

Assessment of Castor Plant (*Ricinus communis* L.) Tolerance to Heavy Metal Stress-A Review

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Abstract: Increased urbanization and industrialization have greatly contributed to the emission of higher amount of heavy metals such as cadmium, nickel, and lead into the environment. These metals are non-biodegradable and toxic, causing much effects on plants and by extension to animals and humans, which have become a major global concern. The inherent ability of plants to resist heavy metal toxicity seems to be the most sustainable and cost-effective strategy. Castor plant is widely studied due to its tolerance to the effects of heavy metal contaminated soils, owing to its large biomass content and high accumulating capacity. Castor plants to some extent can tolerate elevated levels of heavy metals through several developed mechanisms, such as activation of antioxidant enzymes, exclusion, accumulation of proline, compartmentalization, organic acid exudation, and phytochelatins. Molecular studies have identified some stress-responsive to aid the tolerance of heavy metals in castor. Stress caused by heavy metal toxicity affects seedling growth, biomass, photosynthetic pigments, protein level, and nutrient uptake of castor plant. The response of castor, however, to these stresses differs among cultivars, metal type and concentration, and time of metal applied. This review aims to summarize the physiological responses and various defense mechanisms of castor to tolerate and eradicate heavy metal toxicity and some stress-responsive genes identified at transcriptional and posttranscriptional levels that confer metal tolerance in this plant.

Keywords: Castor; metal stress; biomass; proline; tolerance

1 Introduction

Over the period, studies have revealed that pollutants from anthropogenic, industrial, and natural activities are the primary sources of heavy metals (HMs) (Fig. 1) [1–3]. These activities greatly contaminate the environment, affecting the soil, plants, and humans. During nutrient and water absorption from the soil, toxic metals including, cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) found in soil and groundwater are also absorbed by the plants, which in excess become toxic to the plants



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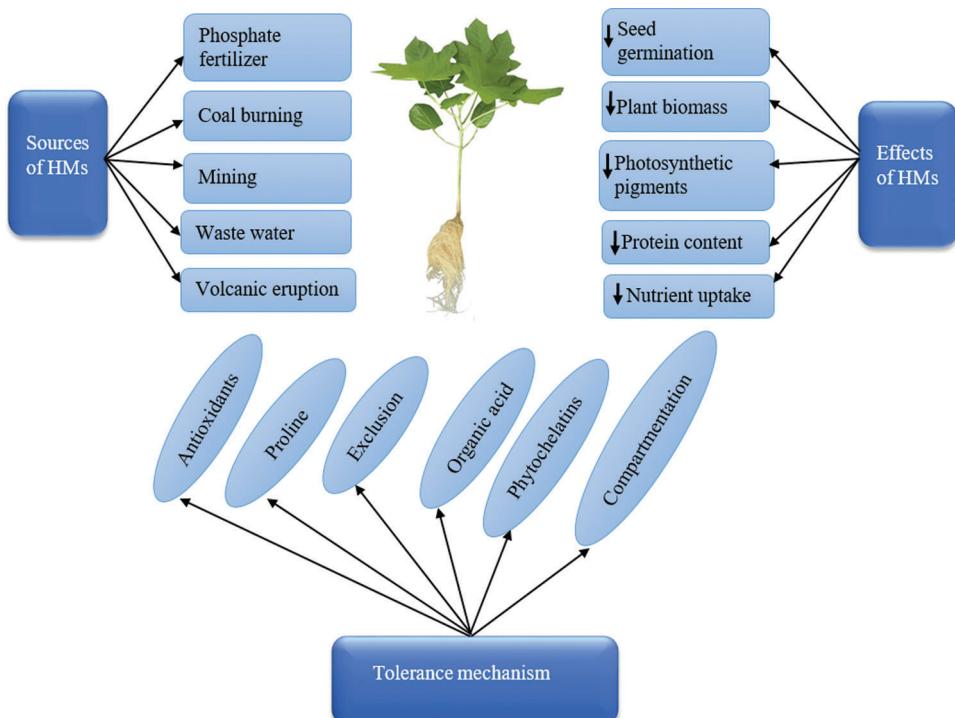


Figure 1: Sources, effects of HMs and tolerance mechanisms of castor plant under heavy metal stress. ↓ represents a decrease

[4–6]. Interactions between the ionized form of these absorbed metals and plant tissues disrupt the normal metabolic processes of the plant, causing cell injuries and death of tissues. Plants affected by these toxic HMs show the following physiological and morphological symptoms; stunted growth, leaf chlorosis, necrosis, decreased plant biomass, poor seeds germination and chlorophyll synthesis, water imbalance, reduced nutrient uptake, reduced enzymatic, and photosynthetic activities, and oxidative stress [7–9].

Tolerance to HM toxicity is the ability of plants to effectively grow on the metal soil without significantly affecting plant growth and productivity [10]. Plants' tolerance to HM can be reflected by their physiological responses, including seed germination, biomass content, photosynthetic pigments, and nutrient uptake [11,12]. Plants possessing large biomass can absorb and accumulate toxic metal ions in the soil. Plants could tolerate metal stress in the environment by employing several physiological and molecular defense mechanisms. This mechanism enhances plant growth and development and overcomes oxidative damage which is due to excessive production of reactive oxygen species (ROS). Some of the mechanisms functions either dependently or in combination with other mechanisms to tolerate metal stress. At the molecular level, ROS induces several stress response genes. These genes trigger the intercellular level of several proteins and transcripts, signifying the role of posttranscriptional and transcriptional regulations in the tolerance of metal stress [13]. The expression of these genes is known to confer stress tolerance in plants but the mechanism behind their role in HM tolerance has not been fully known in several plant species like castor.

Ricinus communis, commonly known as the castor oil plant, has been considered as a tolerant plant to HMs. This is attributed to its rapid growth, deep roots, large biomass production, and high accumulation capacity [14–16]. Castor has greater potential for HMs uptake and tolerance than other plants such as *Brassica juncea*, *Solanum nigrum*, and *Zea mays* [17–19]. Exposure to extremely high concentrations of

heavy metals results in the decrease of castor biomass, seedling growth, photosynthetic pigments, and protein content. Castor plant employs different mechanisms such as production of antioxidant enzymes, exclusion, accumulation of proline, compartmentation, organic acid exudation, and phytochelatins, which enhance its ability to tolerate HMs [20–22]. Also, molecular studies have identified some stress response to aid the tolerance of heavy metals in castor. This review, therefore, summarizes the physiological responses and various defense mechanisms of castor to tolerate and eradicate heavy metal toxicity. This paper also reveals some stress-responsive genes that have been identified at the transcriptional and posttranscriptional level that confers metal tolerance in this plant.

