

Chapter 16

Molecular Mechanisms of Chromium Tolerance in Plants: A Key Role of Antioxidant Defense



Viktor Husak and Maria Bayliak

Abstract Accumulative contamination of soil with chromium-containing compounds generates many adverse effects in plants. The toxic effect is manifested in the retardation of plant development, lower green biomass, morphological defects, and poorer flower quality and crop yield. Stimulation of reactive oxygen species (ROS) synthesis followed by oxidative stress development is considered to underlie the harmful effects of both Cr^{6+} and Cr^{3+} . Since a high intensity oxidative stress may cause the inhibition of photosynthetic mechanisms and oxidative modifications of cellular molecules, plants up-regulate various antioxidant and non-antioxidant defense mechanisms to avoid oxidation, maintain low stable-state ROS levels and repair damages that have already occurred in response to Cr(IV) exposure. These mechanisms include: (i) the antioxidant enzymes that directly scavenge ROS; (ii) the non-enzymatic antioxidant low molecular mass molecules (vitamins, terpenoids, phenolics, etc.) which are able to scavenge ROS and repair oxidized molecules, and some of them (ascorbic acid, glutathione) are used as substrates by antioxidant enzymes, in particular in so-called ascorbate-glutathione pathway; (iii) thioredoxins and glutaredoxins to restore oxidized proteins; (iv) metal-binding proteins such as phytochelatins and metallothioneins which sequestrate toxic metals in the specific plant compartments (vacuoles). In this review, we analyze current data on the involvement of redox regulators, particularly Rap2.4a and NPR1, in the regulation of adaptive antioxidant response of plants under Cr exposure.

Keywords Chromium · ROS · Oxidative stress · Antioxidant defense · Plant

V. Husak (✉) · M. Bayliak (✉)
Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine
e-mail: viktor.husak@pnu.edu.ua

M. Bayliak
e-mail: maria.bayliak@pnu.edu.ua

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbic acid
CAT	Catalase
Grx	Glutaredoxin
HM	Heavy metal
HSP	Heat shock protein
MT	Metallothionein
NPR1	Non-expressor of the pathogenesis-related gene
PC	Phytochelatin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SA	Salicylic acid
TF	Transcription factor
TGA	TGACG sequence-specific binding protein
Trx	Thioredoxin

16.1 Introduction

Metals with variable valence, such as chromium, iron, copper, cobalt, nickel, etc., have a wide practical application in various branches of industry. At the same time, they are one of the most widespread pollutants of the environment, because they are intensively released with industrial emissions and domestic waste (Uddin 2017). Metal retention in soil poses a serious danger to soil organisms, including agricultural plants, reducing their quality and crop yield (Alengebawy et al. 2021; Saud et al. 2022; Wyszowska et al. 2023). Chromium(Cr) is a particularly dangerous metal pollutant for all organisms, from microorganisms to humans (Kubrak et al. 2010; Vasylykiv et al. 2010; Stambulska et al. 2018; Saud et al. 2022). Chromium can exist in eleven valence states, but the main valence states of Cr in the soil are Cr³⁺ and Cr⁶⁺. Hexavalent Cr compounds are widely used in industry. In particular, Cr⁶⁺ compounds are used in the metallurgy (e.g., for the production of different steels, metal materials with high resistance to physical and chemical factors) as well as in the pigment production, leather tanning, etc. (Pellerin and Booker 2000; Shahid et al. 2017). Hexavalent chromium emitted into the atmosphere is airborne for some time in the form of particles or dust. As sediments, it contaminates soil and water. Rain precipitates Cr⁶⁺ particles from the atmosphere, depositing them in the soil, or they can be spread by wind for long distances (Yu et al. 2014; Tumolo et al. 2020). Emissions from diesel vehicles are the main source of Cr air pollution, as Cr is a component of diesel fuel (Wang et al. 2003; Yu et al. 2014). It is generally accepted that Cr⁶⁺ is more toxic than Cr³⁺. However, many studies showed that both Cr⁶⁺ and Cr³⁺ can increase dramatically the amount of so-called reactive oxygen species

(ROS), triggering oxidative stress in different organisms with various harmful effects (Lushchak et al. 2009; Vasylykiv et al. 2010; Sawicka et al. 2021). Mutagenic and genotoxic effects of Cr^{6+} and Cr^{3+} ions are largely a result of ROS-induced damage to DNA (Figgitt et al. 2010; Novotnik et al. 2016; Sawicka et al. 2021).

Plants are able to absorb chromium from the soil and, as a result, excessive accumulation of chromium is detrimental for plants, causing defects in growth and development up to plant death. Chromium toxicity in plants is associated with its inhibitory effect on a number of metabolic processes, in particular photosynthesis, chlorophyll, and protein biosynthesis, as well as with DNA damage (López-Bucio et al. 2015; Stambulska and Bayliak 2020; Wakeel et al. 2020; Kumar et al. 2022; Saud et al. 2022; Wyszowska et al. 2023).

Unlike animals, which can leave the contaminated area, plants are immobile, so they have evolved powerful defense systems to withstand pollution. In response to chromium exposure, many plants up-regulate a battery of enzymatic and non-enzymatic defenses to sequester chromium, keeping redox balance, preventing oxidative damage and repair damaged biomolecules (Anjum et al. 2017; Stambulska et al. 2018; Stambulska and Bayliak 2020; Kumar et al. 2022). If the capacity of defense systems is not enough to overcome the increase in ROS levels and treat the damage, chromium-induced damage to biomolecules can be significantly enhanced, which is manifested in severe oxidative stress with the corresponding detrimental consequences for plants. It should be noted that chromium toxicity is reduced in the presence of associative or symbiotic microorganisms, e.g. nitrogen-fixing rhizobia, which are able to accumulate heavy and transition metals and thus reduce metal uptake by plants (Stambulska et al. 2018; Sharma et al. 2022).

This chapter summarizes the available information on the mechanisms of toxicity of Cr^{6+} ions in plants, focusing on their involvement in the generation of ROS and cellular and tissue oxidative injuries. Studies point out that plants with more powerful antioxidant systems are more resistant to soil chromium contamination. Therefore, we have also analyzed in detail the plant antioxidant compounds contributing to prevention and neutralization of Cr toxic effects. The main molecular players of plant responses to redox perturbations are also discussed.

