Review

Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations

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ABSTRACT

Increasingly, anthropogenic perturbations of the biosphere manifest in a broad array of global phenomena, causing widespread contamination of most ecosystems, with high dispersion rates of many contaminants throughout different environmental compartments, including metals. Chromium (Cr) contamination in particular, is, increasingly, posing a serious threat to the environment, emerging as a major health hazard to the biota. However, although the molecular and physiological mechanisms of plant responses to many heavy metals, especially lead (Pb) and cadmium (Cd), have been focused upon in recent years, chromium has attracted significantly less attention. In this context, this review discusses aspects of Cr uptake and transport, some physiological and biochemical effects of Cr exposure in plants, and molecular defense mechanisms against this metal. Recent advances in determining these responses, in fields of knowledge such as genomics, proteomics and metallomics, are discussed herein.

1. Introduction

Increasingly, anthropogenic perturbations of the biosphere manifest in a broad array of global phenomena, including accelerated industrialization, intensive agricultural activities, extensive mining accompanied by significant increases in the human population and, consequently, rapid urbanization (Emamverdian et al., 2015). This has, in turn, caused widespread contamination of most ecosystems, with high dispersion rates of many contaminants throughout different environmental compartments, including metals.

Chromium (Cr) is the seventh most abundant element in the earth's crust and the sixth most abundant transition metal (Mohan and Pittman, 2006; Panda and Choudhury, 2005). It is present in the ecosystem as a result of the weathering of the earth's crust and deposition of waste from anthropogenic activities, such as the metallurgical (mainly steel and metal) and chemical (pigments, electroplating, leather, among others) industries (Kotaś and Stasicka, 2000; Tchounwou et al., 2012). This element is detected in most of the environmental matrices (air, water, soil) and has, in recent decades, increased exponentially in aquatic and terrestrial ecosystems (Velma et al., 2009). This metal can be detected in several oxidation states ($Cr°$, Cr^{1+}, Cr^{2+}, Cr^{3+}, Cr^{4+}, Cr^{5+}, Cr^{6+}). $Cr°$, Cr^{4+} and Cr^{5+} do not occur naturally. While $Cr°$ is mainly found in metal alloys, such as stainless steel, and is an additives which gives metallic material properties, such as corrosion resistance wear, high temperature and higher color durability (Gomez and Callao, 2006; Zayed et al., 1998), the latter Cr species are unstable intermediate forms in oxidizing and reduction reactions of Cr^{3+} and Cr^{6+} (Kotaś and Stasicka, 2000; Zayed and Terry, 2003). Cr^{+1} is rarely seen except when stabilized in complexes (Lay and Levina, 2012) and Cr^{2+} is relatively unstable and is readily oxidised to the trivalent state which occurs naturally in ores (Zayed and Terry, 2003).

Among all Cr oxidation states, Cr^{3+} and Cr^{6+} are the most stable in aquatic and terrestrial environments (Augustynowicz et al., 2010; Santos et al., 2009; Zayed et al., 1998), although they differ in terms of mobility, bioavailability and toxicity (Panda and Choudhury, 2005). Generally, the oxidation of Cr(III) to Cr(VI) is a very slow process at pH above 5 (Eary and Rai, 1987), and alkaline conditions favor the oxidation of Cr(III) to Cr(VI) (Pantsar-Kallio et al., 2001; Seaman et al., 2001). The reduction of Cr is influenced primarily by the decomposition of organic matter, dissolved reduced sulphates and industrial effluents that may alter the physical-chemical parameters of the environment (Stanin and Pirnie, 2004). As oxygen concentrations are usually low in polluted environments, the reduction of Cr^{6+} to Cr^{3+}

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is favored (Stanin and Pirnie, 2004), this reduction can indirectly influence and change environmental pH to both alkalinity or acidity extremes (Hawley et al., 2004). In soil, this phenomenon might in turn disturb nutrient bioavailability and their sorption by plants (Emamverdian et al., 2015).

Although Cr^{3+} has been shown to be less toxic than Cr^{6+} and is a necessary nutrient for maintenance of metabolic activities in animals (Mohan and Pittman, 2006; Urrutia et al., 2008), both Cr species, when present in high concentrations, can show highly toxic effects on the biota (Dazy et al., 2008; Sreeram et al., 2004). Particularly, the range between Cr^{3+} toxicity and the need for this element is very narrow (Chang et al., 1996). Regarding plants, there is no conclusive evidence of the essentiality of the role of Cr in plant metabolism, and literature results are discrepant in this regard; while some studies indicate that Cr is not essential in plants (Hayat et al., 2012), others have shown that small additions of Cr have stimulating effects on plant growth and productivity (Ghosh and Singh, 2005; Zayed and Terry, 2003).

Common physicochemical treatment technologies that remove Cr pollution are based on reduction–oxidation, precipitation, accumulation, and sorption (Hawley et al., 2004). Plants pose mechanisms of contaminant remediation related to the all of the mentioned strategies, however, the details of these mechanisms are not clear (Augustynowicz et al., 2013). Thus, knowledge on the biological processes that affect the mobility, chemical distribution and speciation of Cr in the physical and chemical environment is essential in order to develop effective preventive and/or remediation strategies to counteract the toxic effects of this metal (Zayed and Terry, 2003).

Plants employ several different types of strategies for metal tolerance or detoxification, as described previously (Emamverdian et al., 2015). Briefly, as a first step, plants adopt avoidance strategies, such as restricting or excluding metal uptake from the soil, thus preventing metal entry into the roots (Viehweger, 2014), by either immobilizing the metal ions by mycorrhizal association or complexing the metals through organic compounds produced and exhuded from the roots (Dalvi and Bhalerao, 2013). If this fails and the metal enters the plant, tolerance mechanisms for detoxification are activated, such as metal sequestration and compartmentalization in different intracellular compartments (Patra et al., 2004), metal transport or binding to the cell wall and biosynthesis and accumulation of several compounds aimed at metal complexation and protection against metal toxicity, such as prolines and metallothioneins (Dalvi and Bhalerao, 2013; John et al., 2009). If all these measures prove unsuccessful and plants begin suffering effects of metal toxicity, activation of antioxidant defense mechanisms is then pursued (Manara, 2012).