2 Overview of Castor Plant

Ricinus communis L., is an oilseed plant of a diploid ($2n = 2x = 20$) species belonging to the family Euphorbiaceae [23]. It is a fast-growing annual crop ranging from 1–4 m in height and can extend to the size of a small tree approximately 12 m, depending on different environmental factors [24]. Ethiopia is speculated to be the center of origin, owing to the presence of high bio-diversity [25]. Currently, castor is widely grown over 30 different countries, with India, China, Brazil, Mozambique, and Ethiopia as the most predominant countries contributing about 90% of the world's castor production [26]. Castor plants have differences in appearance and growth habits that have intensified the selection of different cultivars for oil production.

Castor oil, extracted from the seed, is unique among all other oils because it is the sole source of hydroxy fatty acid; ricinoleic acid (about 85%), conferring a lot of its distinctive industrial properties [27,28]. The use of castor dates as far back as 1500 BC in Egypt where castor oil was to protect the eyes from irritation. Castor oil is widely used for many industrial products like; ointments, fiber optics, nylon, varnishes, airplane engine lubricants, hydraulic fluids, dyes, detergents, plastics, soaps, greases, paints, synthetic leather, cosmetics and perfumes [29,30].

The castor plant is characterized by its hard seed coat with dotted brownish spots that protects the endosperm and the cotyledon from biotic and abiotic stresses [31]. Nevertheless, at the seedling stage, the plant is susceptible to these stresses such as *Botrytis ricini* and *Fusarium* wilt diseases, and low temperatures below 15 °C are capable of inducing frost, affecting its germination and seed yield [23,32]. The castor plant is also characterized by high biomass yield, which enables the root to absorb and accumulate maximum amount of toxic metals in the soil. The high biomass content makes the plant suitable for biofuel production, which has been used as a substitute for fossil fuel. The castor plant can grow in metal polluted soils, even during drought and salinity, of which some tolerant cultivars (RG 1526, RG 1449, RG 2826, RG 2122) have been identified [33–35]. Castor has high photosynthesis (leaf area) capacity which is attributed to the higher amount of chlorophyll pigments, total soluble protein, and rubisco protein [36]. The plant also has high antioxidant properties including high glutathione (GSH) and low malondialdehyde (MDA) and high proline content which protects the plants from excessive exposure to metal and other stresses [37].

3 Heavy Metal Uptake by Castor

Heavy metals are available and can be absorbed by plants in their ionic states in the soil solution. These metal ions primarily enter plants through the roots and their translocation to the shoot. Enhanced metal translocation to plant aerial parts is controlled by certain carrier proteins. Heavy metal transporters, namely natural resistance-associated macrophage proteins (NRAMP) and ATPase assist plants in metal uptake and translocation [38]. These transporter proteins have been studied in other crops but rarely studied in castor [15,39]. The expression of ATPase increased in the roots of castor under increasing concentration of Pb (800 mg/kg) stress with no expression in the leaves [15]. Recently, a study on the differential responses of NRAMP genes in castor genotypes under As stress was investigated. The results

showed that increasing concentration of As at 200 μM increased the expression of RcNRAMP2, RcNRAMP3 and RcNRAMP5 in GCH 2 and WM genotypes when compared to other cultivars under study [39].

Several studies have revealed that the castor plant can absorb metals better than other plant species [12,37,40–44]. It was reported that uptake of Cd concentrations in castor was higher when compared to *B. juncea* [37]. A study that also determined the tolerance and bioaccumulation of 49 different indigenous plant species to As contaminated soil revealed that, only castor and five other species had high translocation factor (TF) and bio-concentration factor (BCF), making them suitable for phytoremediation [40]. Similarly, out of eight energy crops treated with equal concentrations of Cd, four crop species including castor, accumulated maximum amount of Cd [12]. Costa et al. [41] and Pal et al. [15] reported of a rise in the uptake of Pb in castor under elevated levels of the metal concentrations. To access castor tolerance to Cu, Andreazza et al. [42] cultivated the plant on three different Cu polluted soils (Mollisol, Inceptisol, and Cu mining waste). The results showed that, castor plant had higher Cu uptake except for Cu mining waste soil compared to the control soil. Another study revealed that castor plant could be used to decontaminate contaminated soils, as it was able to tolerate and accumulate high proportions of HMs (Cd, Pb, Zn, Ni, and Cu) in fly ash soil [43]. In a recent study, Boda et al. [44] investigated on the phytoremediation ability of castor plant grown on re-vegetating industrial waste sites contaminated with Cd and Pb and observed that the TF for both metals at all sites was greater than one but BCF was greater than one for only Cd.

4 Physiological Responses of Castor under HM Stress

Tolerance of castor to the effects of HMs can be evaluated by its physiological responses which include; seed germination and seedling growth, biomass level, photosynthetic pigments, and protein content [14,21,44,45]. The extent of metal effect on the plant depends on the type of metal, concentration of metal applied, cultivar, and exposure duration.

4.1 Seed Germination and Seedling Growth of Castor under HM Stress

Plant growth and yield are influenced by seed viability, germination rate, and seedling growth which may be affected when grown in a metal contaminated soil. The effect of HM on germination depends on its ability to penetrate the seed coat to reach the embryonic tissues [46]. Studies have revealed that castor at the germination stage could resist metal effects. Castor was unresponsive to Al and Cd stresses at higher concentrations during germination due to the hard seed coat which served as a barrier to the metal [14,47]. Castor plant, however, after seedling emergence, is sensitive to metal toxicity, inhibiting root development and causing disease symptoms. For example, a study on castor under Pb toxicity showed that plant height at seedling stage significantly decreased ($p < 0.05$) at 400 $\mu\text{mol/L}$ of Pb compared to control [48]. Another study showed that the emergence viability and percentage of castor at 10 days of germination were higher, but seedlings were restricted after three weeks of germination when cultivated on Pb-Zn tailing [59]. Hazama et al. [49] reported of growth inhibition with smaller and slightly yellow leaves of castor grown on 10 μM Cd treated soil for three weeks, compared to when grown on 0, 0.1, 1.0 μM Cd treated soils. Wu et al. [14] researched on tolerance of castor plants, screened in Cd polluted soil to assess the plant's effectiveness for phytoremediation. The results showed that, radicle elongation was significantly ($p < 0.05$) inhibited but there was no difference ($p > 0.05$) between mean germination percentage of the Cd treatments and control. Also, leaf chlorosis and reduction in growth of castor attributed to Pb toxicity when Pb was applied at concentrations, 200 and 400 μM , has also been reported [50]. Recently, a study which evaluated the effects of HMs (Cd, Pb, Al, As, Cr, and Cu) at higher concentrations in mine tailing soil cultivated with castor, showed that the growth of roots were highly inhibited ($p < 0.05$) [45].