16.2 Reactive Oxygen Species and Reactive Nitrogen Species as Components of Plant Immunity and Energy Metabolism

In living organisms, reactive oxygen (ROS) and nitrogen (RNS) species are products of incomplete reduction of oxygen and nitrogen and are generated as primary products or either byproducts of basic metabolism. In plants, ROS and RNS as side products of metabolism are generated in plastids, mitochondria, peroxisomes, apoplast, and cytosol (Gupta and Kaiser 2010; Luis and Río 2013; Del Rio and Lopez-Huertas 2016; Mittler 2017; Qi et al. 2017). Intracellular ROS/RNS homeostasis is rigorously

managed to sustain proper cellular functions by a wide range of non-enzymatic (vitamins (A, E, K) organic acids, glutathione, ionic metals (Fe^{2+} , Cu^{2+} , Zn^{2+} , and Mn^{2+}), phenolic and terpenoid compounds), and enzymatic scavengers (superoxide dismutase (SOD), catalase (CAT), enzymes engaged in the reduction-oxidation of non-enzymatic antioxidants (glutathione and ascorbic acid), amine oxidase, etc.) (Chaudiere and Ferrari-Iliou 1999, Apel and Hirt 2004; Choudhury et al. 2017; Mur et al. 2013; Cadet and Davies 2017; Samardzic and Rodgers 2017).

The main ROS formed by the plant cells are the typical for aerobic organisms and include hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\cdot), superoxide anions (O_2^-), and singlet oxygen ($^1\text{O}_2$). They can be signaling molecules in plants, controlling various physiological processes—differentiation, senescence and death of the cells, pathogen defense, acclimation, and abiotic stress survival (Foyer and Noctor 2005; Gechev et al. 2006; Di Meo et al. 2016; Dunnill et al. 2017).

In photosynthesizing chloroplasts of green organs, three main ROS-generated processes can be distinguished (Fig. 16.1). First one is ROS generation in photosystem II via overexcitation of chlorophyll (Fryer et al. 2003). The Mehler reaction in photosystem I, in terms of which O_2 accepts one electron converting to O_2^- , is a second process leading to ROS generation (Badger et al. 2000; Ort and Baker 2002). The third process is related to the Rubisco by the substantial production of H_2O_2 by glycolate oxidase (Douce and Neuburger 1999). Glycolytic acid also undergoes oxidation in peroxisomes, and this is accompanied by H_2O_2 release. Even under favorable conditions, a certain amount of oxygen formed in chloroplasts is metabolized into ROS (Asada 1999). One of the major producers for H_2O_2 are peroxisomes (Noctor et al. 2002). Peroxisomal membrane NADPH oxidase and peroxisomal matrix xanthine oxidase produce O_2^- radicals that are quickly reduced to H_2O_2 (Del Rio and Lopez-Huertas 2016). Enzymes, such as superoxide dismutase and different oxidases (sarcosine, flavin, sulfite copper amine ones) can produce H_2O_2 in peroxisomes (Gilroy et al. 2016; Hasanuzzaman et al. 2020a, b). While in animals, mitochondria are the main producer of ROS, in plants chloroplasts do this function. The rate of chloroplast ROS formation is 20 times higher than mitochondrial one (Foyer and Noctor 2003). At low levels, ROS are responsible for the coordinated functioning of cellular organelles, cellular homeostasis, and adaptability to rapidly changing external conditions (Gechev et al. 2006). Mitochondrial respiratory complexes I and III are the major sites for the emergence of ROS, superoxide, and H_2O_2 , but the factors that lead to the intensification of ROS production in mitochondria are mostly unknown (Rhoads et al. 2006). In apoplast, oxalate oxidase, amine oxidase, and peroxidases can produce ROS (You and Chan 2015; Mittler 2017; Qi et al. 2017).

In addition, the excessive production of ROS is an effective component of the plant immunity under pathogenic infections. In plant–pathogen interactions, the major site of elevated ROS levels is the apoplast (Qi et al. 2017). NADPH oxidase produces ROS (O_2^- and H_2O_2) and thus provides innate plant immunity. Also, the redox networks of cells (peroxiredoxins, thioredoxins, glutaredoxins) affect very strongly ROS levels. The enhanced ROS production reduces bacterial and fungal growth (Suzuki et al. 2011; Mittler et al. 2011). The increasing concentration of ROS can

