induced under long-term exposure of excess Cu and its expression is suppressed by Cu deficiency. In mature root zone, OsHMA4 is localized at the pericycle [81]. OsHMA4 regulates the cellular Cu concentration before loading to the xylem depending on its environmental concentration. OsHMA3 is localized in all root cells [75]. In future, the mechanism responsible for the transporter substrate specificity of the HMAs needs to be studied.

## 7. CDF/MTP

The cation diffusion facilitator (CDF) family of proteins plays an important role in the maintenance of cation homeostasis in all forms of life from bacteria, yeast, plants to mammals [82]. Generally, CDF proteins are involved in binding and efflux of cations such as Zn, Fe, Co, Cd, and Mn from the cytoplasm either by sequestering into internal organelles like vacuole or effluxing from the cell [82, 83]. CDF transporters also influence the cation accumulation, metal ion tolerance, signal transduction cascades, oxidative stress resistance, and protein turnover in cells [84]. In plants, CDF members are called Metal Tolerance Proteins (MTPs) and as Solute carrier family 30 (SLC30) in vertebrates [84].

The first plant CDF protein identified was ZAT (zinc transporter of *Arabidopsis thaliana*), because of its role in heavy metal tolerance in Arabidopsis. Later it was renamed as AtMTP1 (Metal Tolerance Protein 1) [85]. MTPs are a group of proteins that play an important role in heavy metal homeostasis in plants [82, 83]. MTP members are present in all the three kingdoms (Archaea, Eubacteria, and Eukaryotes). The plant CDF family can be classified into three subgroups phylogenetically: Zn-CDF, Fe/Zn-CDF, and Mn-CDF [83] based on their main substrate transported: Zn, Zn, and Fe, or Mn [82, 84].

Rice genome has 10 MTP genes [84]. Studies have shown that Rice Metal Tolerance Protein1 (OsMTP1) gene expression is induced by Cd and OsMTP1 belongs to the Zn-CDF subgroup. In rice, there are five Mn-CDFs, three Zn-CDFs, one Fe/Zn-CDF, and one unclassified CDF.



*OsMTP1* was characterized recently [86, 87]. In mature leaves and stem, it is highly expressed [86]. Generally, *OsMTP1* transports Zn but can also transport Co, Fe, and Cd. Earlier *OsMTP1* has been shown to transport Ni [86]. The vacuolar localization of *OsMTP1* in the tonoplast, compartmentalizes primarily Zn, but also Co, Fe, and Cd, and serves as a detoxification system when these metals are available in excess. *OsMTP1* is expressed constitutively and upregulated by Cd [86]. *OsMTP1*, *OsMTP5*, and *OsMTP12* belong to the Zn-CDF subgroup [82, 84]. Expression of *OsMTP1* in leaves, stems, roots, and flowers is relatively low and spatially and temporally regulated during development of rice. Also, it shows differential response to Cd stress. Transgenic assays in rice have shown that *OsMTP1* expression levels can change plant cation absorption and in turn has affect on Zn, Ni, and Cd contents [86].

In rice, *OsMTP8.1* is the first Mn-CDF member to be identified. It is localized on to the tonoplast, and its over expression in rice enhances Mn accumulation and tolerance [88]. *OsMTP9* is also a Mn-CDF member, which is involved in the uptake and translocation of Mn in rice plant [89]. Mn and other heavy metals induce the expression of *OsMTP11* in rice. In rice plant, *OsMTP1* is involved in Zn and Cd homeostasis/stress and mediates their translocation from roots to the aerial parts [86]. In most rice plant tissues *OsMTP11* is constitutively expressed.

*OsMTP8.1* is localized to the tonoplast and involved in the detoxification of manganese by sequestering excess manganese to the vacuoles [90]. In rice root, *OsMTP9* is polarly localized at the proximal side of both exodermis and endodermis opposite to *Nramp5*. The cooperative transport by *Nramp5* and *MTP9* efficiently transport Mn leading to its high accumulation in rice [91].