4.2 Plant Growth and Biomass of Castor under HM Stress

Plant biomass is an essential factor for evaluating castor tolerance levels to HMs. Several studies have analyzed the effects of HM stress on castor plant growth and biomass [18,41,51,52]. In a pot experiment to examine castor plant growth and biomass levels under Cd polluted soils, the results showed that the average height and total dry weight were 54 cm and 8.3 g respectively per pot. A significant difference ($p < 0.05$) was seen in the shoot and root biomass of the plant when compared to control. Hence, it was concluded that, castor could be suitable for remediating Cd-polluted soils due to its massive roots system which penetrates deeper than other herbaceous plants [51]. Costa et al. [41] revealed that a higher concentration of Cd (16 mg/L) and Pb (96 mg/L) respectively decreased and increased both root and shoot dry mass in a hydroponic system. Costa et al. thus concluded that castor could be used for phytoremediation, an indicator for Cd, and tolerance for Pb in the contaminated growth medium. In another study, the root and shoot biomass and yield of castor decreased by 10% when treated with Ni at increasing concentrations of 200 mg/kg soil [52]. Bauddh et al. [18] reported a reduction in dry biomass weight of castor and *B. juncea* when treated with Ni. However, the reduction in *B. juncea* was higher than that of castor which occurred at the root. Another pot experiment that examined castor tolerance under Zn, Cd and Cu polluted soils for 4 months revealed that the plant had a high biomass level with an average height of 136 cm. Comparing to the control, the biomass of castor grown in Cd single treatment (40 mg/kg) of soil decreased by 22.9%. At 200 mg/kg and 600 mg/kg Cu of soil, the biomass increased (116.9%) and decreased (92%) respectively, over control. Similarly, biomass under Zn-polluted soil increased (113.6%) and decreased (85%) at 200 mg/kg and 800 mg/kg. Meanwhile, aside treatment M4-5, there was no significant difference ($p > 0.05$) in biomass content in M4-1 to M4-4 when compared to control in co-contaminated soils (Fig. 2) [53]. They concluded that castor possesses a high tolerance to these metal soil. A pot experiment of 46 castor cultivars was grown to assess their tolerance and phytoremediation potential in Cd contaminated soil [14]. The results showed that, varieties with relatively high Cd accumulation, including 92-2812, Z02, L93, BJH, and 93-4 had high roots and shoots biomasses, whereas varieties with relatively high Cd concentration, including Y11 and Z44 had a low amount of Cd in both roots and shoots. In a recent study, the presence of Pb toxicity at a concentration of 200 $\mu\text{mol}/\text{L}$ did not affect the dry matter yield in the leaves, roots, and stems of castor when grown in hydroponic solution [54]. Based on the above findings, it can be concluded that the increase or decrease in castor biomass is based on the metal type, castor cultivar, and the concentration of the metal applied.

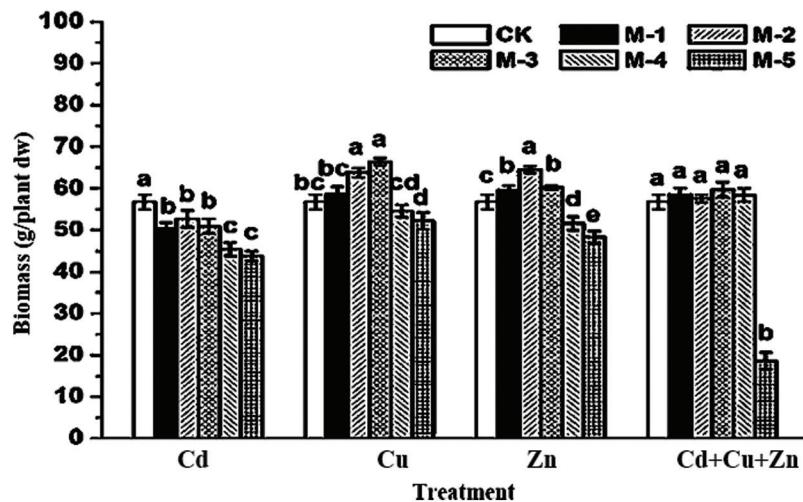


Figure 2: Dry biomass content of castor plant treated individually with Cd, Cu or Zn and co-contaminated soil (Cd + Cu + Zn). Data are seen as mean \pm SD, $n = 3$. Different letters represent significant differences, ($p < 0.05$) [53]

4.3 Photosynthetic Pigments of Castor under HM Stress

The adverse effects of HM on photosynthetic pigments (chlorophyll, carotenoid) could inhibit plant growth and yield. Chlorophyll (Chl) is an essential parameter used to measure photosynthetic injury caused by HM toxicity and other environmental stresses to plant. Several studies in castor plant have revealed that increased or decreased in Chl content depends on the metal concentration [12,15,21,44,55–57]. A study by Shi et al. [12] observed the highest levels of Chl α fluorescence and total Chl content in castor and *Cannabis sativa* grown in Cd toxic soil at a concentration of 50 and 100 mg/kg. Romeiro et al. [48] experimented on the effect of castor plant cultured in hydroponic solution polluted with Pb at different concentrations (0, 100, 200, and 400 $\mu\text{mol/L}$). It was found that, the stomatal conductance was higher than the net photosynthetic value and a minimal reduction in photosynthesis level only at 400 $\mu\text{mol/L}$ occurred. In another study, a decrease in Chl content in castor was recorded at increasing Pb concentrations (300 mg/kg, 800 mg/kg) in the soil for 30 days [15]. Also, a reduction of Chl content of castor plant grown on co-contaminated (Cd, Pb, Fe, Zn, and Mn) soil when compared to the control was observed. This reduction in Chl levels was attributed to either the replacement of Mg^{2+} with metal ion in the leaf tissues or reduction of chlorophyll synthesis [44]. Additionally, Chl (a and b) and total chlorophyll content of castor decreased by 30% due to elevated level of Pb (400 μM) [50]. In a recently reported study, Chl and carotenoid content of castor and three other species (*Melia azedarach*, *Reutealis trisperma*, and *Jatropha curcas*) decreased when cultivated on gold mine tailings contaminated with Pb at different levels (0%, 50%, and 100%) [57]. *Medicago sativa* (*M. sativa*) is considered as a hyperaccumulator and capable of remediating Cd contaminated soil [58]. Co-planting of *M. sativa* with castor significantly enhanced the Chl content of castor in the presence of Cd contamination [21].

4.4 Protein Content of Castor under HM Stress

Different studies have evaluated the level of protein content in castor exposed to HM stress. A study revealed an increase in total protein content and a higher absorption ratio at A250/A280 in the roots of castor plant when exposed to increasing amount of Ni. These were due to the induction of some protein (cysteine) under Ni stress involved in metallothionein (metal ion homeostasis) [52]. Contrarily, Bauddh et al. [18] reported a decrease in protein content (33.79%) in castor leaves due to Ni toxicity (150 mg/kg soil) compared to control after 60 days of sowing which was attributed to the low level of protein synthesis and protein degradation. Additionally, in Cd contaminated soil, castor leaves had decreased protein content under Cd stress which increased later with the application of inorganic fertilizer [59]. Also, the protein level in castor leaf decreased by half when compared to the control [44]. In another study, the protein content decreased by 80% due to increasing concentration of Pb toxicity at a concentration of 200 and 400 μM Pb [50]. From the above studies, it can be said that protein content in roots is higher than in leaves of the castor plant under HM stress which needs to be confirmed in further studies.