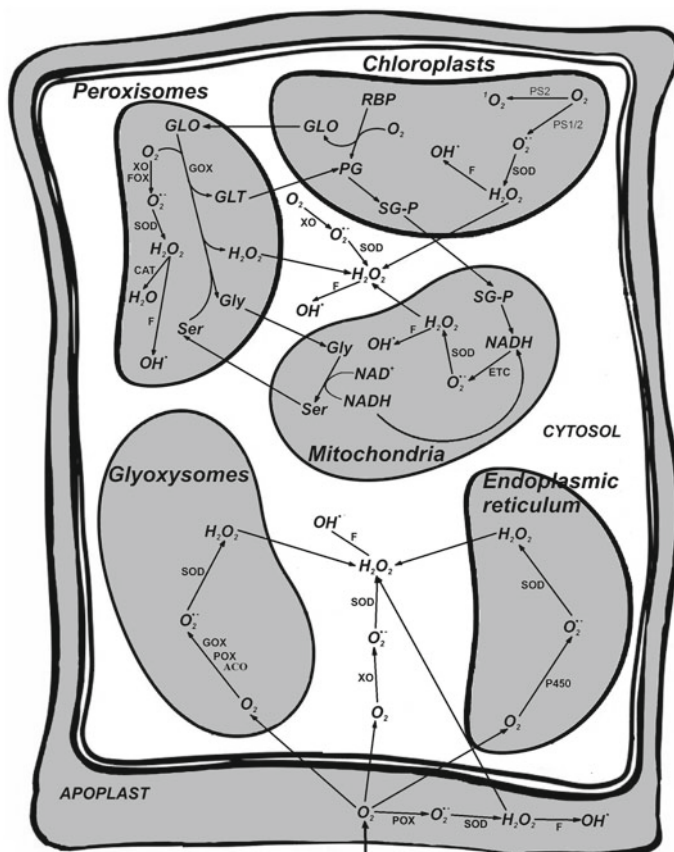


Fig. 16.1 Schematic overview of the ROS generating sites in the plant cell and their activity in various cellular components. The model is based on results described by various authors (Karuppanapandian et al. 2011; Hasanuzzaman et al. 2020a, b; Mandal et al. 2022). In chloroplasts, during photosynthesis, sunlight energy is captured and transferred to photosystem I (PS1) and photosystem II (PS2), where superoxide radical (O_2^-) can be formed. Then O_2^- is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Under excess light conditions, PS2 is able to generate singlet oxygen (1O_2). The glycolate (CLO), produced in chloroplasts, moves to peroxisomes, where it is oxidized by glycolate oxidase (GOX) with formation of H_2O_2 . In peroxisomes, H_2O_2 can also be formed from O_2 by xanthine and flavin oxidases (XO and FOX, respectively) coupled to SOD. In the mitochondrial electron transport chain, O_2^- production is likely to occur in complex I—the flavoprotein region of NADH dehydrogenase segment reduces O_2 into superoxide O_2^- . Acyl-CoA oxidase (ACO), GOX, peroxidases (POX), and SOD are the primary enzymes responsible for O_2^- and H_2O_2 generation in glyoxysomes. The endoplasmic reticulum-mediated ROS generation takes place by means of cytochrome P_{450} . Cell-wall-associated peroxidase and SOD are the main sources of O_2^- and H_2O_2 in apoplast of plant cells. In the presence of redox-active metals, the extremely reactive hydroxyl radical (OH) can be formed from H_2O_2 through the Fenton reaction (F). Other abbreviations used: CAT, catalase; GLO, glycolate; GLT, glycerate; Gly, glycine; PG, phosphoglycolate; RBP, ribulose 1,5-bisphosphate; Ser, serine; SG-P, sugar-phosphates

cause increase in synthesis of phytoalexins and other secondary metabolites which have defensive roles (Thoma et al. 2003). ROS may limit the spread of pathogens or play a signaling function (Mur et al. 2008).

Reactive nitrogen species ($\cdot\text{NO}$, ONOO^- , NO_2), like ROS, are signal molecules in plants. S-nitrosation and nitration are the main RNS-modifying mechanisms of plant cell behavior (Khurma et al. 2019; Corpas and Palma 2020; Corpas et al. 2021). S-Nitrosation is the reaction between $\cdot\text{NO}$ and cysteine, giving S–N=O bound. This mechanism is important for posttranslational modification of proteins and $\cdot\text{NO}$ signal transmission. As a result of alterations in the functional activity of proteins, changes also occur in the proteome of the cells (Corpas and Palma 2020). For example, S-nitrosoglutathione GS–N=O is obtained as a result of glutathione S-nitrosation. The irreversible transformation of GS–N=O into oxidized glutathione (GSSG) occurs with the participation of S-nitrosoglutathione reductase (GSNOR) (Corpas et al. 2021). Then glutathione reductase catalyzes NADPH-dependent reduction of GSSG to GSH (Fig. 16.2).

S-nitrosoglutathione reductase determines cellular concentrations of GSNO (Feechan et al. 2005), since this metabolite is a stable supply of $\cdot\text{NO}$ in plant cells. Nitric oxide performs immune functions, since cellular $\cdot\text{NO}$ concentrations enhance salicylic acid levels (SA, plant immune activator) followed by the increased expression of SA-dependent genes (Feechan et al. 2005; Loake and Grant 2007). Salicylic acid modulates the activity of proteins (SA binding protein 3, SABP3) that perform immune functions in plants (Slaymaker et al. 2002; Kumar and Klessig 2003). Therefore, $\cdot\text{NO}$ is a key regulator of SA-dependent immune responses against pathogens.

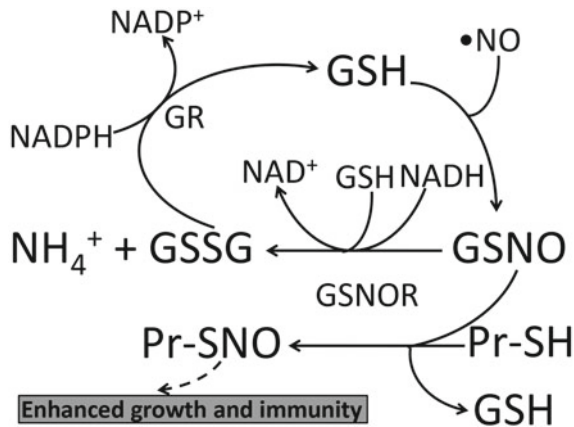


Fig. 16.2 S-nitrosylation of reduced form of glutathione (GSH) in plant cells. More details in the text. GSNO, S-nitrosoglutathione; GSNOR, S-nitrosoglutathione reductase; GSSG, oxidized form of glutathione; GR, glutathione reductase; Pr-SH, thiol-containing proteins; Pr-SNO, S-nitroso-containing proteins; NADH, nicotinamide adenine dinucleotide (reduced form); NAD⁺, nicotinamide adenine dinucleotide (oxidized form); NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NADP⁺, nicotinamide adenine dinucleotide phosphate (oxidized form)

Nitration is an addition of a nitro group (NO_2) to different biomolecules, especially proteins. The result of this type of protein nitration is a formation of nitrated amino acid residues (e.g., tyrosine, cysteine, tryptophan, methionine). These chemical modifications entail a loss of functional activity of the entire protein molecule (Corpas and Palma 2020). In addition, the interaction of metal ions with NO (metal nitrosylation) can occur in metalloproteins. Such an interaction can also lead to changes in protein metabolic activity (Corpas and Palma 2020). Reactive nitrogen species react also with fatty acids. Nitro-fatty acids act as messenger molecules, that modulate gene expression during developmental processes and stress events (Di Fino et al. 2021; Kolbert et al. 2019; Medrano-Macías et al. 2022).

16.3 Antioxidant System of Plants: Overview

As described above, ROS/RNS and other reactive species are continually being formed in plants as by-products of photophosphorylation and respiration. Reactive species are highly damaging molecules that rapidly oxidize protein and lipid molecules, nitrogen bases in DNA, exerting a strong damaging effect on individual organelles, cells, organs and, the plant organism as a whole. Therefore, cells have evolved sophisticated defense mechanisms to neutralize ROS/RNS. At the same time, complete neutralization of ROS/RNS does not occur, as they perform signaling functions within a single cell and at a distance. ROS/RNS regulate some cell functions and processes such as cell division, regeneration, organogenesis, cell death, immune responses, resistance to UV radiation, etc. Temporary increase of ROS/RNS levels is a component of plant immune defense against disease agents (viruses, microbes, fungi, parasites), mechanical injuries, and physical stressors (water deficit, temperature changes, etc.) (Hasanuzzaman et al. 2020a, b; Mandal et al. 2022). Thus, the cell strictly regulates the synthesis and degradation of ROS in accordance with its needs, so that the level of reactive forms allows for the effective performance of their regulatory functions and the amount of oxidative damage caused by them is minimized. Maintenance of low stationary ROS/RNS concentrations is achieved through the regulation of systems that produce reactive species, as well as systems that scavenge reactive species and restore /degrade oxidatively damaged biomolecules.