The Mn-CDF group in plants is further clustered into two subgroups, Groups 8 and 9 [82]. In the rice genome, there are three members (*MTP9/11/11.1*) of Group 9. *MTP9* shows much higher expression in the roots than in the basal region and shoots. The expression is unaffected by the deficiency of iron, zinc, copper, and manganese. The expression of *MTP9* in roots is eightfold higher in the basal parts than that in apical parts. At the reproductive growth stage, *MTP9* is also expressed in other organs such as nodes and leaf sheath in addition to the roots. *MTP9* is polarly located at the proximal side of the exodermis and the endodermis, which is in opposition to *Nramp5* [71]. Therefore, *MTP9* at the proximal side of the exodermis releases manganese taken up by *Nramp5* to the apoplast of a spoke-like structure in the aerenchyma, whereas *MTP9* at the proximal side of endodermis further releases manganese toward the apoplast of stele including xylem vessels. Thus polar localization of transporters plays an important role in the directional transport of minerals. Recently, a number of transporters have been found to show polar localization. However, our understanding of the molecular mechanism underlying polar localization is still very poor. Also, *MTP9* is different from other members of the Mn-CDF group as it shows a distinct expression pattern in tissue and subcellular localization. *MTP9* is mainly expressed in the roots, but *MTP8.1* from rice [90] in the same group is mainly expressed in shoots rather than roots. Different from other members, rice *MTP9* is localized to the plasma membrane. These differences are associated with the role of *MTP9* in manganese uptake in rice roots. In conclusion, *MTP9* is a plasma membrane-localized efflux transporter for manganese uptake and translocation in rice roots.

The polar localization of MTP9 and Nramp5 at both the exodermis and the endodermis leads to efficient and unidirectional flux of manganese from the soil solution to the stele.

## 8. Root-to-shoot translocation and metal chelation in cytoplasm

Plants that prevent or limit the entry of metals from roots to shoots are categorized as excluders. On the contrary, plants that can transfer metals from root-to-shoot via the xylem along the transpiration stream by increasing the uptake of metals in roots, thus increasing the sequestration of metals in the aerial parts are considered as accumulators/hyperaccumulators. The transfer of metals from the roots to the aerial parts in plants helps to reduce the damage caused by the heavy metals on their root. The translocation of metals from root to the aerial parts is an essential process for the overall growth and development of the plant.

Studies of long-distance root-to-shoot metal transport within plants mainly emphasizes on transporters that are localized to either xylem parenchyma cells or phloem companion cells, as they are directly associated with xylem and phloem loading or unloading thus majorly contribute to the metal redistribution process within the plant. The movement of heavy metals from roots to shoots is facilitated when metals are chelated with ligands such as organic acids, amino acids, and thiols. The movement of metal cations across the xylem cell wall is restricted when the metals are not chelated by ligands due to high cation exchange capability of the xylem wall.

The chelation of metals with NA provides improved tolerance against the restriction by the xylem cell wall. NA facilitates the chelation and transport of divalent ions of metal Ni, Cu, and Zn [92]. Synthesis of NA by trimerization of S-adenosylmethionine is facilitated by nicotianamine synthase (NAS) [93]. In rice plant, increased accumulation of Fe, Zn, and Cu is associated with over expression of the gene *NAS3* [94].

During the process of long-distance transport of metals, some chelators, like nicotianamine [41], glutathione (GSH), and phytochelatins [95], also play a vital role.

Metals pass through xylem unloading process before their distribution and detoxification in the shoot and followed by their redistribution via the phloem. After unloading, the metals either enter into the nearby cells or are symplastically transported or they are apoplastically distributed throughout the leaf tissue [96]. For the symplastic transport of metals across the leaf via the YLS transporter proteins, chelation of metals to NA is required [97].

Excess metals in the plant are sequestered in various aerial plant parts, such as trichomes, leaf epidermal cell vacuole, and mesophyll vacuole. Not much study has been done on the transport of metals through the phloem sap. Nicotianamine is the only molecule to be identified as a phloem metal transporter which is associated with the transport of Fe, Cu, Zn, and Mn [98].

In plants, the vacuole sequestration capacity (VSC) also plays an important role in the long-distance transport and sequestration of metals. Vacuolar metal sequestration capacities are

automatically adjusted to the varying availability of metal ions in the environment, due to the cooperation between tonoplast-localized transporters and ion chelators. Hence vacuoles work as a buffering zone.