4.5 Nutrient Uptake of Castor under HM Stress

Essential mineral nutrients (macro and micronutrients) are needed by plants to facilitate their growth and development. Macronutrients (N, K, Ca, Mg, P, and S) are required by plants in larger quantities whereas micro (Fe, B, Mn, Zn, Cu, and Mo) are needed in smaller amounts. The ability of plants to absorb these nutrients may be affected when grown on metal contaminated soils. The uptake of both macro and micro-nutrient was affected in *Cocos nucifera*, *Oryza sativa*, and *Amaranthus viridis* upon their exposure to Cr (VI) and Cr (III) [60]. This decrease was attributed to the reduction of H^+ ATPase activity and the removal of essential nutrients from physiological binding sites. Similarly, in castor, a decrease in nutrient uptake have been reported by several researchers [18,41,42,61]. A study showed that elevated levels of Cd and Pb affected the translocation and uptake of Ca, Cu, Fe, Mg, Mn and Zn in the root and shoot of castor [41]. It was concluded that, Ni stress decreased the concentrations of mineral uptake in castor [18].

Another study reported that, though not significant, there was a decrease in Fe and Cu uptake when castor was grown in a hydroponic solution polluted with increased concentrations of Cd [49]. It was revealed that increasing As concentrations $\leq 5000 \mu\text{g/L}$ positively correlated with Ca, Cu, N, P, Mn, and Zn concentrations in roots and shoot of castor plant [61]. It has been reported that increasing concentrations of Cu increased and decreased the uptake of Ca, Mg, K, and S in the roots and shoot of castor respectively when cultivated in different Cu contaminated soils [42]. Recently, a hydroponic study revealed that addition of Cd in the growth medium increased the concentrations of Al, Ca, Cu, Fe, Pb, Mg and Zn in the roots of castor than in *S. nigrum* [62].

4.6 Fatty Acids and Oil Content of Castor under HM Stress

Few studies have been conducted on the fatty acid composition and oilseed crops growing on metal contaminated soils. Seeds growing on metal contaminated soils are smaller in size compared to those on non-contaminated soils which might be due to metal effects on plants. The seed coat and cake of castor have been found to accumulate higher amount of metals [63], which could be the reason for smaller seed size to reduce the metal effect on the plant fatty acid and oil content. In *Lactuca serriola* and *Lycopersicum esculentum*, the accumulation of Cd, Cr, Cu and Ni negatively affected fatty acids content and membrane lipid of these plants [64]. Boda et al. [44] also revealed that the fatty acid composition of castor exposed to mine tailing site was unaffected and higher when compared to those growing on non-contaminated soils. The percentage of the various fatty acid compositions were ricinoleic acid (75.1%–84.94%), linolenic acid (7.76%–11.87%), linoleic acid (4.06%–8.47%), palmitic acid (1.71%–2.53%), and stearic acid (1.52%–2.89%).

Ruiz et al. [65] observed 41–64% oilseed weight of castor when cultivated on mine tailing soil and a negative linear correlation was observed for oil content and Cd, Cu, Pb and Zn concentration in root and shoot, whereas Mn and Ni showed a positive correlation in roots. This study confirms that the oilseed weight of castor under metal soil remains unaffected. González-Chávez et al. [63] also reported that the castor oil properties (palmitic and oleic acids) from tailing site exhibited no difference when compared to control, but a higher ($p < 0.05$) amount of linoleic acid content was recorded and low metal concentration (Cd, Mn, Ni, Pb, and Zn) was detected in the oil content but the presence of Cu was not detected. One desirable quality for plant biofuel is the high amount of oleic acid, therefore the high content of oleic acid in castor under metal stress indicates that their biofuel quality remains unaffected.

The above studies demonstrate that, the oil content and fatty acid composition of castor seeds grown on metal soils are unaffected, feasible, safe, and could be used for biofuel production. In addition, all of the above studies were conducted under mine tailing sites, therefore studies on soil treated with metal concentrations at different levels are required to ascertain the metal effect on the fatty acid composition and oil content of castor.

5 Tolerance Mechanisms of Castor under HM Stress

To tolerate metal stress, plants modify their physiological processes to enhance their growth in the polluted environment. At the physiological and molecular level, plants adapt certain mechanisms to tolerate HMs including; exclusion, induction of antioxidant defense, accumulation of proline, compartmentation, organic acids exudation and phytochelatins (Fig. 3) [20,54,66–68]. These mechanisms play vital roles to overcome metal-induced stress due to the extreme production of ROS, thereby enhancing plant tolerance. Some of the mechanisms operate either singly or in combination with other mechanisms to enhance their detoxification against overproduction of ROS. At the molecular level, ROS induce several stress-responsive genes. These genes trigger the intercellular level of several proteins and transcripts, signifying the role of posttranscriptional and transcriptional regulations in plants [13,20]. The expression of these genes is known to confer stress tolerance in plants but the exact mechanism behind

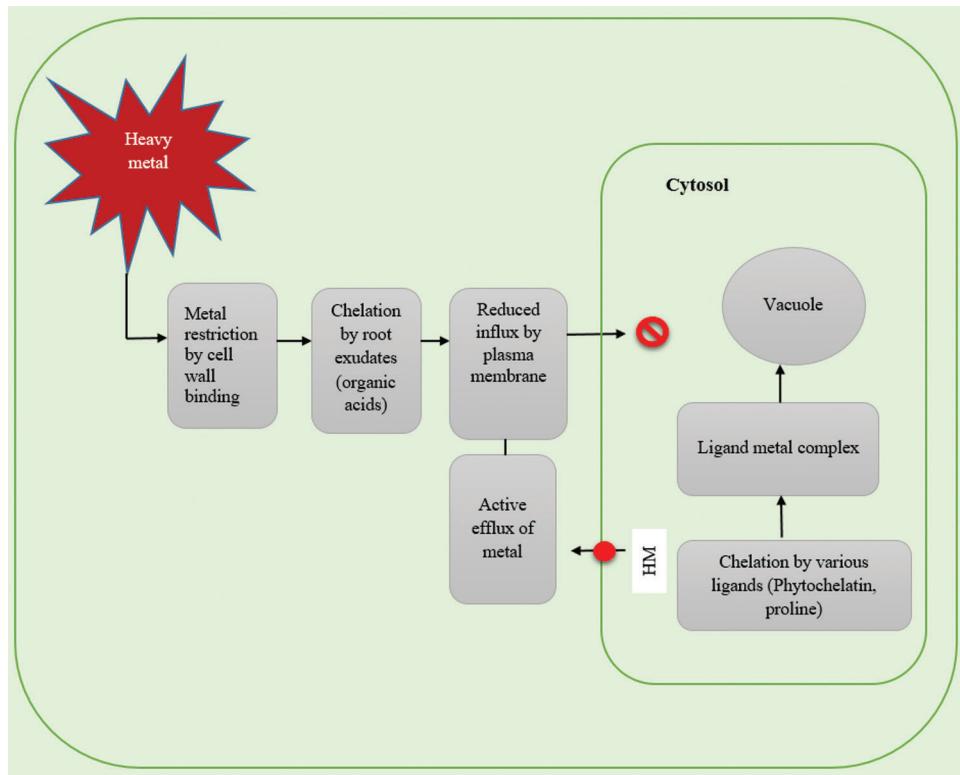


Figure 3: Cellular mechanism for detoxification and tolerance of metals by plants