In plant cells, the system that protects against damaging effects of ROS/RNS is multilevel and includes: (1) antioxidant enzymes that are able directly to neutralize ROS/RNS—superoxide dismutases and various peroxidases; (2) ascorbate–glutathione cycle enzymes, destroying H_2O_2 and utilizing NADPH as a donor to reduce oxidized glutathione; (3) enzymes involved in NADPH generation (e.g., ferredoxin-NADP-reductase); (4) non-enzymatic low molecular mass antioxidants, such as redox-active vitamins, glutathione, phenolic compounds, terpenoids etc.; (5) enzymes that reduce peroxides and thiol-containing compounds (glutathione reductase, thioredoxin reductase, etc.); (6) enzymes involved in detoxification of xenobiotics (e.g., glutathione-S-transferase); (7) peptides/proteins capable of binding metals via SH-groups (phytochelatins and metallothioneins); (8) heat shock proteins

(HSPs) involved in formation of the native protein structure (folding) and restoring native structure of denatured proteins (Cobbett and Goldsbrough 2002; Michalak 2006; Anjum et al. 2017; Kapoor et al. 2019; Jedelská et al. 2020; Stambulska and Bayliak 2020; Fedenko et al. 2022). Since ROS and RNS are synthesized in different organelles and cytosol of the plant cell, a sophisticated network of defense mechanisms is present not only in the cytosol but also in the organelles of the plant cell, especially in mitochondria, chloroplasts, and peroxisomes (Geigenberger et al. 2017; Noctor et al. 2018; Martí et al. 2020). Also, the composition of the antioxidant system is slightly distinct in different parts of the plant (roots, flowers, fruits, leaves).

16.4 Low-Molecular Mass Antioxidants

16.4.1 Tocopherols

Tocopherols (collectively called vitamin E) are a lipophilic compounds that are synthesized in thylakoid membranes of plastids; therefore, they are found mostly in the green plant organs. There are four isomers of tocopherol (α , β , γ and δ) (Szewczyk et al. 2021). According to their antioxidant activity, the α isomer is the most active and δ one has the lowest antioxidant activity. The antioxidant activity is related to degree of methylation (Dumanović et al. 2021). Chromanol ring of tocopherols possesses the ability easily to donate electrons to reduce oxidized molecules that determines the antioxidative effects of this vitamin. Due to their structure, tocopherols and tocotrienols are incorporated into the phospholipid bilayer of cell membranes, where they perform both antioxidant and structural functions (Szewczyk et al. 2021). The membrane of chloroplasts contains predominantly α -tocopherol; therefore, green parts of the plant are rich in α -tocopherol. At the same time, seeds contain mainly γ -tocopherol (Dumanović et al. 2021). In chloroplasts, tocopherols protect membrane lipids against oxidation by trapping singlet oxygen $^1\text{O}_2$, thus ensuring the normal functioning of photosystem II. The $^1\text{O}_2$ scavenging activity of tocopherols is extremely effective: in vitro it was calculated that more than 100 molecules of $^1\text{O}_2$ can be neutralized by one molecule of α -tocopherol until the latter degrades (Gill and Tuteja 2010). Alpha-tocopherol also interrupts lipid peroxidation in plasma and intracellular membranes, reacting with the lipid radicals ($\text{LO}\cdot$, $\text{L}\cdot$ and $\text{LOO}\cdot$), which arise from the oxidation of polyunsaturated fatty acids at the membrane-water surface (Dumanović et al. 2021). By giving protons to a radical molecule, tocopherol is converting to a tocopheroxyl radical, which is not reactive enough to initiate membrane peroxidation by itself (Das and Roychoudhury 2014). In model plant *Arabidopsis thaliana*, inactivation of genes, encoding enzymes of tocopherol biosynthesis, led to a strong oxidative state and lowered content of photosynthetic pigments in green plant parts (Semchuk et al. 2009, 2011). In plants, alpha-tocopherol was also found to perform protective functions contributing to the

tolerance to salt stress (Semchuk et al. 2012), high/low temperature (Bergmüller et al. 2003), and xenobiotics (Semchuk et al. 2011).

16.4.2 Ascorbic Acid

Ascorbic acid, or ascorbate, is a common compound found in all cellular compartments. The concentration of ascorbate (AsA) is the highest in cytosol but is also substantial in chloroplasts (Smirnoff and Wheeler 2000). Under physiological conditions, AsA is implicated in photosynthesis serving a cofactor of enzymes (including those involved in the synthesis of phytohormones and phenolic compounds) and in the control of the cell growth. Some fruits (e.g. black currant, pepper) show a high concentration of ascorbic acid, which may be the result of its compartmentalization and stabilization in acidic vacuoles (Smirnoff and Wheeler 2000).

Glucose, synthesized in chloroplasts during photosynthesis, is a precursor of ascorbic acid. Metabolic conversion of glucose occurs mostly via formation of GDP-mannose first and then L-galactose, which is oxidized to L-galactono-1,4-lactone with conversion of the latter to AsA by specific dehydrogenase located in mitochondrial membrane (Siendones et al. 1999). Plant mitochondria are involved in both de novo synthesis of AsA and the regeneration of oxidized forms of AsA such as dehydroascorbic acid (Gill and Tuteja 2010). Ascorbate is considered to be the most powerful ROS scavenger due to its ability to easily give electrons and protons. It is able to directly react with O_2^- , H_2O_2 and $\cdot OH$, converting them to non-radical molecules, thus contributing to maintenance of membrane integrity. In addition, ascorbate regenerates α -tocopherol by reducing its oxidized form—tocopheroxyl radical. Moreover, ascorbate is a component of the ascorbate-glutathione cycle, described in more detail below. In reduced form, AsA maintains the activity of enzymes containing transition metal ions as prosthetic groups, preventing the oxidation of these metal ions (Gill and Tuteja 2010). Important function of ascorbate is serving as cofactor for several peroxidases and other enzymes like methionine sulfoxide reductases (Noctor et al. 2018). AsA-dependent enzymes are called ascorbate peroxidases (APXs). They are polygenic group of proteins. In particular, there was identified eight genes encoding APXs in *Arabidopsis* (Narendra et al. 2006) and 11 APX genes in poplar tree (Leng et al. 2021).