2. Cr absorption, transport and distribution in plants

Many factors influence metal absorption in plants such as environment, temperature, pH, aeration, electrical conductivity, competition between species, type of plant, plant size, root system, element availability, type of leaf, and soil and plant moisture content (Yamamoto and Kozlowski, 1987). The phytotoxic effects of Cr are primarily dependent on the speciation of the metal, which determines its uptake, translocation and accumulation (Shanker et al., 2005). Cr absorption and distribution mechanisms in the vegetative and reproductive organs of plants, however, are still not fully understood (Hayat et al., 2012). It has been reported that Cr is transported and accumulated in plants via carrier ions, such as sulfate or iron, and is not directly absorbed by plants (Gajalakshmi et al., 2012; Singh et al., 2013). It is also known that Cr can be absorbed both as Cr^{3+} and Cr^{6+}, but no specific mechanism for Cr absorption has yet been postulated (Oliveira, 2012; Singh et al., 2013).

Most studies have demonstrated excessive accumulation of Cr in roots, and the immobilization of this metal in the vacuoles of plant root cells is suggested as the main reason for this bioaccumulation (Nematshahi et al., 2012; Oliveira, 2012), and is proposed as a means
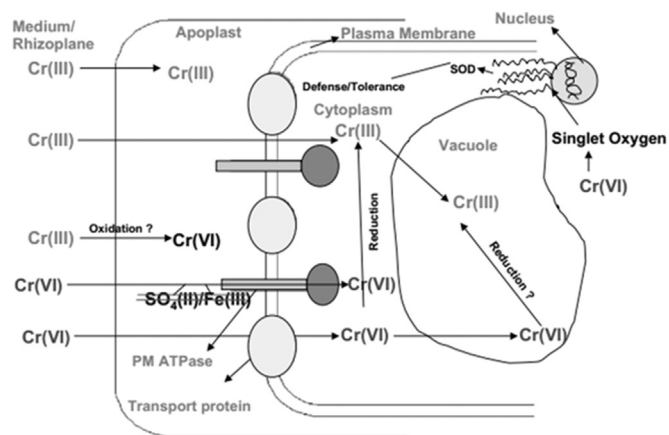

Fig. 1. Hypothetical Model of Cr transport and toxicity in plant roots (adapted from Shanker et al. (2005).

of protecting the photosynthetic apparatus in leaves (Brune et al., 1995). In roots, Cr^{6+} absorption occurs actively, while Cr^{3+} absorption occurs by osmosis (Barros et al., 2006). The plasma membranes of roots are the first functional structure to come into contact with metals (Fig. 1), and play a crucial role in metal tolerance (Hayat et al., 2012). It is possible that the entry of this element in root cells occurs through entry channels of essential ions (Liu et al., 2011).

In a study with *L. hexandra*, the absorption of Cr^{3+} was shown to be dependent on metabolic energy, with no relation to Ca^{2+} and K^+ uptake channels. However, higher amounts Cr^{3+} in plants that received Fe^{3+} were observed (Liu et al., 2011). This suggests that Cr^{3+} absorption by plant roots may be mediated in part through Fe^{3+} complex carriers (Liu et al., 2011). Moreover, other studies indicate that, in addition to Fe, S and P also compete with Cr for the binding site in the carrier complex (Fig. 1) (Cervantes et al., 2001; Shanker et al., 2005). This was also corroborated by the fact that, in maize, chromate inhibited sulfate absorption when supplied for a short period of time (López-Bucio et al., 2014).

Following Cr entry through the roots, transport by translocation to the shoots occurs very slowly, another reason why Cr is retained preferentially in roots (Paiva et al., 2009; Singh et al., 2013; Sundaramoorthy et al., 2010). Cr in roots also inhibits cell division and shortens the overall length of roots, which may lead to severely restricted water and nutrient absorption processes, in turn leading to decreased shoot growth (Shanker et al., 2005). Metal ions can also be actively absorbed by root cells through the plasmalemma and adsorbed on cell walls by passive diffusion and delivered via acropetal transport in aquatic plants (Mishra and Tripathi, 2009).

In order to study Cr bioaccumulation, a study was conducted with *Brassica chinensis* L., investigating effects of increases in the concentrations of $CrCl_3$ medium (0, 2.5, 5 and 10 mg L^{-1}). Results showed that, after increasing exposure, Cr concentrations in the cell wall, plastids, nuclei and mitochondria also increased. The authors deemed it noteworthy that Cr in roots increased two-fold (5.43, 1.44, 2.35, 3.79 and 4.43 mg L^{-1}) compared to shoots (2.55, 1.63, 3.01 and 3.43 mg L^{-1}) (Wu et al., 2013). In several macrophytes (*Alternanthera philoxeroides, Borreria scabiosoides, Polygonum ferrugineum* and *Eichhornia crassipes*) exposed to 25 and 50 mg L^{-1} of $CrCl_3{\cdot}6H_2O$, higher Cr concentrations in roots were also observed when compared to the stem, and with the exception of *E. crassipes* (9.02 mg Cr kg^{-1} dry weight (d.w.)), almost negligible amounts of Cr were found in leaves (0.15 A. *philoxeroides*; 0.13 *B. scabiosoides*; 0.04 *P. ferrugineum* mg Cr kg^{-1} d.w.) of Cr in the leaves (Mangabeira et al., 2011). In a previous study, Mangabeira et al. (2004), observed, by ion microscopy, large amounts of Cr in the vascular cylinder of *E. crassipes* roots and leaves exposed to 25 and 50 mg L^{-1} for 30 days. Cr was mainly located in the cell wall of roots, as well as in the parenchyma of the roots. Furthermore, the

authors suggested that the parenchymal cells of the roots are involved in Cr transport to the shoots.

After absorption, Cr has been shown as transported mainly though plant xylem (Hayat et al., 2012). When Cr^{6+} passes through the endoderm via symplasts, it is reduced to Cr^{3+}, which is retained in the root cortex cells (Hayat et al., 2012). Furthermore, it has been suggested that the conversion of Cr^{6+} to Cr^{3+} can also occur in the aerial part of the plant (Cervantes et al., 2001). In this regard, a study with *Gynura pseudochina* (L.) DC., Cr^{6+} was reduced to Cr^{5+}, which was then reduced to Cr^{3+}, the less toxic form. This Cr^{3+} was then transported through the xylem through the symplastic system, and was subsequently distributed in the cytoplasm of cortical cells (Mongkhonsin et al., 2011).

3. Physiological effects of Cr in plants

Although there is no conclusive evidence of the essentiality of Cr in plant metabolism, some studies have shown that small additions of Cr have stimulating effects on growth and plant productivity (Zayed and Terry, 2003). Beneficial effects, such as antifungal and growth stimulation have also been observed in plants grown in soil or in solutions containing Cr (Barceló et al., 1993). For example, in one study, the presence of 1 μmol L^{-1} Cr was found to stimulate plant growth (Ghosh and Singh, 2005), and, according to El-Bassam (1978), low concentrations of Cr^{3+} stimulate chlorophyll synthesis and photosynthesis activity. Increases in carbon assimilation were also observed in a study conducted with *E. crassipes* collected from the river Imbé, in South-eastern Brazil and exposed to 1 mmol L^{-1} Cr_2O_3 (Paiva et al., 2009).