their role in HM tolerance has not been fully known in several plant species like castor. In the following subsections, some identified physiological mechanisms and stress-responsive genes that confer HM tolerance in castor under metal stress have been discussed.

5.1 Exclusion

The idea behind exclusion deals with the removal of metal ions from soils with accumulation in specific parts of plants to eliminate the effects of the metals on the overall physiology and growth of plants. Under this mechanism, plants retain most of the accumulated metals in roots and prevent excess translocation of toxic metal ions to the shoot [69,70]. In the roots, the bioavailability of the accumulated metals is restricted. This may be due to the formation of metal complexes hence limiting effects of the metal on growth and physiology of roots. Mehes-Smith et al. [71] also suggested that the high accumulation of metals in plant roots might be due to differences in metal electronegativity. Metals with high electronegativity can accumulate higher amount of metals ions in the roots compared to those with low metal electronegativity.

Research has shown that the accumulation of metals in roots differs from plant species and genotypes [72]. Plant species like *B. juncea*, *Salix viminalis*, *Solanum nigrum*, and castor have been reported to accumulate high levels of metals ions in roots [19,73]. Recently, a study in castor reported that Ni accumulation in the roots and leaves of the castor plant increased as the metal stress increases. However, higher Ni was found in the roots than in leaves [20]. At a concentration of 0.1 and 0.2 mM, Ni respectively increased in leaves by 4.0 and 8.0 fold and increased by 90 and 39.0 fold respectively in the roots, compared to the control. Elevated levels of Cd were also found in the roots (1328.47 µg/g dwt.) than in the shoots (189.92 µg/g dwt.) of castor plant when cultivated on Cd polluted soil [37,74]. Similarly, Huang et al. [51] in their study identified higher concentrations of Cd (37.63 µg/g dwt.) in

castor roots with minimal translocation to the stem (2.27 µg/g dwt.) and leaves (1.22 µg/g dwt.). The above studies confirm that castor could tolerate metal stress by accumulating larger proportions of the metal ions in the roots. The castor plant has a very thick root system; and hence this may be a reason for its higher accumulation in this part.

Research has reported that the high accumulation of metals in roots can help in the stabilization of metal soils [65] hence, the ability of castor plants to accumulate highly in roots proposes it as a potential player in phytostabilization.

5.2 Induction of Antioxidant Defense

Oxidative damage caused by several abiotic stresses in plants is as a result of excessive production of ROS such as hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2), and superoxide radicals (O_2^-) [75,76]. Plants have unique mechanism to maintain ROS at the physiological limit, counteracting their overexpression beyond a certain limit, thereby playing a vital role in the acclimation process against an imposed stress [77]. This mechanism is called plant antioxidant defense system which involves enzymatic and non-enzymatic antioxidants and controls ROS at the cellular level (Fig. 4). The enzymatic components are ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GR), peroxidase (POD), and superoxide dismutase (SOD) whereas the non-enzymatic antioxidants are ascorbate, glutathione (GSH), salicylic acid (SA), vitamin E, and α -tocopherol [76,78]. The high or low expression of antioxidant in stressed plants are often correlated to an enhanced or declined stress tolerance. The antioxidants control ROS concentration preventing them from causing detrimental effects to promote plant growth and development. Also, antioxidants help to remove, neutralize, and scavenge ROS. GSH eliminates H_2O_2 and reacts with O_2^- and OH^- to promote plant tolerance to drought, salinity, metal stress, and extremely high or low temperature [2,4]. SOD catalyzes the disproportionation of O_2^- radicals into H_2O_2 and O_2 [79]. CAT also catalyzes the reduction of H_2O_2 molecules into H_2O and O_2 during cell metabolism [79]. APX and POD catalyze the decomposition of H_2O_2 molecules into H_2O . The various mechanisms of operation of the above stated antioxidant enzymes enable them to prevent oxidative damage due to excess ROS production.

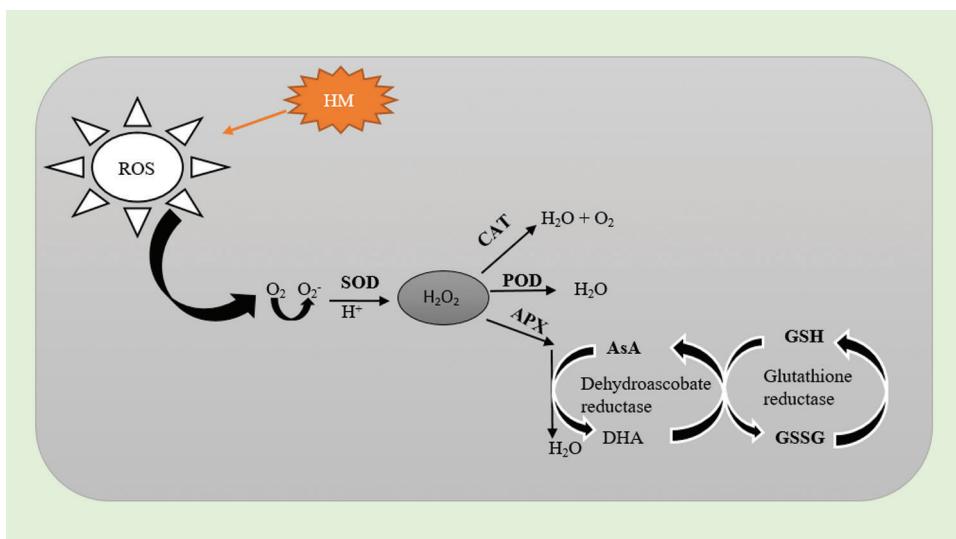


Figure 4: Antioxidant enzyme responses to metal stress. AsA-ascorbic acid, APX-ascorbate peroxidase, POD-peroxidase, CAT-catalase, MDHA-monodehydroascorbate, GSH-glutathione (reduced), GSSG-oxidized glutathione, H_2O -water molecule, H_2O_2 -hydrogen peroxide, O_2 -oxygen molecule, O_2^- -superoxide radicals, ROS-reactive oxygen species, SOD-superoxide dismutase, HM-heavy metal