Plants with higher ascorbate content demonstrated the enhanced tolerance to stressors whose action leads to oxidative stress (Ding et al. 2009; Akram et al. 2017). Exposure to heavy metals was found to decrease ascorbate levels with increasing content of its oxidized forms (dehydroascorbic acid) (Gill and Tuteja 2010). In particular, Cd exposure decreased ascorbate levels in *Cucumis sativus* chloroplasts (Zhang et al. 2002), leaves of *A. thaliana* (Skórzyńska-Polit et al. 2003) and pea (Rodríguez-Serrano et al. 2006), roots and nodules of *Glycine max* (Balestrasse et al. 2008). Exposure to hexavalent chromium decreased ascorbate levels in tomato roots, while the addition of exogenous ascorbate improved tolerance of tomatoes to Cr (Al-Huqail et al. 2020). Decrease in ascorbate levels under Cr and Cd exposure was shown to

be connected with up-regulation of APXs which use AsA a substrate to counteract a toxicant-stimulated ROS production (Sinha et al. 2018).

16.4.3 Glutathione

Glutathione (GSH) is a tripeptide (L- γ -glutamyl-L-cysteinylglycine) found in all living organisms, including plants. It is synthesized by γ -glutamylcysteine synthetase and glutathione synthetase (Lushchak 2012). In plants, glutathione synthesis occurs in the chloroplasts and cytosol and then GSH can be recruited also in other organelles, including nucleus (Dumanović et al. 2021). In plant cells, 85–90% of GSH is cytosolic (Kosakivska et al. 2021). Glutathione has two forms in the cell, an reduced one (GSH) and an oxidized one formed by disulfide binding of two molecules of glutathione (GSSG) (Noctor et al. 2018). In the cell, glutathione is largely present as GSH, when GSSG consists of about 1% of the total content (Lushchak 2012). For the cell, it is important the GSH/GSSG ratio which determines whole redox status of the cell. In plants, excessive ROS production may cause oxidation of GSH and decrease in GSH/GSSG levels indicating more oxidative state of the cell (Queval et al. 2011).

Glutathione has a variety of functions in the cell; in particular, as we mentioned above, it is a key maintainer of redox cellular state in the range of values essential for adequate functioning of redox-sensitive signaling pathways controlling processes connected with growth and aging (seed development, formation of organs and fruits, senescence) (Noctor et al. 2018). This is evidenced by growth retardation and higher vulnerability to oxidant compounds in *A. thaliana* mutants, defective in glutathione synthesis (Cobbett and Goldsbrough 2002). As an antioxidant, GSH can directly react with ROS such as hydroxyl radical (HO^\cdot) or peroxynitrite (ONOO^-) (Lushchak 2012) Also, GSH is involved in eliminating peroxides (H_2O_2 , organic peroxides) with the help of appropriate enzymes and is involved in the regeneration of the oxidized form of AsA, which is formed during H_2O_2 reduction (Foyer and Halliwell 1976). In detail, ascorbate-glutathione cycle is described in the section below. Glutathione is also able to conjugate with molecules having free SH-groups with forming respective disulfides in a reaction called S-glutathionylation (Lushchak 2012). So, GSH reacts with cysteine-rich proteins. This process is reversible and affects the protein function. In particular, S-glutathionylation inhibited the activity of the chloroplastic glyceraldehyde-3-phosphate dehydrogenase, an enzyme of the Calvin-Benson cycle in plants (Zaffagnini et al. 2007). S-glutathionylation is accepted to be a cellular protective strategy to protect cysteine residues in proteins against irreversible oxidation (Lushchak 2012). GSH is a substrate of glutathione-S-transferases to conjugate and eliminate different xenobiotics (Lushchak 2012). Also, GSH can interact with certain metal ions, especially cadmium, copper, zinc, mercury, arsenic, and lead (Lushchak 2012; Kosakivska et al. 2021). Chelating Cu^{2+} ions by GSH prevented their participating in the Fenton and Haber-Weiss reactions. In addition, GSH seems to be involved in Cr^{6+} reduction to Cr^{3+} that is considered as a way to decrease chromium toxicity (Lushchak 2012). However, some studies proposed that CSH

participates in detoxification of heavy metals not directly but as a precursor molecule for phytochelatin (Cobbett and Goldsbrough 2002; Kosakivska et al. 2021).

Short term treatment of certain plants (maize, tomato and cauliflower) with Cr^{6+} increased GSH levels in the leaves and roots (di Toppi et al. 2002). Enhanced GSH levels were also reported in sorghum (*Sorghum bicolor* L.) treated with Cr^{6+} (Kumar et al. 2019). Some studies report that supplementation with exogenous glutathione improved plant growth and tolerance to Cr toxicity (Zeng et al. 2012; Sharma et al. 2020; Wen et al. 2022). The addition of exogenous GSH improved the growth of soybean and rice exposed to chromium and increased antioxidant capacity in the treated plants (Zeng et al. 2012; Wen et al. 2022). Treatment with exogenous glutathione resulted in chromium accumulation in the roots of plants rather than in the green part. This indicates that glutathione compartmentalizes chromium ions in plants, thus reducing chromium toxicity (Zeng et al. 2012; Wen et al. 2022).

16.4.4 Carotenoids

Carotenoids are isoprenoid polymeric compounds with diverse roles in plant metabolism, including those related to confer resistance to oxidative stress. The known carotenoids include lutein, β -carotene, zeaxanthin, lycopene, etc. (Pérez-Gálvez et al. 2020). As photosynthetic pigments, carotenoids absorb light at 400–500 nm and transfer it to chlorophyll (Havaux et al. 1998). Secondly, they protect the photosynthetic apparatus by quenching singlet oxygen $^1\text{O}_2$ and $\cdot\text{OH}$ formed during light stage of photosynthesis (antioxidant function) (Stahl and Sies 2003). Antioxidant function of carotenoids also include prevention of the formation of $^1\text{O}_2$ by reacting with excited chlorophyll (Chl^*) and breakdown of the chain reactions of lipid peroxidation (Das and Roychoudhury 2014). Thirdly, they as lipid-soluble molecules are components of the photosystem I and provide stability of the proteins of the photosynthetic complex and the thylakoid membrane as a whole (structural role) (Gill and Tuteja 2010). In *Capsicum annuum*, exposure to Cr-containing compounds was reported to result in increased carotenoid levels (Sharma et al. 2020). This suggests that carotenoids can contribute to Cr tolerance in plants.