Toxic effects of Cr on the growth and development of plants, however, are more apparent, and have been increasingly investigated. These include several metabolism changes, such as modifications to the germinating process and the growth of the roots, stems and leaves (Shanker et al., 2005). Cr also causes harmful effects on physiological processes, such as photosynthesis, water relations and mineral nutrition (Nagajyoti et al., 2010; Shanker et al., 2005), and can also generate morphological changes (Daud, 2014; Rodriguez et al., 2012; Singh et al., 2013).

3.1. Effects on plant morphology

Plant exposure to Cr has been shown to reduce certain root parameters, such as diameter, surface area, and number of hairs, and has also been determined as the cause of wilting and plasmolysis in root cells (Ali et al., 2011; Moral et al., 1995; Panda and Choudhury, 2005). Cr^{6+} has been proven to reduce the number of root cells as it enters the cell through the cellular membrane, and can be responsible for cytotoxic effects and damage to DNA (Chidambaram et al., 2009). Cr may also cause alterations in the ultrastructure of chloroplasts in cell membranes and necrosis in plant leaves (Chidambaram et al., 2009; Choudhury and Panda, 2005; Ghani, 2011; Tiwari et al., 2009). For example, in a study conducted with *E. crassipes* after 2 and 4 days

exposure to 1 and 10 mmol L^{-1} Cr_2O_3 and $K_2Cr_2O_7$, it was observed that after 2 days of exposure to 10 mmol L^{-1} plants exposed to Cr^{6+} plants were completely necrosed, while plants subjected to 1 mmol L^{-1} Cr^{6+} showed necrosis only after 4 days of metal exposure, indicating lesser toxic effects to lower Cr concentrations. After 4 days of exposure to 10 mmol L^{-1} of Cr^{3+}, plants showed healthier conditions when compared to the control plants and the plants exposed to 1 mmol L^{-1} Cr^{3+} (Paiva et al., 2009).

It has also been demonstrated that Cr^{6+} is more phytotoxic than Cr^{3+}, since, at high Cr^{6+} concentrations (1 mmol L^{-1}), complete distortion of the chloroplastidic membrane alongside severe thylakoid disarrangement were observed (Choudhury and Panda, 2005). Cr has also been shown to be toxic to bean plants, causing changes in morphology compared to control plants (Azmat and Khanum, 2005), including reduced root length with increasing Cr concentrations (0, 5, 10, 50, 100, 150, 200 and 250 mg kg^{-1}). Canopy size, however, was less affected. Changes in morphology of corn leaves after one day of exposure to 300 mg L^{-1} of $K_2Cr_2O_7$ have also been observed, where young leaves suffered epinasty after 6 h of exposure, and significant wilting was observed after 12 h, possibly due to water stress caused by Cr (Wang et al., 2013). Toxicity symptoms of Cr^{3+} were also observed in *Ocimum basilicum* plants exposed to $CrCl_3 \cdot 6H_2O$ concentrations of 0, 2, 4, 6, and 8 mg L^{-1}. Alterations were also observed in the cytoplasm, such as cytoplasm deorganization, alterations in the ultrastructure of chloroplasts, an underdeveloped lamellar structure with widely spaced thylakoids and less amounts of grannae (Bishehkolaei et al., 2011). These morphological changes can, in turn, severely affect photosynthetic pigments and photosynthesis (Bishehkolaei et al., 2011), discussed in the following section.

3.2. Effects on photosynthesis metabolism

Photosynthesis inhibition during stress caused by metals is one of the main consequences in plants, since these elements invariably, directly or indirectly, affect the photosynthetic apparatus (Sytar et al., 2013). Metals alter the functions of the chloroplast membrane and components of the electron transport chain in mitochondria (Ventrella et al., 2011) and, thus, inhibit part of the energy transfer from one level to another (Sytar et al., 2013). According to Dixit et al. (2002), the change in redox reactions of Cu and Fe carriers (Fig. 2), allows for Cr^{6+} to be transferred via cytochrome mitochondria, allowing this element to bind to the heme group of cytochrome, interfering with the transport electrons. Cr^{6+} can also bind to the cytochrome a_3, as well as the Complex IV of cytochrome oxidase (E.C. 1.9.3.1.), thus causing a severe inhibition of the activity of this enzyme (Dixit et al., 2002).

Metals can also affect the activity of the photosystem I (PSI) and the photosystem II (PSII), located in the thylakoid membranes. PSII has been shown to be more susceptible to toxic metal effects in comparison to PSI (Sytar et al., 2013). For example, in thylakoids isolated from *Brassica juncea* individuals exposed to 200 and 400 mmol L^{-1} Cr^{6+}, greater activity of PSII was observed when compared to control plants
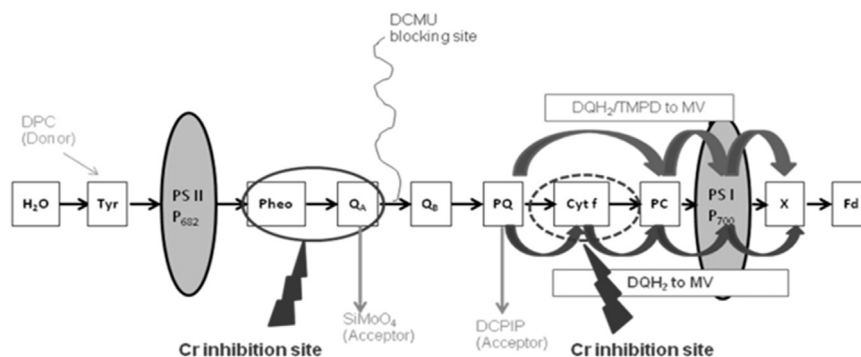

Fig. 2. Schematic diagram of Cr inhibition sites in electron transport in photosynthesis in chloroplasts isolated from spinach (Pandey et al., 2013).

(Gupta et al., 2009). The PSI activity in thylakoids of seedlings exposed to 200 µmol L^{-1} Cr^{6+} was similar to controls, whereas the PSI activity in plants exposed to over 200 mmol L^{-1} Cr^{6+} concentrations were lower compared to control seedlings (Gupta et al., 2009). In research conducted with chloroplasts isolated from *Beta vulgaris L.* submitted to Cr^{6+} exposure, a significant inhibition of electron transport activity in both PSI and PSII was observed. Within the PSII, the pheophytin and plastoquinone regions were more affected (Fig. 2) (Pandey et al., 2013). The water oxidation complex, however, was unaffected by exposure to Cr^{6+}.