### 8.1. Metal sequestration in vacuoles by tonoplast transporters

Plants have developed several defense mechanisms like chelation, excretion, and subcellular compartmentalization to combat heavy metal toxicity as it severely affects its overall growth and development [99].

A large lytic vacuole (LV) is present in most of the plant cells which occupies about 80% of the cell volume. In plants, LVs undergo less metabolism and acts as a store house which can accumulate a huge amount of minerals and water thus play a major role in turgor generation. LVs also function as a store house for other xenobiotic and toxic compounds and also reduce their impact in the cytoplasm where several sensitive processes take place. LV also store plant secondary metabolites and proteins involved in plant defense against pathogens and herbivores and release them when subjected to cellular damage. LV has acidic pH around 4–5, and this acidic environment helps in the degradation of both exogenous and endogenous compounds [100].

Plant LVs are equivalent to lysosomes in animal and vacuoles in yeast and acts as degradation and waste storage compartments. The vacuolar tonoplast of higher plants and fungi as well as lysosomes of animal cells share very similar H<sup>+</sup>-ATPases that acidify the lumen. Apart from these, inner organellar membranes also contain multidrug resistance-associated protein (MRP) type ATP binding cassette (ABC) transporters, chloride channels (CLC) type ion channels, cation channels, and aquaporins [99].

Storage of nutrient minerals in the LV is important in buffering any variations in the supply of nutrients like when plants are grown in nutrient-rich conditions, they will deposit large quantities of such nutrients in vacuoles of vegetative tissues. This helps the plant to survive during subsequent periods of nutrient deficiency by remobilizing from the vacuolar store. Thus, the solute composition of vacuoles is highly dynamic and reflects changes in the environment and the plant developmental stage [101].

The vacuole is a major organelle in higher plants functioning as a store for metabolites, mineral nutrients, and toxicants. Studies have shown that in addition to its storage role, the vacuole also contributes to long-distance transport of metals, through the modulation of vacuolar sequestration capacity (VSC) which is basically controlled by cytosolic metal chelators and tonoplast-localized transporters, or the interaction between them [102].

VSC regulates the long-distance transport of mineral nutrients in plants. Zhang et al. [103] isolated the two vacuolar membrane-localized metal transporters OsVIT1 and OsVIT2 in rice. Both OsVIT1 and OsVIT2 primarily function to sequester Fe/Zn into vacuoles across the vacuolar membrane. In rice plant, flag leaves show a high expression of OsVIT1 and OsVIT2. OsVIT1 and OsVIT2 along with VSC play an important role in Fe and Zn long-distance translocation between flag leaves and seeds.

Studies have also suggested that the long-distance transport of nonessential toxic metals and their detoxification is regulated by VSC thus making the plants highly tolerant to metals [102]. Generally, the VSC of certain metal varies between different plant tissues to ensure proper metal distribution.

Nonessential metals like Cd and As have adverse effects on plants, either through oxidative stress or competitive inhibition of essential mineral nutrient involved in any biological pathways. In order to protect the aerial parts which are associated with important biological processes like photosynthesis, plants have developed several mechanisms to regulate metal distribution between roots and shoots, VSC is one such mechanism.

In rice plant, *OsHMA3* is involved in Cd accumulation in the rice shoots. In rice root, *OsHMA3* is localized to the vacuolar membrane, and it mediates the transport of Cd into vacuoles. In rice roots, VSC along with *OsHMA3* plays a major role in the long-distance transport of Cd from roots to shoots [75, 76].

## 9. Biochemical processes modulated by heavy metal stress

Both abiotic and biotic stresses have several effects on plant growth as well as productivity. Plant vigor and crop yields are strikingly influenced by these abiotic stresses. In order to combat these stresses, plants have evolved many responses. Plants have developed and used various strategies to cope with and also to adapt to these stress conditions. It depends on variation in protein relative abundance of stress-responsive proteins, resulting in changes in the whole proteome, transcriptome, and metabolome levels [104].