16.4.5 Phenolic Compounds

Phenol compounds are ubiquitous in plant kingdom. Up to now, there are identified over eight thousand phenolic compounds in plants. Depending on the structure, phenolic compounds are divided into several classes: simple phenols, phenolic acids (gallic acid, caffeic acid, ferulic acid, etc.) stilbenes (e.g., resveratrol), lignans, coumarins, and flavonoids (including simple flavonoids (e.g., quercetin, luteolin, apigenin, catechins) and condensed tannins (Dai and Mumper 2010). Phenols are

secondary metabolites in plants and are synthesized from intermediates of carbohydrate metabolism. In the cell, phenols are mainly accumulated in vacuoles, as well as in apoplast. Phenolic compounds confer pigmentation of flowers and fruits, germination of pollen, defense against UV radiation and different pathogens (Das and Roychoudhury 2014).

Plant phenols are well-known as powerful antioxidants. Antioxidant properties of plant phenols include:

- (i) direct scavenging of free radicals (superoxide radical anion O_2^- , hydroxyl radical $\cdot OH$, peroxyxynitrite $ONOO^-$);
- (ii) inhibition of lipid peroxidation through interaction with peroxide radicals ($RO\cdot$, $ROO\cdot$) and reduction of hydroperoxides ($ROOH$);
- (iii) quenching of excited molecules (e.g., singlet oxygen 1O_2);
- (iv) chelation of transition metal ions through aromatic hydroxyl groups (prevention of the Fenton reaction);
- (v) reduction of oxidized low molecular weight antioxidants (e.g., tocopheroxyl radical);
- (vi) direct binding followed modulation of pro-/antioxidant enzymes, as well as the impact on cellular signaling pathways at biochemical and molecular level (Rice-Evans et al. 1997; Michalak 2006; Dai and Mumper 2010; Bayliak et al. 2016b; Fedenko et al. 2022).

Similarly, to other non-enzymatic antioxidants (e.g., ascorbic acid and carotenoids), plant phenols may also demonstrate pro-oxidant properties, which depend on many external factors—pH, oxygen availability and presence of transition metals (Dai and Mumper 2010; Bayliak et al. 2016a, b). Exposure to heavy metals, including Cr^{6+} , leads to increased levels of phenolic compounds in the treated plants, suggesting a defensive role of phenolics against Cr toxicity (Michalak 2006; Stambulska and Bayliak 2020; Alsherif et al. 2022).

16.5 Protein Non-enzymatic Antioxidants

16.5.1 Metal-Binding Proteins

In animals and fungi, heavy metals (HM) induce the increase in content of metal-binding proteins so-called metallothioneins (Zhou and Goldsbrough 1994). Metallothioneins are small Cys-rich proteins that binding HM make them metabolically inactive, conferring heavy metal tolerance. Like animals and fungi, plants possess several metal-binding proteins, which function to sequester metals with variable valence (Cobbett and Goldsbrough 2002; Pal and Rai 2010). Among the plant metal-binding proteins, the phytochelatin (PCs) and metallothioneins (MTs) are the best described (Cobbett and Goldsbrough 2002). PCs are enzymatically synthesized peptides, whereas MTs are proteins. PCs are a polymer based on glutathione

monomers. The main role of PCs is to detoxify xenobiotic metals (Ag, Cd, Hg) and maintain essential metal homeostasis (Cu, Zn, Se, Ni) (Cobbett and Goldsbrough 2002; Kosakivska et al. 2021). It should be noted that overexpression of phytochelatin synthase gene confers contrast tolerance to HM in transgenic *A. thaliana*, in particular, both enhanced cadmium (Cd) tolerance and Cd hypersensitivity was observed dependently on experimental conditions (Wang et al. 2012). Also, phytochelatin synthase had contrasting effects on cadmium and arsenic accumulation in rice grains dependently on experimental conditions (Uraguchi et al. 2017).

Plant MTs are low molecular mass proteins, rich in cysteine residues and are capable of binding mono- or divalent metal ions, in particular Cu ions (Domènech et al. 2006). Moreover, a correlation between MT gene expression and copper tolerance has been observed in *A. thaliana* (Murphy and Taiz 1995). Plants contain several types of MT which are distinguished by their structure and subcellular localization (MT1-MT4) (Kosakivska et al. 2021). In sorghum, Cr stress was shown to induce MT transcription but did not induce PC synthesis, suggesting a role of MT in Cr detoxification (Panda and Choudhury 2005). On contrary, another study showed that the PC content was significantly up-regulated in green parts of plants exposed to Cr⁶⁺. Also, plants with higher PC content accumulated chromium predominantly in roots and cell walls, that was proposed to be a mechanism improving plant tolerance to Cr (Saud et al. 2022).

16.5.2 Heat Shock Proteins

Heat shock proteins (HSPs), termed also chaperons, are ubiquitous in plant and animal cells. They were originally identified as proteins whose levels increase in the response to heat stress (Vierling 1991; Park and Seo 2015). To date, it has been established that the functions of HSPs are much broader. The integrity of cells is maintained by heat shock proteins which participate in correct formatting of native structure of de novo synthesized proteins, restoration of structure of misfolded or denatured proteins, preventing aggregation of damaged proteins and directing to degradation of the proteins which cannot be repaired under different stresses (Vierling 1991; Park and Seo 2015; Waters and Vierling 2020). Increased HSP90 was found in rice seedlings which were exposed to Cr⁶⁺ (Zeng et al. 2014). It was shown that copper, cadmium and chromium induced the synthesis of HSPs in *A. thaliana* (Cui et al. 2019; Appiah et al. 2021).

16.6 Enzymatic Antioxidant Defense

16.6.1 Thioredoxins and Glutaredoxins

Thioredoxins (Trx) and glutaredoxins (Grx) belong to families of small oxidoreductases, containing SH groups in the active site, that enable the reduction of disulfide intramolecular bridges in a specific set of proteins (Meyer et al. 2012). Thus, like glutathione, these proteins help to maintaining the more reduced state of the cell. Proteomic approaches identified the putative Trx and Grx target proteins, which are components of many physiological processes and stress adaptive responses in plants (Meyer et al. 2012; Martí et al. 2020). Transgenic plants defective in Trx and Grx allow to identify that these redox proteins are components combating with pathogen and are induced by pathways mediated by phytohormones (Meyer et al. 2008, 2012).