Studies have also demonstrated that Cr interferes with gas exchange parameters consisting of CO_2 assimilation (*A*), evapotranspiration (*E*), stomatal conductance (g_s) and internal carbon (*C*i) (Rodriguez et al., 2012). In this context, Cr^{3+} causes water imbalance in plants and affects stomatal opening, leading to g_s changes (Barbosa et al., 2007). Cr^{3+} in high concentrations also affects photosynthesis in terms of carbon assimilation, electron transport, photophosphorylation and alterations in Rubisco activity (Pandey and Sharma, 2003). Additionally, several enzymes are inhibited at high Cr concentrations (Vazques et al., 1987), which leads to reduction of photossynthetic yield (Nagajyoti et al., 2010).

When comparing the effects of Cr^{3+} and Cr^{6+} on photosynthetic parameters, Paiva et al. (2009) found that the Cr^{3+} can increase carbon assimilation in *E. crassipes*. However, the same study observed that Cr^{6+} caused a decrease in carbon assimilation and chlorophyll a content, and fluorescence parameters. One explanation for the reduction in photossynthetic yield caused by Cr^{6+} can be ascribed to the disorganization of the ultrastructure of chloroplasts (Van Assche and Clijsters, 1983) and inhibition of the electron transport processes due to a deviation of electrons from the PSI electron donor side (Shanker et al., 2005). It is possible that the electrons produced by the photochemical process are not used for carbon sequestration, as evidenced by the low photosynthetic yield observed in plants exposed to Cr^{6+}. Thus, it is hypothesized that part of the electrons may be used for the reduction of oxygen molecules, which may explain oxidative stress caused by Cr^{6+} (Shanker et al., 2005).

A decrease in photosynthetic rates, transpiration and stomatal conductance of *Oryza sativa* L. cultured with 50, 100, 150, 200, 300, 400 and 500 mg kg^{-1} of Cr^{6+} was observed, compared to control plants. The results indicate that gas exchange parameters and chlorophyll a, b and carotenoids were reduced with increasing concentration of Cr^{6+} (Ahmad et al., 2011).

The reduction of photosynthetic pigments by metals occurs by inhibition of the activity of enzymes involved in the biosynthesis of chlorophyll, as well as by substitution of the central Mg ion of the chlorophyll molecule by the metal, impairing the reception of light and leading to the collapse of photosynthetic activity (Küpper et al., 2002; Prasad and Strzałka, 1999). For example, Dhir et al. (2009) found a significant decrease in the activity of ribulose bisphosphate carboxylase oxygenase (RuBisCO, E.C. 4.1.1.39.) induced by exposure to wastewater rich in Cr in *Salvinia natans*, and suggested that this result may be explained by the substitution of Mg^{2+} in the active site of RuBisCO by subunits metal ions. This would, in turn deplete chlorophyll content as suggested by other authors (Vajpayee et al., 2000).

In *Phaseolus vulgaris* plants exposed to 10^{-6}, 10^{-4} and 10^{-2} mol L^{-1} of $CrCl_3·6H_2O$, it was observed that at the lower and moderate Cr^{3+} concentrations, pigment content in leaves increased. However, the plants under irrigation at the highest Cr^{3+} concentration showed significant decreases in chlorophyll a, b and carotenoids (Zeid, 2001). Photosynthesis was also reduced in tomato plants (*Lycopersicon esculentum* Mill. Cv. Juncal) exposed for two weeks to 0, 10, 20, 30, 40 and 50 µmol L^{-1} Cr^{3+} and Cr^{6+}. The reduction in photosynthetic yield caused by Cr^{3+}, however, was gradual and slow, while the decreases caused by Cr^{6+} were much more pronounced (Henriques, 2010). In that study, Cr^{3+} caused no significant effect on stomatal conductance, while Cr^{6+} from 20 µmol L^{-1} upwards significantly reduced this parameter.

Under the 50 µmol L^{-1} Cr^{6+} concentration, stomatal conductance values were reduced to 1/3 in comparison with the leaves of control plants. The tomato plants exposed to Cr^{3+} showed significant increase of internal carbon, possibly caused by a decrease of CO2, but the plants subjected to Cr^{6+} was an initial increase in internal carbon, followed by a decrease at concentrations of 30, 40 and 50 µmol L^{-1}. The author suggested that Cr^{6+} in moderate and high concentrations significantly decreases the maximum PSII efficiency, measured by the maximum fluorescence quantum variable (Fv/Fm) in leaves. This reduction shows that the Cr^{6+} directly or indirectly causes deficiencies in PSII through its toxic effects (Henriques, 2010). According to Appenroth et al. (2001) the number of inactive PSII units increases in the presence of Cr^{6+} as a consequence of the reduction in the number of plastoquinone B bonding sites.

4. Oxidative stress induction

Plants have developed strategies to tolerate adverse conditions and their negative effects (Liu and Yao, 2007), and excess of Cr and other metals can initiate a variety of metabolic responses leading to changes in plant development (Hayat et al., 2012). Redox metals, such as Cr, can directly generate oxidative injury via the Haber-Weiss and Fenton reactions, which in turn lead to the production of reactive oxygen species (ROS) in plants (Flora, 2009). Reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-•}$), hydrogen peroxide (H2O2), hydroxyl radicals (OH˙) and oxygen singlets ($^1O_2$) are normally produced in small amounts in aerobic organisms. However, under stress conditions, their production is dramatically increased (Miller et al., 2010). This overproduction of ROS in plants can result in cell homeostasis disruption, DNA strand breakage, defragmentation of proteins, or cell membrane and damage to photosynthetic pigments, which may trigger cell death (Flora, 2009).

Production of ROS in plants exposed to Cr has been demonstrated, and has been shown to result in oxidative stress leading to DNA, protein and pigment damage, as well as the initiation of lipid peroxidation (Choudhury and Panda, 2005; Panda, 2003). Absorption of Cr is facilitated by a carrier membrane, thereby ROS generation and their impact on the plasma membrane are very important (Maiti et al., 2012).

ROS are usually eliminated in their production sites by antioxidant compounds (Hossain et al., 2012), such as antioxidant enzymes. The main antioxidant enzymes studied in plants include catalase (CAT, E.C. 1.11.1.6), guaicol peroxidase (E.C. 1.11.1.7), glutathione reductase (E.C. 1.8.1.7), ascorbate peroxidase (E.C. 1.11.1.11) and superoxide dismutase (SOD, E.C. 1.15.1.1) (Gill and Tuteja, 2010; Panda and Choudhury, 2005). Besides enzymes, increases in the synthesis of other compounds have also been reported in response to metal-induced stress, such as polyamines (PAs) (Hussain et al., 2011), proline (Pro) (Kishor et al., 2005), nitric oxide (NO) (Delledonne, 2005; Liu and Yao, 2007) and metallothioneins (MT) (Teixeira et al., 2013).