The presence of heavy metals in plants have been reported to induce the production of ROS hence the antioxidant system is essential in preventing oxidative stress and damage which the above stated antioxidant enzymes are key players. Different studies have reported on the levels of antioxidant activities in castor under metal stress [21,66,80,81]. Zhang et al. [80] investigated the tolerance and accumulation of two castor cultivars (Zibo No. 5 and Zibo No. 8) under Cd stress by exploring its antioxidant system. The results showed that Zibo No. 8 accumulated more Cd than Zibo No. 5 and the activities of GSH, POD, and SOD were higher in root than in leaf in both cultivars under Cd stress when compared to control. Comparatively, GSH levels significantly ($p < 0.05$) increased in the root of Zibo No. 8 than in Zibo No. 5 at increasing concentrations. This proposes that, despite the high accumulation of metals in roots, the roots exhibit an effective system that eliminates the metal, an instance observed by the up-regulation of antioxidants in the root of Zibo No. 8. A recent study also evaluated the tolerance mechanisms of two castor cultivars (JX-22 and ZB-9) and the role of GSH and NPT grown in hydroponic system under Cd stress. The outcome revealed a higher concentration ($p < 0.01$) of GSH in the xylem of ZB-9 cultivar than in JX-22. In the leaves, however, the levels of NPT and GSH were significantly lower ($p < 0.01$) in ZB-9 than in JX-22, suggesting that JX-22 is highly tolerant to Cd than ZB-9 [66]. The low tolerance of ZB-9 may be explained by the fact that, the xylem is a water transport system in plants and may not accumulate heavy metals. Hence the high levels of GSH may be due to high availability of Cd during water uptake from the hydroponic system. The SOD activity in the leaves of castor plant was also observed to significantly increase with an increasing concentration of Cd treatment (0, 10, 50 $\mu\text{mol/L}$) when compared to control [81]. Activities of CAT, APX, and PDX enzymes also significantly increased in castor plants intercropped with *M. sativa* under Cd toxicity [21]. The increase in these activities indicates that this plant has effective antioxidant systems in tolerating metal stress.

The above results suggest that castor could tolerate metal stress by employing several antioxidant enzymes. The increase in these antioxidants in castor plants under Cd stress may be related to the difference/types of cultivar cultured and the dose of metal available or metal concentration and the part of tissues analyzed.

5.3 Accumulation of Proline

Biosynthesis of amino acids, such as proline, is one of the strategies plants adapt to chelate metal ions in the cytosol (Fig. 5) [82]. Proline is a stress metabolite that plays a major role in protecting plants against HM stress. Proline serves as a cell wall plasticizer and osmoprotectant, which protects the cell membrane and preserves plant cells from dehydrating [83]. Proline also scavenges ROS, stabilizes protein, chelate metal, inhibits lipid peroxidation, and acts as a redox signaling molecule. The high accumulation of proline in stressed plants is governed by the ex-novo synthesis of proline. Proline undergoes oxidation to produce NADP/NADPH cycle and later influences the reduction of GSSG/GSH by GSH reductase [82,84]. The high level of proline limits ROS effect to PSII which enables PSII catalyzed electron transport activities of thylakoid [84]. Proline acts as an electron donor to PSII when uncoupling of oxygen-evolving complexes occurs as a result of HM stress and hence protect the plants by enhancing the production of NADPH.

Proline has also been reported to induce the activities of antioxidant enzymes to withstand oxidative stress by decreasing denaturation of enzymes. The accumulation of proline in the castor plant has been reported to demonstrate a positive correlation with the stress intensities. Studies have demonstrated that increasing levels of Cd and Pb in the growth media increased the proline levels in castor plants [15,44]. Comparative studies on *B. juncea* and castor also showed that castor exhibited stronger defense by accumulating high proline content than *B. juncea* by increasing concentration of Cd in drought and saline conditions [74,85]. This suggests that castor has a strong defense towards several abiotic stresses as compared to *B. juncea*. There was a rise in osmoprotectant proline in the leaves of castor when cultivated

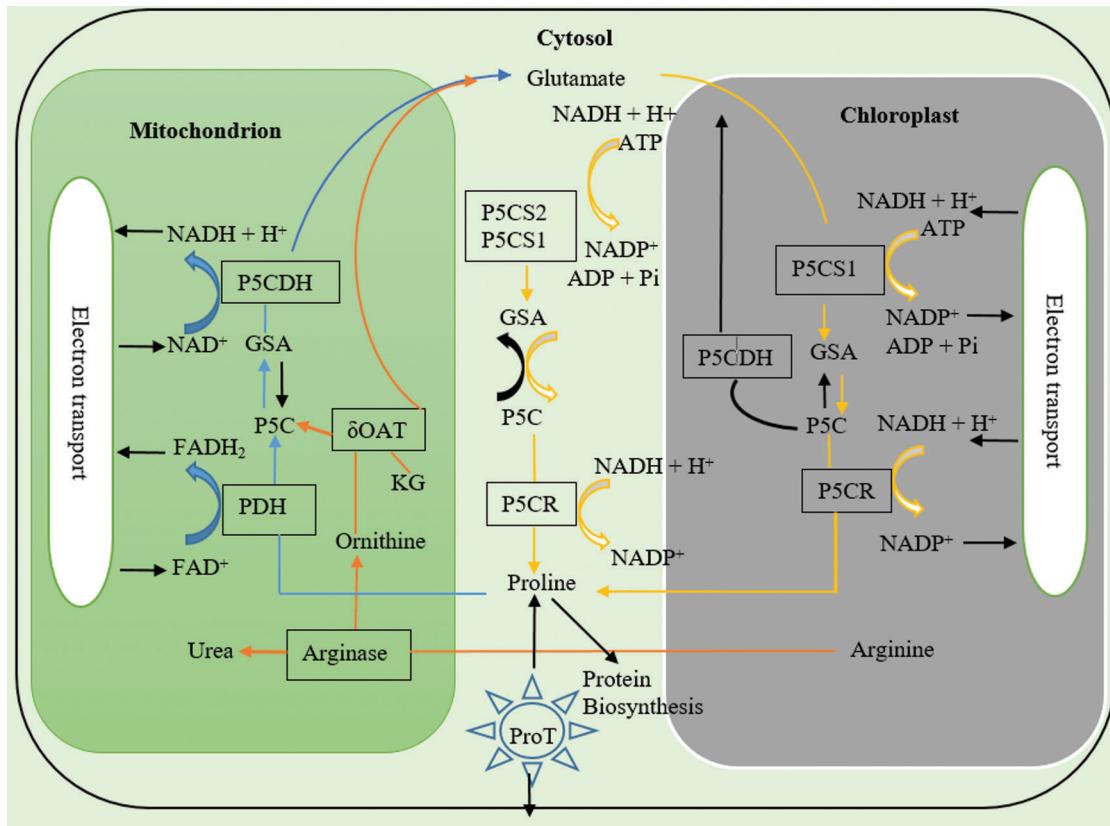


Figure 5: Metabolic pathway of proline in stress plants. The Cytosol or chloroplast are site for proline synthesis and its degradations also takes place in the mitochondria. GSA-glutamic semialdehyde, PDH-Proline dehydrogenase, P5C- δ -pyrroline-5-carboxylate, P5CS-pyrroline-5-carboxylate synthase, P5CR-pyrroline-5-carboxylate reductase, ProT-Proline transporter, KG-Ketoglutarate, OAT-Ornithine aminotransferase [82]