In plants, the thioredoxin and glutaredoxin families include a large number of isoforms. Thus, at least 20 genes encoding thioredoxins and about 50 genes encoding glutaredoxins are identified in *A. thaliana* (Gelhay et al. 2005; Meyer et al. 2008; Martí et al. 2020). Thioredoxins and glutaredoxins are classified depending on their primary structure and subcellular localization. By reducing intramolecular disulfide bonds in target proteins, Trx and Grx are oxidized themselves, forming intramolecular disulfides. Plant cells use different ways to reduce disulfide bounds to free thiol forms in the oxidized Trx/Grx. In chloroplasts, oxidized Trx can be reduced by a ferredoxin-thioredoxin reductase, whereas NADPH-dependent thioredoxin reductases catalyze reduction of Trx in mitochondria, nucleus, and cytoplasm (Smiri et al. 2010; Jedelská et al. 2020). Oxidized Grx can be reduced by glutathione (Meyer et al. 2008). Besides that, Grx is able to reduce oxidized Trx (Gelhay et al. 2005). Diversity of metabolic pathways of regeneration of oxidized thioredoxins identified emphasizes the important role of these proteins in protection against stressors, especially oxidants (Gelhay et al. 2005; Jedelská et al. 2020). Knock-out mutants in specific chloroplastic Trx showed defects in chloroplast structure and were more susceptible to stressors associated with increased ROS generation (Noctor et al. 2018). Heavy metals were found to affect thioredoxin reductase/thioreoxin system (Smiri et al. 2010, 2013). Moreover, it was shown that Trx possesses metal-chelating property, in particular regarding Cd²⁺ (Smiri and El Ghouli 2012).

16.6.2 Antioxidant Enzymes

Enzymatic antioxidant defense system includes of several antioxidant enzymes such as superoxide dismutases, catalases, and peroxidases (glutathione-ascorbate-dependent, GPx and APX, and non-specific for substrates ones, POX); enzymes of GSH and AsA redox metabolism—glutathione reductase (GR), glutathione S-transferase (GST), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR).

Superoxide dismutase (SOD) is one of the most effective enzymes for protecting plant cells from $O_2^{\cdot-}$ toxicity. Three main types of this metalloenzyme are described in plants—Cu, Zn-SOD, Mn-SOD, and Fe-SOD ones. The SOD isoforms differ in protein structure and subcellular distribution. Fe-SOD is located in plastids, Mn-SOD is in mitochondria, apoplast, and peroxisomes, and Cu, Zn-SOD occurs in peroxisomes, cytosol, and plastids (Pilon et al. 2011; Miller 2012). By catalyzing of $O_2^{\cdot-}$ dismutation to H_2O_2 , SODs reduce the probability of oxidizing effects of the superoxide radical and the likelihood of its interaction with transition metal ions with the appearance of $\cdot OH$ (Gill and Tuteja 2010).

Catalase is the heme-containing oligomeric enzyme, which catalyzes disproportionation of the product of the SOD reaction H_2O_2 to water and oxygen. In this reaction, one molecule of H_2O_2 is a donor of electrons and the second is the electron acceptor. Catalase (CAT) activity has been detected in mitochondria, peroxisomes, and cytosol of different plants (Sofo et al. 2015). Catalase isozymes exhibit developmental stages and tissue specificity in plants. The one CAT molecule can convert 6 million H_2O_2 molecules per minute (Mehla et al. 2017). This is the maximum rate of substrate transformation into a product among antioxidant enzymes.

Hydrogen peroxide can be also scavenged by different peroxidases, which use H_2O_2 for reduction of different substrates. Phenol peroxidase (POX) can generate phenoxyl radical ($PhO\cdot$) by oxidizing phenols with concomitant H_2O_2 reduction to water, and two molecules of H_2O are formed in this reaction. The polyphenol oxidase (PPO) found in the chloroplast thylakoid membrane can then interact with peroxidase, which helps to scavenge ROS. In PPO-catalyzed reaction, reduced phenols are oxidized to the quinone Q_A and H_2O (Boeckx et al. 2015).

Glutathione peroxidase (GPX) is the non-heme peroxidase family and protects cells from oxidative damage by reducing of the lipid hydroperoxides (LOOH) and H_2O_2 into non-toxic products or H_2O (Bela et al. 2015). GPX has been found in many cellular compartments (Koua et al. 2009). Currently, the functions of plant GPXs, which contain Cys residues in their active sites, are not completely understood. In stress conditions, GPXs can maintain H_2O_2 homeostasis, modify nuclear signaling proteins, and contribute to crosstalk between different signaling pathways (immune responses to pathogens, H_2O_2 -mediated and the abscisic acid-mediated signaling). The plant GPXs also contribute to hormone-stimulated growth of main and lateral roots, plant regeneration, flower- and seed development, development of leaves (Bela et al. 2015).

Glutathione reductase (GR) is a protein that reduces of GSSG to the sulphhydryl form GSH (Hasanuzzaman et al. 2017). In higher plants, three types of GR are predominantly localized in chloroplasts (about 80% of total CR activity), but its small amount is also found in peroxisomes, mitochondria, and cytosol (Edwards et al. 1990). In plants, maintaining a high ratio GSH/GSSG by chloroplastic GR is necessary important for normal oxygenic photosynthesis (Korniyev et al. 2003). Different GR types are activated by specific abiotic signals that ensure the specificity of the plant responses to stressors (Stevens et al. 1997).

Glutathione S-transferases (GST) are group of detoxification enzymes regulating different functions in plant cells such as the elimination of toxic substances, mitigation of oxidative stress, tyrosine degradation and hormone transport (Oakley 2011; Dong et al. 2016). Some GSTs can also scavenge toxic lipid hydroperoxides. GSTs catalyze conjugating of glutathione with metals/metalloids to reduce their toxicity (Dixon et al. 2011). In plant development, glutathione S-transferases also have functions, comprising formation of ion channels, modulation of regulatory kinases, and the glutathionylation of proteins (Dixon et al. 2010). GSTs are found in mitochondria, cytosol, and microsomes. In contrast to mitochondrial and cytosolic forms of GSTs, microsomal GSTs are identified as the integral membrane proteins that participate in eicosanoid and glutathione metabolism. Expression of GSTs was shown can be induced by fungal elicitors and pathogen attack, cold, drought, hormone treatments, phosphate starvation, heavy metals, salt, and wounding (Kim et al. 2011; Chen et al. 2013; Xu et al. 2015; Shukla et al. 2015; Jia et al. 2016).