Interestingly, despite the harmful effects of ROS, it has been proposed that H2O2, in itself a ROS species, can act in signaling mechanisms in response to stress (Mittler et al., 2004; Sharma and Dietz, 2009), and has been proposed as a key molecule to elicit signal transduction for metal tolerance in plants, since it is immediately produced under stress by metals (Seth et al., 2012). Further discussion in this regard shall follow.

Many antioxidant enzymes have been demonstrated as significantly altered in plants exposed to metals. SOD, for example, acts as a first line of defense against oxidative stress in all aerobic organisms and all subcellular compartments prone to oxidative stress mediated by ROS (Gill and Tuteja, 2010). This enzyme catalyzes the dismutation $O_2^{•-}$ to H2O2 and O2 (Gill and Tuteja, 2010), and is found in almost all cell compartments, as well as in the water and ascorbate-glutathione cycles in chloroplasts and in the cytosol, mitochondria, peroxisomes and apoplasts (Bhaduri and Fulekar, 2012). CAT is also an essential enzyme for the detoxification of ROS in plants (Panda and Choudhury, 2005).

This enzyme catalyzes the dismutation of H_2O_2 into O_2 and H_2O (Mhamdi et al., 2010), is located in the peroxisomes, and is indispensable for detoxification during stress. However, the complete mechanism of catalase is not well understood (Bhaduri and Fulekar, 2012). Regarding peroxidase, two classes exist in plants, ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) (Jouili et al., 2011). APX has been identified in many plants, and APX isoenzymes have been observed in the cytoplasm, chloroplast stroma and thylakoids (Shigeoka et al., 2002). GPX is a heme protein able to oxidize some substrates at the expense of H_2O_2, in order to rid cells of excess peroxides produced by cell metabolism under both normal and stress conditions (Karuppanapandian et al., 2011). GPX decomposes 3 indole acetic acid and plays an important role in the biosynthesis of lignin and defense against stress by H_2O_2 consumption in the cytosol, vacuole, cell wall and extracellular space (Karuppanapandian et al., 2011). GPX prefers aromatic electron donors, such as guaiacol and pyrogallol (Sharma et al., 2012). Peroxidases are still not well defined, since they catalyze the oxidation of various substrates, including phenolics, such as guaiacol (Jouili et al., 2011), and the stimulation of peroxidase activity under metal stress is not always obvious, because in high metal concentrations peroxidase activity may be reduced due to damage caused in organelles and tissues (Jouili et al., 2011).

In a study on the toxicity of Cr^{3+} in chamomile plants, after seven days of exposure to metal Cr^{3+} accumulated preferentially in roots, which presented high concentrations of ROS, NO and thiols. SOD mainly increased in the roots of plants exposed to higher Cr^{3+} concentrations, whereas H_2O_2 content showed a discontinuous trend for the different Cr^{3+} concentrations, which has been postulated as due to variation in the activities of different peroxidases (Kováčik et al., 2013). In maize plants exposed to different Cr^{6+} concentrations (50, 100, 200 and 300 mmol L^{-1}), increases in the production of H_2O_2 and lipid peroxidation, as well as increasing activities of SOD and GPX in plants were observed when compared to control plants (Maiti et al., 2012). In yet another study, conducted with *Camellia sinensis* L. exposed to Cr^{3+}, SOD, peroxidase and CAT activity all decreased with increasing Cr concentrations (Tang et al., 2014). It is possible that plant resistance in this case exceeded the defense response threshold for high Cr concentrations, causing the observed decreases in enzyme activities. Increases in proline content with increasing Cr^{3+} concentrations were also observed.

Polyamines are low molecular weight aliphatic nitrogen compounds, with two or more amino groups, present in all living organisms (Groppa and Benavides, 2008). They have positive charges at their nitrogen atoms, facilitating their interaction with DNA and RNA molecules and phospholipids (Baron and Stasolla, 2008). Studies show the important role of PAs in various biological processes associated with the growth and development of plants under conditions of biotic and abiotic stress (Kaur-Sawhney et al., 2003; Kusano et al., 2008; Nayyar and Chander, 2004). PA are also involved in gene regulation, stabilization of cell proliferation, membrane and cell-signaling modulation and the modulation of the activity of certain sets of ion channels (Kusano et al., 2008). Diamine putrescine, triamine spermidine, tetramine and spermine, are the main APs found in cells (Groppa and Benavides, 2008). In plants, APs are found in the cytoplasm, vacuoles, mitochondria and chloroplasts (Kumar et al., 1997). However, the concentration of APs in plants varies according to species, organs and tissues, in addition to the development phase (Kuznetsov and Shevyakova, 2007).

Several studies show the activity of PAs associated with abiotic stresses (Bitrián et al., 2012), and it has been shown that AP content can be modified in response to exposure to metals (Hussain et al., 2011; Sharma and Dietz, 2006). In this sense, the reduction of suspended particulate matter, for example, especially, has been shown to improve the photosynthetic efficiency of plants under stress conditions (Hamdani et al., 2011). However, the specific mechanism of action of PAs in plants under stress caused by metals has still not been completely unraveled (Sharma and Dietz, 2009). In *Pterogyne nitens,* for example,

polyamine concentrations (Put, Spd and Spm) were higher in leaves compared to roots, while, in leaves, Spd was the most abundant polyamine. Although the pattern of polyamine production was similar between the control plants and the plants treated with Cr^{+6}, lower concentrations were found in Cr^{+6} exposed plants (Brito et al., 2014). On the other hand, in the roots, polyamine levels increased in the presence of Cr^{+6}. This increase was greater for Spd, followed by Spm and Put. Analyzing the different free polyamines, Put presented a significant decrease in the leaves and increases in roots of the plants exposed to Cr^{+6}. Spd showed no variation in the leaves, while, in the roots, an increase of about 6 times was observed in the presence of Cr^{+6}. For Spm, an inverse behavior was observed, of decreases in leaves and increases in roots compared to the control samples. Studies have suggested the interaction of polyamines with thylakoid proteins during stress, imparting a greater tolerance to the plant (Hamdani et al., 2011).