in Ni contaminated soils and was concluded that the increase of proline under Ni stress depends on plant species and concentration of metal toxicity [18]. It was revealed that castor plant exposure to Pb toxicity at the concentration of 400 μ M Pb increased the level of proline when compared to the control [50]. The above studies indicate that, increasing levels of proline enhances castor plant tolerance to metal stress.

5.4 Compartmentation

Plants can tolerate metal stress by accumulating large amount of metals ions in different compartments without the plant showing any toxic symptoms. To achieve detoxification, plants either transport the metal ions out of the cell or sequestrate in cell wall or vacuole thereby limiting the metal effect on other sensitive metabolic activities in the cell. In castor, different studies have reported compartmentalization of Cu/Pb mainly in the cell wall of roots [22,54,86,87]. Recently, Nascimento et al. [54] reported that Pb distribution was more pronounced in the cell wall of castor roots than in the leaves. It was found that, at 200 μ mol/L in the roots were 382 times more than that in leaves. A study also revealed that Cu was mainly bound to the cell walls of castor with less amount present in the cytoplasm and nucleus [87]. Likewise, a higher amount of Cu was distributed in the cell wall of roots followed by organelles and cytoplasm of castor [22]. These studies hence confirm the sequestration of metal ions in the cell wall of the accumulating organs, mainly the roots.

Some metal transporters reported to assist plants in detoxification or sequestration of metal ions out of the cell or into vacuoles include ABC transporters (ATP binding cassette), copper transporter (COPT) family proteins, zinc regulated transporter AtMTP1, and yellow-stripe-like (YSL) [88]. It has been reported that AtMTP3, AtIREG2, and ZTP1 helped in detoxification and sequestration of Cd, Ni, and Co into vacuoles [88].

However, metals that cross the cell wall and plasma membrane to the cytosol distribute around the various cytosolic ligands and subcellular compartment (intracellular organelles) [89]. The subcellular compartment reflects the internal detoxification mechanism in plants. The subcellular partitioning can be classified into five major sections based on the differential centrifugation of tissues. These partitions are cellular debris, organelle, heat-stable protein (HSP), metal-rich granules (MRG), heat denature proteins (HDP) [78]. This partitioning gives an idea of metal tolerance in plants. Collectively, the soluble fraction, which are, HSP and MRG are termed as the biologically detoxified mechanism (BDM), whereas HDP is the metal sensitive fractions (MSF). The BDM fraction acts as a primary detoxification mechanism but at very high concentrations, metals binds to MSF to achieve detoxification. For example, Zhang et al. [78] demonstrated that Cd distribution was found in the BDM of castor leaves (Zibo 9) at a concentration of 2 mg/L of Cd whereas, at a higher level (5 mg/L), Cd was bound to the MSF. A recent study also revealed that Cd distribution in young leaves of castor cultivar JX-22 was bound to BDM when compared to ZB-9 [66]. Hence, the detoxification of metal ions by BDM could be linked to the type of cultivar and dose of metal applied.

These, therefore suggest that accumulation and detoxification of metal ions do not just rely on the various compartments but an interplay between biological mechanisms, cultivars, metal concentration and the cell structure of the compartments like the cell wall of roots.

5.5 Exudation of Organic Acids

Exudation of organic acids is involved in metal storage, tolerance, and detoxification. To combat metal toxicity in the soil solution, plant roots secrete low molecular weight organic acids which may form stable ligand metal complexes with metal ions and change their bioavailability and mobility, in order to prevent metals from penetrating plants or circumvent their accumulation in sensitive parts of roots [90]. Organic acid may also chelate metal ions that reach the cytosol to render them into non-toxic or less toxic forms. Plants form an array of ligands for metal such as Cd, Cu, Ni, and Zn. Organic acids including citric acid (CA), malate acid (MA), and tartaric acid (TA) are some possible ligands for toxic metals and these ligands promote plant tolerance in contaminated soils. The CA has a high affinity to chelate several metals especially Fe, Cd, Cu and Ni [91]. Studies conducted on Ni hyper-accumulator have shown that Ni mainly binds to CA, proposing that accumulation of CA had a positive correlation with Ni stress. For example, Bauddh et al. [18] found that both CA and MA increased in the roots of castor plant, which chelated excess Ni when grown on increasing concentrations of Ni contaminated soil. Currently, organic acids have been found to be associated with several metals as reported in many plant species [92]. In a study, the role of root exudates from castor treated with Cu at five different levels (0, 100, 250, 500, and 750 µmol/L) was investigated [67]. The results showed that increasing Cu (750 µmol/L) significantly ($p < 0.01$) increased the concentration of TA (329.13 µmol/g dwt.) and a positive linear correlation was seen between the concentrations of TA, CA, and succinic acid and Cu in root castor. It was concluded that the high TA and CA, low cysteine and oxalic in the root exudation enhanced the plant tolerance to Cu stress [67].

5.6 Phytochelatins

High-affinity ligands such as phytochelatins (PCs) help in alleviating metal stress. Phytochelatins are cysteine-rich metal binding proteins produced by plants with the general structure (c-Glu-Cys) n -Gly ($n = 2-11$) [93]. Phytochelatins are known to be restricting metals to plants roots. Adhikari et al. [52]

observed that increasing concentration of Ni in roots increased PCs, which bounded the excess Ni in the root of castor. Plants form metal phytochelatin complexes upon higher levels of metal exposure, and these ions are then sequestered into vacuole for detoxification [3,93]. The translocation of metal phytochelatin complexes into vacuole is governed by a set of organic solute transporters. It has been found that at elevated levels of Cr, phytochelatins form complex with Cr in *B. juncea*, *Vigna radiata*, and *Oryza sativa* as well as decreasing their root to shoot translocation [3]. The enzyme phytochelatin synthase (PCS), (a constitutive enzyme which requires post-translational activation by metals), catalyzes the synthesis of phytochelatins [93]. This enzyme aids in the removal of Glu-Cys residue from glutathione (Glu-Cys-Gly) and binds to another glutathione. In *Nicotiana glauca*, it was revealed that the expression of phytochelatin synthase gene extracted from *T. aestivum* enhanced Cd, Cu and Pb accumulation in this plant [94]. Aside from metal detoxification, it has been found that PCs play a vital role in metal homeostasis and thus controlling the bioavailability of metal ion in plant cells.