Expression patterns of these protein and transcript levels are influenced by the intensity and duration of stress apart from the usual post-translational regulatory mechanisms such as RNA stability and protein degradation [105]. In addition, the intensity and duration of stress can have a substantial effect on the complexity of the stress response. Recent progress in different areas of rice research such as analysis of interactome analysis, transcriptome, metabolome etc., have given us a better insight of abiotic stress response in rice plant [106–109]. The study of these differential changes in proteome profiles in response to abiotic stress is an approach to better understanding the physiology and molecular mechanisms that underlie rice stress responses. Most common response to all stresses is the induction of oxidative stress [110] and modulation of gene expression.

### 9.1. ROS production and modulation of antioxidant system during heavy metal stress

In an environment of metal toxicity, the elevated activities of antioxidant enzymes and non-enzymatic constituents play important role in the plant tolerance to stress. Metal tolerance is enhanced by the plant's antioxidant resistant mechanisms. The harmful effects of heavy metals in plants are due to the production of ROS and induction of oxidative stress. Increased levels of reactive oxygen species such as singlet oxygen ( $^1\text{O}_2$ ), superoxide radical ( $\text{O}^{2-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl radical ( $\text{OH}^\cdot$ ) result in oxidative stress [111]. ROS are strong



oxidizing agents which cause oxidative damage to biomolecules, like lipids and proteins and can eventually lead to cell death [112]. It has been shown that plant tolerance to metals is correlated with an increase in antioxidants and activity of radical scavenging enzymes [113]. Plants respond to oxidative stress by activating antioxidative defense mechanisms, which involves enzymatic and nonenzymatic antioxidants. The enzymatic components include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and enzymes of ascorbate glutathione cycle, while the nonenzymatic antioxidants include ascorbate and glutathione and atocopherol [3, 113]. These antioxidants play an important role in the elimination and destruction of the reactive oxygen species [3].

An imbalance between the detoxification of the ROS products and the antioxidative system results in oxidative damage [113]. The tolerance of deleterious environmental stresses, such as heavy metals, is associated with the increased capacity to scavenge or detoxify activated oxygen species [113].

Studies using comparative analysis suggest that each heavy metal is accumulated differentially in root tissues. Heavy metal stress induces production of reactive oxygen species (ROS) which promotes cell death by apoptosis, necrosis, or mechanisms with both features. Methods like  $H_2O_2$  staining are used to measure production of ROS.

SOD, APX, and glutathione peroxidase (GPX) are the ROS scavenging antioxidant enzymes. They play a very important role in scavenging ROS like superoxide radical,  $H_2O_2$ , hydroxyl radical, peroxy radical, and singlet oxygen species. SOD acts as the first line of defense against ROS as it converts superoxide radical to  $H_2O_2$  [114]. Ascorbate, which is present inside the cell, plays an important role as an antioxidant either by participating in ascorbate-glutathione cycle or by directly quenching the ROS [115]. Ascorbate is also utilized by APXs as a reducing agent to catalyze the conversion of  $H_2O_2$  to water and GPX breaks down  $H_2O_2$  to  $H_2O$  and  $O_2$ . Similar modulation of the antioxidant system upon exposure to heavy metal stress has been observed in studies of different rice cultivars [110, 116].

Glutathione is an important antioxidant in plant cells which is involved in scavenging of free radicals and  $H_2O_2$ , which are formed as a result of oxidative stress along with ascorbate [117]. Glutathione (GSH) is the precursor of phytochelatin (PC), which bind above the optimal concentrations of heavy metals [118]. It also serves as a substrate for GSTs which catalyzes the conjugation of GSH with xenobiotics like herbicides [119]. In silico studies and over expression of GSTs have shown to provide tolerance toward different heavy metals [120, 121]. Glutathione levels in plant tissues are known to accelerate under stress induced by heavy metals.

In rice plants, the root tissue shows variable response of antioxidant enzymes during growth with different heavy metals of varying concentration.