In addition to Aps, proline (Pro) also aids in combating metal-induced stress (Tripathi et al., 2013). Pro is an essential five-carbon α-amino acid mainly synthesized from glutamate that acts as a compatible and metabolic osmolyte, a constituent of cell wall, free radical scavenger, antioxidant, and macromolecules stabilizer (Pavlíková et al., 2007; Szabados and Savoure, 2010). Pro functions as a molecular chaperone and can protect the integrity of proteins and improve the activity of various enzymes (Szabados and Savoure, 2010). Most plants accumulate osmolytes such as Pro to ensure protection for osmotic adjustment and membrane stability (Kumar and Yadav, 2009). Increases in Pro content occurs under various stress conditions including droughts and metal exposure (Kishor et al., 2005), salinity (Huang et al., 2009), pathogens (Fabro et al., 2004), and temperature (Hayat et al., 2012), among others. Pro accumulation usually occurs in the cytoplasm, where it acts in stabilizing protein structures (Hayat et al., 2012). It has been suggested that metal-induced Pro accumulation in plants does not result directly from metal-induced stress, but from water balance disorders due to metal excess (Clemens, 2006). However, some studies have observed ROS scavenging by Pro through detoxifying hydroxyl radicals and quenching singlet oxygens (Mourato et al., 2012; Tripathi et al., 2013). In addition, ROS scavenging has also been observed as a results of increases in antioxidant enzyme activities, maintenance of cellular redox homeostasis (Mourato et al., 2012), and chlorophyll reconstruction, as well as regulation of intracellular pH (Rastgoo and Alemzadeh, 2011) due to Pro activity.

Free proline content of wheat seedlings were shown to be significantly affected by Cu exposure at 40 °C (Muslu and Ergün, 2013) and a substantial increase in proline content in wheat leaves was reported with increasing Cr concentrations (Panda, 2003). Rice seedlings treated with Cr^{+6} (100 μmol L^{-1}) and *Cucumis sativus* L. and *Macrotyloma uniflorum* Lam exposed to increasing Cr^{6+} concentrations also showed significant increases in proline content (Mohanty and Patra, 2011), although not many studies are available in this regard conducted with Cr^{3+}.

Ocimum tenuiflorum L. exposed to 0.0, 10.0, 20.0, 50.0, 100.0 μM de Cr^{6+} showed increases in proline content in leaves, that act as antioxidants, protecting against Cr toxic effects (Rai et al., 2004). According to Ganesh et al. (2009), proline is apparently the only amino acid that accumulates to a great extent in the leaves of plants under stress. Its accumulation starts under mild water stress and the magnitude of accumulation is proportional to the severity of stress. Thus, these authors indicate that proline accumulation under such conditions may also be operative as usual in osmotic adjustment, while accumulation of proline in tissues can be taken as a dependent marker for genotypes tolerant to stress (Ganesh et al., 2009). Proline has multiple functions such as osmoticum, growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential (Ganesh et al., 2009).

Another compound that confers stability against metal-induced stress is nitric oxide (NO) (Sandalio et al., 2012). Additionally, it has been suggested that NO is an important regulator of Cr toxicity in plants

(Kováčik et al., 2013). NO is a free radical that acts as a signaling compound in plants, being involved in many physiological processes including germination, root growth, stomatal closure, and adaptive response to biotic and abiotic stress (Delledonne, 2005; Delledonne et al., 1998; Desikan et al., 2004). NO protects plants from damage from oxidation, regulating general mechanisms for homeostasis and promotes the conversion of $O_2^{\bullet-}$ to H_2O_2 and O2 and increases the activity of H_2O_2 (Hossain et al., 2012). Recently, an increasing number of articles have reported the effects of exogenous NO on alleviating metal toxicity in plants. However, compared with the current understanding of the relationships between NO and other abiotic stresses, knowledge of the molecular and physiological mechanisms by which NO alleviates deleterious metal effects is still limited and discrepant results are found in the literature (Xiong et al., 2010). In chamomile plants subjected to 3, 60 and 120 μmol L^{-1} Cr^{3+}, for example, the intensity of NO activity was directly correlated to Cr concentrations, although the increases observed were not sufficient to counteract the oxidative damage caused by Cr exposure (Kováčik et al., 2013). In *Pterogyne nitens* Tul NO fluorescence emission from roots exposed to Cr^{+6} for two hours was more intense when compared with the control, and compared to plants exposed to Cr^{+6} for seven days. The authors postulate that the greater NO fluorescence after two hours of treatment with Cr^{+6} may be associated with the rapid synthesis of this compound, resulting in the activation of protective responses (Brito et al., 2014).

Another plant defense mechanism against Cr toxicity is the induction of metallothionein (MT) synthesis. These are low-molecular weight metal-binding proteins, or metalloproteins, involved in essential element homeostasis and detoxification of toxic metals and metals present in excess in the organism (Memon et al., 2002), although they have also been known to exhibit free radical scavenging ability (Wong et al., 2004) and play a role in the maintenance of the redox level, repair of plasma membrane, cell proliferation and repair of damaged DNA (Emamverdian et al., 2015; Macovei et al., 2010).

In plants, these metalloproteins are classified into four types: MT1 (subtypes a, b, c), whose gene expression is higher in roots than shoots, MT2 (subtypes a, b, c, d), in which gene expression occurs mostly in shoots, MT3 (subtypes a, b, c), that has a specific accumulation of their transcripts in fleshy fruits as they ripen, and MT4, whose gene expression is restricted to developing seeds (Teixeira et al., 2013). Evidence indicates that all four types of plant MTs and their isoforms are able to bind to metals and act as metal chelators, although recent data suggests that plant MTs show distinct treatment towards varying types of metals and that their functionality and metal-binding and metal-affinity characteristics, as well as tissue localization, might be different within a plant species or among species (Emamverdian et al., 2015). MT roles in plant metal homeostasis however, are still poorly understood.

In a recent study, *Solanum nigrum* L. plants were exposed to Cr^{3+} and Cr^{6+} for 4 weeks of low concentrations and 1 week of higher concentrations. In addition to several of the morphological alterations linked with metal toxicity and previously discussed, such as reduction of root and shoot growth and fresh, mass, increased free proline content in shoots from plants exposed to both Cr^{6+} treatments and to a prolonged 375 μmol L^{-1} Cr^{3+} exposure, as well as in roots from shock treatments to both metals, MT mRNA analyses demonstrated that Cr^{3+} induced the synthesis of MT2a-related transcripts in both roots and shoots, and of MT1- and MT2d-related transcripts only in roots, whereas Cr^{6+} induced the accumulation of MT2a- and MT2d-related transcripts only in roots during exposure to higher concentrations, and the accumulation of the MT2c-related transcripts only in shoots, suggesting that these MTs are related to the Cr homeostasis in *S. nigrum* (Teixeira et al., 2013).