5.7 Molecular Studies Associated with HM Tolerance in Castor

Over the period, studies on physiological mechanisms of HM tolerance and detoxification have received considerable attention, but only a few have been conducted at the molecular level. Several stresses such as drought, low temperature, or salinity induce expression of a group of genes in plants [95]. The role of these stresses has been well studied in many plant species while little is known about their role in the tolerance of castor to HMs. Assessing plants' ability and tolerance to HM stress at the molecular level is very key in revealing the gene expression profile for biological processes. Stressors activate huge number of genes and different proteins in order to combine their signaling pathways that confer stress tolerance [77]. These genes can be grouped into two; functional genes or regulatory genes [77,95]. The regulatory genes (transcription factors (TFs) as the name depicts, regulate either a group or single stress-responsive genes, while the functional genes (metabolic compounds like amines, alcohol, or sugars) play a major role in HM tolerance. The expression of gene clusters and group of multigene families are usually controlled by TFs, thus the master regulators. It has been reported that the expression of many target genes could be controlled by a single TF through binding of the TF to the *cis*-regulatory element in the promoter of its target genes [96]. A number of the TFs involves DNA-binding that interrelates with *cis*-acting elements in the promoters and by a protein-protein coordination domain that aids in oligomerization with other regulators [96]. This form of transcriptional regulatory system is termed as regulon [96,97]. Among the members of TF families responsible for controlling plant response to stresses include; AREB/ABF, ARF, ABI3VP1, CPP, C2C2-Dof, DREB1/CBF, EMF1, E2F-DP, MYB, MADS, NAC, and TUB [13,77,95]. These TFs, however, differ among plant species and metal type. It has been demonstrated that the expression of FIT with AtbHLH38 and AtbHLH39 activated the expression of certain Fe transporters (HMA3, IRT2, and MTP3) in *A. thaliana* when exposed to Cd stress [98]. DREB transcription factor was reported to be down-regulated in *A. thaliana* root under metal stress, which was attributed to the fact that DREB might have been enhanced in the normalization of osmotic potential in order to reduce the movement of contaminated water in this plants [97].

In a recent transcriptomic study, four TF genes (DREB-1A, DREB-1B, DREB-1F and CBF) in castor were associated with Cd tolerance and were influenced by a micronutrient, specifically molybdenum (Mo) [13]. Different factors induce different gene expression whiles multiple/combination of factors induces the relative expression of genes. For instance, DREB-1B, DREB-1F, and CBF genes were expressed only upon Cd exposure whereas only CBF expressed when Mo alone was applied. However, when castor was associated with soils exposed to both Mo and Cd the relative expression level of the above genes increased as well as the phenolic and proline content [13], indicating that Mo might have an enhancing effect on expression of the CBF like genes.

At the posttranscriptional level, modulators such as microRNAs (miRNAs) and their putative target genes could be useful in detecting the signaling pathway in plant tolerance to different stresses. miRNAs are small non-protein coding RNAs transcribed by endogenous RNA polymerase II and are involved in the both down and up-regulation of gene expression [99]. Diverse miRNA has been reported to regulate HM stress in plants. Some of these includes; miR398, miR171, miR838, miR395 among others, which have also been observed in the castor plant [20,99]. According to post-transcriptional regulation study in castor, four HM stress-responsive miRNAs and their target genes were observed under Ni stress [20]. These miRNAs have been observed to play diverse functions in plants.

miR171 is connected to regulating biosynthesis of photosystem II [100]. Its expression after HM stress in castor proposes that, despite the exposure to HM, photosynthetic activity of the plant might not be destroyed. miR319 and miR396 regulate senescence which intends to regulate plant growth and development [101], hence its expression in castor may promote its growth despite HM stress. miR395 controls sulphate uptake by regulating sulphate molecule such as GSH [102]. The expression of miR395 may explain the enhanced antioxidant ability of castor to HM stress. miR159 and its target, as well as their secondary metabolite, enhances plant survival under metal stress [99]. miR838 is found in intron 14 of DICER-LIKE1 (DCL1) and plays a major role in miRNA biogenesis to initiate the DCL1 miRNA degradation [99]. miR838 also controls DCL1 mRNA with a negative feedback mechanism which is supposed to induce miRNA maturation. The high tolerance of castor to HM stress can then be attributed to the various functional roles of miRNA.

The transcriptional regulation of some miRNA has been reported to be regulated based on the plants' tolerance ability to HMs, type of metal, and accumulated tissues. In *B. juncea*, *B. napus*, and *Oryza sativa*, the expression of miR171, miR159 were down-regulated and miR395 was up-regulated under metal stress such as Cd, As and Hg [100]. miR398 and its target gene Cu-Zn/SOD was down-regulated in castor plants under Ni stress [20]. In plant tissues under the same stress treatments, a differential expression of some miRNAs was observed. The expression levels of miR159 and miR319 were significantly expressed only in the roots tissue of castor after Ni^{2+} stress [20]. Also, in assessing the relation between miR395 level and its target genes; sulphate adenyl-transferase and sulphate transporter were found only in the leaf, but the regulatory level of sulphate transporter showed a significant relation in the root. This study then confirms our earlier assertion that HM stress (Ni^{2+}) in castor depends on the part of tissue analyzed.

However, some miRNA can express irrespective of tissues. An instance was observed through qRT-PCR analysis whereby miR838 highly responded to Ni^{2+} stress and the target gene was up-regulated in both leaves and roots of castor plant [20].

6 Conclusion and Prospects

Products of castor oil plant are widely used in the manufacture of many industrial, agricultural, and pharmaceutical products. Its growth, yield, and production can be influenced by HM which causes several damages to the plant. However, castor adapts several mechanisms to combat and tolerate the effects of HMs through activation of antioxidant enzymes, exclusion, accumulation of proline, and organic acid exudation. Also, some metal responsive genes specifically at the transcriptional and posttranscriptional levels have been identified in relation to metal tolerance in castor. Castor tolerance to HMs is dependent on the duration of metal exposure, concentration of the metal, and cultivar of the plant. The tendency of castor to retain maximum concentration of toxic metals in the root system suggests that castor would be a potential player for phytostabilization. Further molecular and genetic studies are required to understand the mechanism and principle of the stress-responsive genes that confer metal tolerance in this plant. These studies combined with some of the already identified tolerant cultivars will help in breeding super tolerant cultivars and these cultivars can be used for remediation of contaminated agricultural and

industrial sites. Also, other agricultural management practices such as exogenous application of plant growth regulators could be employed to enhance the plant's tolerance ability under extremely high contaminated soil.

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