## 9.2. Differential expression and modulation of genes during heavy metal stress

Heavy metal stress in plants severely modulates the gene expression pattern. In plants, many genes are downregulated due to heavy metal stress. They are related to the energy metabolism, carbohydrate metabolism, lignin biosynthesis, phenylalanine metabolism, cell growth and death, lipid metabolism, biodegradation of xenobiotics, amino acid metabolism, etc. Among the



upregulated genes, the majority of affected genes are associated with the biosynthesis of secondary metabolites, specially flavonoid biosynthesis, lipid metabolism, amino acid metabolism, carbohydrate metabolism, biodegradation of xenobiotics, ascorbate, and aldarate metabolism, membrane transport especially multidrug resistance protein, major facilitator superfamily, ABC transporters, glutathione metabolism, MAPK (mitogen-activated protein kinases) signaling pathway, a large number of GST, etc. In a study using rice seedlings grown in metal supplemented media in comparison to control, genes which significantly modulated were filtered. It was found that 17 and 83 genes are commonly upregulated and downregulated under different heavy metal stress. One each of cytochrome P450, Proton-dependent oligopeptide transporter (POT) family protein, heat shock protein, and two NAC domain-containing proteins are commonly upregulated during heavy metals stress, they play important role in detoxification of heavy metals. On the other hand, one heavy metal-associated domain-containing protein, zinc finger protein, cytochrome P450, ring-H2 zinc finger protein, and catalase-1 are commonly downregulated during heavy metal exposure [122]. Plants have developed cellular mechanisms to tolerate and regulate the uptake of heavy metals [123]. However, molecular mechanisms and networks involved in the uptake and detoxification of heavy metals remain poorly understood. Phytochelatins (PCs), a class of cysteine-rich heavy metal-binding peptides, bind to heavy metals, and detoxify by vacuolar sequestration [123]. Sulfur homeostasis in plants results in the increased production of S-rich metal-binding peptides (such as GSH, PCs), which provide metal tolerance [116].

From the expression data, it was demonstrated that the upregulation of a unique cytochrome P450s in different heavy metal stresses is a major detoxification mechanism. In plants, cytochrome P450s plays a major role in the metabolism of several biosynthetic pathways such as flavonoids, coumarins, anthocyanins, isoflavonoids, phytoalexins, salicylic acid, jasmonic acid, and many others [124]. Previously, it has been reported that cytochrome P450 is involved in the metabolism of toxic compounds, indicating their role in heavy metal detoxification. These results indicate that metabolism of plant biosynthetic pathways are very much affected during metal exposure and different cytochrome P450s are involved in the metabolism of different heavy metals. A large number of transporter genes are differentially up- and downregulated under different heavy metal stresses, which include major facilitator genes, sulfate transporters, peptide transporters, nitrate transporters, ABC transporters, multidrug resistance proteins, zinc transporters, Nramp6, and multidrug and toxic compound extrusion (MATE) efflux family proteins. One of the essential nutrient required for plant growth is sulfur that enters the cell via sulfate transporters as inorganic sulfate, it may induce the production of S-rich metal-binding peptides (such as GSH, PCs) and thus provide defense against heavy metal stress [125]. It is clear from their study that each heavy metal has induced specific sulfate transporters. The peptide transporters [126] have been shown to transport nitrate and tripeptides such as glutathione which is a major component in sulfur metabolism and plant defense during stress [127]. It is suggested that nitrate transporter plays a role in root-to-shoot translocation of nitrate thus plays a role in Cd toxicity [128]. ABC transporter proteins play an important role in the transport of various substances like lipids, phytohormones, carboxylates, heavy metals, chlorophyll catabolites, etc. across various biological membranes [129]. It has been shown that Nramp proteins are conserved bivalent metal transporters [130]. NRAMP3 and NRAMP4 are reported to be responsible for Cd<sup>2+</sup> efflux from the vacuole [131]. MATE proteins bind to a variety of potentially toxic compounds and function as proton-dependent

efflux transporters to remove toxic compounds from the cell [132]. Various methyltransferases are differentially modulated under different stresses. In plants, O-methyltransferases constitute a large family of enzymes that are involved in stress tolerance as reported by Lam et al. [133]. Specific methyltransferases catalyze the transfer of methyl groups which are involved in several pathways that lead to the accumulation of methylated inositols, quaternary amines, and tertiary sulfonium species, which play a significant role in stress tolerance [134]. Therefore, modulation of these transcripts must play a secondary role in different heavy metal toxicity.