In another study, the ameliorating effects of hydrogen peroxide (H_2O_2, 200 μmol L^{-1}) on Cr^{6+} toxicity in canola (*Brassica napus* L.) were investigated. Besides several morphological alterations and increases in antioxidant enzyme activities, the expression level of BnMP1 mRNA was increased after 1 day of treatment, and decreased at 7 days in Cr^{6+}-stressed seedlings. At 1 day of treatment, pretreatment with H_2O_2 before Cr^{6+} stress reduced the expression of BnMP1 mRNA when compared to Cr^{6+} stress alone, although non-significantly. At 7 days, H_2O_2 pretreatment alleviated Cr^{6+} stress-mediated decrease in the expression of BnMP1 mRNA. The authors postulate that these results indeed indicate that H_2O_2 may act as a signal that triggers defense mechanisms, as cited previously in the present paper, which in turn protects canola seedlings from Cr^{6+}-induced oxidative damage by inducing MT synthesis (Yildiz et al., 2013). MT3 gene expression has also been investigated in roots after exposure of 100 μmol L^{-1} Cr^{6+} for 5 days in 15-day-old seedlings of two sorghum cultivars, one susceptible and one tolerant to metals. The results demonstrated that the tolerant cultivar showed higher MT transcription rates under Cr stress, again indicating that the reactive oxygen species and H_2O_2 produced under Cr stress act as a signal to induce MT mRNA transcription for plant defense (Shanker et al., 2004).

5. Recent advances in investigations regarding Cr stress responses in plants

In spite of many previous studies having been conducted on the effects of chromium stress, the precise molecular mechanisms related to both the effects of chromium phytotoxicity, the defense reactions of plants against chromium exposure as well as translocation and accumulation in plants in general remain poorly understood (Dubey et al., 2010). With the advances in recent years in the 'omics fields, however, investigations in this regard can now be conducted with far more precision and analyzing a greater number of variables linked to physiological responses to Cr stress. In fact, "omics" fields have great potential to address the underlying mechanisms toxicological effects of chemical pollutants and, consequently, the identification of new biomarkers of effect (Dowling and Sheehan, 2006; López-Barea and Gómez-Ariza, 2006).

5.1. Genomic investigations

Recent studies applying high-throughput genomic technologies have allowed for novel insights regarding the mechanisms that allow plants to cope with chromium stress, since it is possible to analyze the expression of thousands of genes at a time (Dubey et al., 2010). For example, one study applied a microarray assay to analyze the transcriptomic profiles of rice roots in response to Cr(VI) stress. A total of 2688 Cr-responsive genes were involved in binding activity, metabolic process, biological regulation, cellular process and catalytic activity. Exposure time to Cr was shown to modify transcriptomic profiles, since more transcripts were responsive to Cr during long-term exposure (24 h, 2097 genes), than short-term exposure (1- and 3-h results pooled, 1181 genes). Long-term Cr exposure regulated genes were involved in cytokinin signaling, the ubiquitin-proteasome system pathway, DNA repair and Cu transportation. In addition, many kinases were upregulated with short-term Cr exposure. Expression of reactive oxygen species and calcium and activity of MAPKs (E.C. 2.7.11.25) and CDPK-like kinases (E.C. 2.7.1.123) were induced with increasing hexavalent concentration (Huang et al., 2014).

Another recent study conducted in tobacco plants by genomic methodologies investigated Cr-responsive microRNAs (miRNAs) and their targets in roots of Cr-treated (Cr) and Cr-free (control) for 2 contrasting tobacco genotypes one Cr-sensitive and one Cr-tolerant. Comparative genomic analyses of 41 conserved Cr-responsive miRNA families indicated 11 miRNA families up-regulated in the Cr-tolerant species but unaltered in the Cr-sensitive species, while 17 miRNA families were up-regulated only in the Cr-sensitive species under Cr stress. Only 1 family, miR6149, was down-regulated in this plant, but remained unchanged in the Cr-tolerant species. Of 29 novel miRNA families discovered, 14 expressed differently in the 2 genotypes under

Cr stress, providing valuable information on the function of miRNAs in Cr tolerance (Bukhari et al., 2015).

In yet another study, Cr altered the methylation level of rape genomic DNA, by MSAP and immunolabelling techniques, where hypermethylation levels correlated positively with the stress dosage of chromium (Yang et al., 2007), suggesting *de novo* synthesis of methylated cytosine. However, other studies have indicated the contrary, where Cr stress reduced cytosine methylation levels in clover and hemp by 20–40%, also being proportional to Cr concentrations (Aina et al., 2004). These dissimilarities suggest that singular methylation mechanisms for chromium resistance are present in different plant species (Peng and Zhang, 2009).

Thus, with genomic advances, it has been increasingly possible to provide new insights into understanding Cr toxicity and tolerance mechanisms in several plant species, furthering understanding in this regard.

5.2. Proteomic investigations

However, although genomic techniques have been very useful regarding the investigation of stress responses to Cr as described above, changes in gene expression are not always reflected at a protein level (Gygi et al., 1999), so other methodologies, such as proteomics, are more adequate for more in-depth analysis of the protein content of cells and tissues and show greater value in studying stress responses by identifying proteins that aid in detoxifying metals in plants (Hossain and Komatsu, 2012). Proteomic analyses give valuable information when comparing the variations that occur in the proteomes of organisms as a consequence of biological disturbances or external stimuli, resulting in the expression of different proteins or redistribution of specific proteins within cells (Martin et al., 2001, 2003; Tyers and Mann, 2003).

In this context, a recent study investigated the time-course of changes in the protein expression profile induced by short-term hexavalent Cr exposure (1, 6 and 24 h) in maize leaves. Of over 1200 protein spots detected by two-dimensional electrophoresis, 60 were differentially accumulated during Cr exposure, and 58 were identified by tandem mass spectrometry. The identified proteins were mainly involved in ROS detoxification and defense responses, photosynthesis and chloroplast organization, post-transcriptional processing of mRNA and rRNA, protein synthesis and folding, DNA damage response and cytoskeleton functions, and some novel proteins were revealed that may play important roles in the Cr stress response (Wang et al., 2013).

In another study, proteomic responses of rice seedlings to hexavalent chromium stress were conducted using two rice genotypes, differing in Cr tolerance and accumulation. The study demonstrated that the response of rice proteome to Cr stress is genotype- and Cr dosage-dependent and tissue specific. Sixty-four proteins were successfully identified, involved in several cellular processes, such as cell wall synthesis, energy production, primary metabolism, electron transport and detoxification (Zeng et al., 2014).

Yet another report investigated the molecular mechanisms that regulate the response of *Miscanthus sinensis* roots to elevated level of chromium. Protein profiles analyzed by two-dimensional gel electrophoresis revealed that 36 protein spots were differentially expressed of which, 13 were up-regulated, 21 down-regulated and 2 spots were newly induced. These proteins were then identified by MALDI-TOF and MALDI-TOF/TOF mass spectrometry, and included known heavy metal-inducible proteins such as carbohydrate and nitrogen metabolism, molecular chaperone proteins and novel chromium-responsive proteins such as inositol monophosphatase (E.C. 3.1.3.25), nitrate reductase (E.C. 1.7.99.4), adenine phosphoribosyl transferase (E.C. 2.4.2.7), formate dehydrogenase (E.C. 1.2.1.2.) and a putative dihydrolipoamide dehydrogenase (E.C. 1.8.1.4), suggesting that Cr toxicity is linked to heavy metal tolerance and senescence pathways, and associated with altered vacuole sequestration, nitrogen metabolism and lipid peroxidation (Sharmin et al., 2012).

In addition, investigations regarding synergic and antagonic effects of Cr and other elements in plants have also been conducted from a proteomic point of view. For example, a comparative proteomic approach investigated differences in protein abundance between Cr-tolerant and Cr-sensitive cultivars. Germinated seeds were grown hydroponically in S-sufficient (+S) nutrient solution for 7 days and then subjected to S-deficiency (−S) for 7 days. S-deficient and +S seedlings were then exposed to 100 μM Cr(VI) for 3 days. Protein patterns analyzed by two-dimensional electrophoresis indicated 58 differentially regulated protein spots, of which 39 were identified by MALDI-TOF/TOF mass spectrometry. The identified proteins showed functions in photosynthesis, energy metabolism, stress defense, protein folding and stabilization, signal transduction, redox regulation and sulfur metabolism, thus further aiding in the characterization of proteomic plant responses to Cr (Yildiz and Terzi, 2016).

However, as stated previously, plant proteomic modifications in response to Cr are still relatively unknown and studies are still scarce, indicating the potential for this field of knowledge in this regard.

5.3. Metallomic investigations

The very recently developed field of metallomics considers that biomolecules that bind to metals and metalloids constitute a substantial proportion of all molecules involved in cell metabolism and behavior, and identifying a metal cofactor of a protein can greatly assist its functional assignment and positioning in the context of known cellular pathways (Haraguchi, 2004). These metal-binding proteins, or metalloproteins, are increasingly being used successfully as environmental exposure biomarkers (López-Barea and Gómez-Ariza, 2006), although the number of discoveries and studies in metallomics is still much lower than in proteomics, due to several unique issues that must be considered when analyzing metalloproteins. These include the absence of any protein amplification reaction similar to PCR (polymerase chain reaction) in the area of genomics, the occurrence of post-translational changes in the biological entities, and, finally, low concentrations of trace-elements in biological tissues (generally lower than $1\ mg\ g^{-1}$) and the complexity of the matrices (Gomez-Ariza et al., 2004). These factors make the analysis of metals bound to biomolecules very difficult and challenging. However, the continued development of techniques combining atomic spectroscopy and biochemical or proteomic techniques such as gel electrophoresis, capillary chromatography or multi-dimensional nanoflows, as well as the development of strategies for the additional elemental applications and techniques in molecule detection, such as mass spectrometry approaches like inductively coupled plasma (ICP-MS) and electrospray (ESI-MS), have led to new possibilities in this field of research (Prange and Profrock, 2005).

A range of metalloproteins has been used as biomarkers of effect situations, including exposure to different metals, where metalloproteins differ not only in their relative abundances, but in which metals are bound to them, and also the amount of each metal (Garcia et al., 2006). Metalloproteins linked to oxidative stress, for example have been indicated as relevant biomarkers because their expression and abundance relative are modified in different situations, and, with metallomic approaches, can be further investigated not only regarding concentrations, but also speciation and concerning different isoforms (Arruda et al., 2011).

In this context, it has been stated that a large number of the more recent reports on metals do not provide any new information on metal-induced oxidative stress, for example (Arruda and Azevedo, 2009). Besides the precise metal/metalloid that is present in a plant, it also paramount to know in what form it is present (speciation), the biomolecules to which it is bound and the coordination groups involved (Arruda and Azevedo, 2009).

In this context, some studies have already been conducted, such as the determination of Cr in different oxidation states. One of these

studies applied X-ray absorption near-edge structure to pine and beech samples treated with Cr^{6+}, and observed that the samples still contained a measurable content of Cr in this oxidation states after four weeks conditioning, while experiments conducted with heat exposure Cr^{6+} was no longer detected, indicating complete reduction to Cr^{3+} (Strub et al., 2008). Another study analyzed the uptake of Cr^{6+} in free living floating aquatic macrophytes *Eicchornia crassipes* cultivated in non-toxic chromium-doped hydroponic solutions by PIXE, and demonstrated that Cr^{6+} mass uptake by the macrophytes reached up to 70% of the initial concentrations (Espinoza-Quiñonesa et al., 2009). Another successfully applied technique is anion exchange fast protein liquid chromatography (FPLC) with AAS detection for the simultaneous determination of Cr^{3+} complexes and Cr^{6+} in the sap of cabbage plants exposed to various concentrations of chromate and Cr^{3+}-EDTA (Milačič and Štupar, 1994), while an HPLC separation procedure has also been applied for the Cr speciation leaves of *Persea americana* sampled from Taiwan, indicated as a better alternative to the existing procedures for the routine monitoring of plants as it is simple, rapid and easy to adopt and gives further metallomic information on Cr species (Kuo et al., 2007).

However, as with proteomic studies, plant metallomic studies are still scarce, and much more remains to be explored in this context.

6. Conclusions

Cr contamination is, increasingly, posing a serious threat to the environment, emerging as a major health hazard to the biota. This requires further understanding on mechanisms of plant defense against this metal. The present review discussed the different detrimental effects of Cr exposure in plants, from both morphological and physiological points-of-view. Cr is capable of inducing several toxic effects on plants, including changes to the germinating process and the growth of the roots, stems and leaves, as well as harmful effects on morphological and physiological processes, such as photosynthesis, water relations and mineral nutrition. From a molecular perspective, Cr is also capable of inducing oxidative stress in plant cells, disrupting redox equilibrium. Several of the defense mechanisms employed by plants were discussed herein, and, although many of these mechanisms are still poorly understood, recent advances in molecular and cellular biology, such as genomics, proteomics and the recently developed field of metallomics, are increasingly shedding new light on the complex strategies plant employ to this end. However, studies in this regard are still scarce, since these fields of knowledge have only recently been applied to environmental issues. Some limitations, such as the need for advanced mass spectrometry equipment and its hiphenations in the case of proteomics and metallomics may still curb development in this area, but these are, increasingly, becoming cheaper and more available, with further research in this area just around the corner.

Conflict of interest statement

The authors declare no conflict of interests for the present study.

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