During different heavy metal stresses, these metals induce damage to the thylakoid membrane leading to increased lipid peroxidation and thus cause downregulation of peroxidases. These specific peroxidase family genes might play a key role in the enzymatic defense of plant cells by scavenging ROS during stress conditions [135]. Heat shock proteins (HSPs) in particular play important role in protecting plants against stress by re-establishing normal protein conformation and thus cellular homeostasis [136].

It has been observed that various transcription factors like WRKY, MYB family, zinc finger protein, RING-H2 finger protein, and basic leucine zipper (bZIP) are differentially expressed under heavy metal stress [122]. In plants, WRKY transcription factors are linked to various processes associated with different biotic and abiotic stresses and regulation of differential transcription a response to stress in plants [137]. Similarly, MYB TFs play very important roles in many physiological processes under normal or unfavorable growth conditions [138] and also in defense and stress responses [139]. During heavy metal stress various stress-related genes are transcriptionally regulated such as GSTs, dehydrin, sulfite oxidase (SO), L-ascorbate peroxidase, L-ascorbate oxidase, and germin-like proteins. GSTs are a superfamily of multifunctional, dimeric enzymes. It induces the conjugation of GSH a tripeptide glutathione to electrophilic xenobiotics and this is followed by sequestration of this complex into the vacuole for detoxification [140]. Recently, it has been reported that a particular class of GST gene family, that is, Lambda GST plays an important role during heavy metal stress [120, 121]. During drought stress, cold stress, and other defense processes dehydrins are produced in plants [141]. SO catalyzes the transformation of sulfites to the nontoxic sulfate. It has been reported earlier that sulfur is an essential nutrient that is taken up as sulfate by plants and chemical compounds which contain S, such as glutathione (GSH), phytochelatins (the polymers of GSH) play a prominent role in arsenic detoxification [142]. Similarly, L-ascorbate peroxidase plays an important role in defense against oxidative stress as it has been studied that APX is an important antioxidant enzyme which detoxifies  $H_2O_2$  by converting into water. Germin-like proteins have been reported to play a significant role in germination and defense response [143] during Cd toxicity.

Thus transcription factors play a significant role during different heavy metal stress response and indirectly modulate several genes responsible for stress. Further study of these TFs would help to understand the difference in the network of pathways during different heavy metal stresses.

## 10. Conclusion and future prospective

Though different heavy metals are detoxified through similar mechanism their uptake from soil by root system differs for each metal. There are several families of transporters, which

play important role in metal ion uptake from the soil as well as redistribution within the plant and its sequestration in various organelles and plant parts.

Apart from the mineral elements described above, rice requires several other mineral elements for its growth. The transporters associated with the uptake of those mineral elements are yet to be identified in rice. Rice plant because of its distinct root anatomical characteristic requires a pair of influx and efflux transporters for the transport of mineral elements from the soil solution to the stele, thus help to surpass the two casparian strips present in the root exodermis and endodermis. As described above, some of the rice plant transporters associated with mineral uptake have been studied and characterized but there exists no clear understanding about their influx-efflux transporter pairs. More transporters associated with metal uptake and sequestration have to be identified and characterized using different ways like genetics (both forward and reverse), expression pattern, functional characterization in yeast or oocytes, phenotypic analysis using mutants, and so on in future. Each heavy metal modulates specific pathways in addition to common networks such as expression of a specific member of gene families including several transporters, different members of cytochrome P450 and transcription factors are modulated in different heavy metal stresses. In future, these complex responses have to be elucidated using various functional genomic approaches along with proteomic and metabolomic analyses. Also, many plant vacuolar membrane transporters and channels have been identified. Still, there is a dearth of knowledge about the regulation of these networks and how all these transporters and channels interact with each other in order to maintain a cytosolic ion homeostasis. Our understanding about the response of the rice plant to abiotic stress needs to be further refined.

Therefore, further studies on mineral transporters, antioxidant system, and differential gene expression regulated by heavy metals in rice is required to get deeper insight of the abiotic stress caused by heavy metals on rice plant which will in turn help to reduce the abiotic stress caused by heavy metals on rice plant ultimately leading to increased and better crop production for the benefit of mankind.

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