Ascorbate peroxidase (APX) is a heme-containing peroxidase which scavenges H_2O_2 in the presence of ascorbate (Hasanuzzaman et al. 2019). APX has several isoforms with different cellular localization—cytosolic (cAPX), mitochondrial (mitAPX), chloroplastic (chAPX), and peroxisomal/glyoxisomal (mAPX) ones (Del Rio and Lopez-Huertas 2016). All APX isoforms participate in detoxifying H_2O_2 with oxidation of AsA to monodehydroascorbate (MDHA) and then to dehydroascorbate (DHA) (Fig. 16.3).

Monodehydroascorbate reductase (MDHAR) is a NADH/NADPH-dependent FAD-containing enzyme (Fig. 16.3). MDHAR is involved in AsA regeneration from

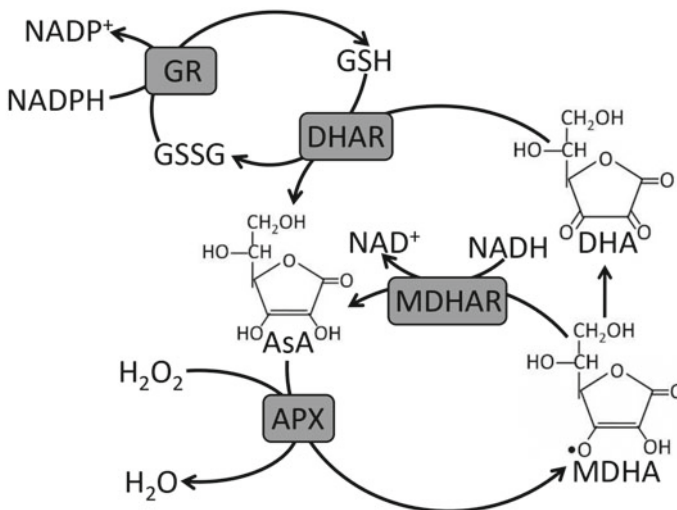


Fig. 16.3 The glutathione-ascorbate cycle in the chloroplasts (Foyer-Halliwell-Asada pathway). APX, ascorbate peroxidase; AsA, ascorbate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; GR, glutathione reductase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase

MDHA and the phenoxyl radical reduction (Hasanuzzaman et al. 2019). Isoforms of this enzyme are in different plant cell components like chloroplasts, mitochondria, peroxisomes, glyoxysomes, and cytosol (Leterrier and Cagnac 2018).

Dehydroascorbate reductase (DHAR) can regenerate AsA by the oxidation of GSH to GSSG (Das et al. 2015). This reaction proceeds by a ping-pong mechanism, in which dehydroascorbate connects to the free reduced form of DHAR with the binding of GSH (Fig. 16.3).

DHAR can also be involved in the regulation of normal plant growth and development (Ding et al. 2020). Increased DHAR activity may be one of the mechanisms that regulate the regeneration of AsA from DHA under stress conditions accompanied by increased ROS formation (Locato et al. 2009). Therefore, increasing AsA content may restrict the detrimental effects of ROS accumulation (Wang et al. 2010).

16.7 Toxicity of Heavy Metals in Plants: Overview

Industrial waste, fertilizers, smelting, and wastewater discharges cause leaching of heavy metals (HMs) such as copper (Cu), manganese (Mn), lead (Pb), nickel (Ni), cadmium (Cd), cobalt (Co), iron (Fe), chromium (Cr), zinc (Zn), silver (Ag), arsenic (As), and platinum (Pt) into soil or groundwater (Gupta and Ali 2002; Dağhan and Ozturk 2015; Basheer 2018a). Plants need for their growth and development in small amounts some HMs (Co, Cu, Fe, Mn, Mo, Ni, V, and Zn). However, excessive amounts of these elements can become harmful to them. For example, Zn and Mn in excessive amounts impair the growth of plants and compete with Fe. The Fe^{2+} excess in cells participates in the Fenton reaction by forming $\cdot\text{OH}$ (Shanmugam et al. 2011). HMs chelate by low molecular mass compounds (GSH, AsA, cysteine or others) and sequester in plant vacuoles. Other HMs (Pb, Cd, Hg, and Cr) are toxic for plants because of intensive protein denaturation and DNA damage. Most HMs are immobile, although some of them can be taken up by root system via endocytosis, diffusion, or through metal transporters (Krzyszowska 2011; Ali et al. 2015a, 2017; Basheer 2018b). The plant species accumulate HMs at different rates. Heavy metals impair virtually all molecular and biochemical processes in plants causing growth defects, root browning, chlorosis, and death (Gupta and Ali 2002; Dağhan and Ozturk 2015).

Heavy metals can bind to proteins, leading to inactivation or denaturation of the latter. The binding of HMs to membrane proteins disrupts the integrity of membranes, which is mainly reflected in the disruption of the transport of various substances. Ultimately, this leads to impaired photosynthesis and ion homeostasis (Hossain et al. 2012). The result of HMs stress is the overproduction of ROS including $\cdot\text{OH}$, O_2^- , OONO^- , $^1\text{O}_2$, $\cdot\text{NO}$, H_2O_2 , hypochlorous acid (HOCl), and then resulting in oxidative stress (Rascio and Navari-Izzo 2011). This process is considered one of the primary mechanisms of HM toxicity in plants (Lushchak 2011; Sharma et al. 2019). Cu, Cd, Ni, Cr, and As ions exceeding permissible limits in plant cells lead to increased ROS amounts (Husak 2015; Shahzad et al. 2018). Depending on the bioactivity, HMs can

be grouped into redox-active (Cr, Cu, Fe) and non-redox-active (Cd, Hg, Ni, Zn). Redox active metals may be oxidized in Haber-Weiss or Fenton reactions yielding highly dangerous free radicals having the capacity to produce oxidative injuries in plant cells. This results in excessive ROS generation and then disturbs the balance between prooxidant and antioxidant levels. Regarding redox-inactive metals, they form covalent bonds with sulfhydryl groups of protein.

All plant responses of HMs toxicity can be broadly classified as those that ensure the acquisition of tolerance to HMs or those reducing the uptake of metals (Krzesłowska 2011). The detoxification of HMs can occur by the chelation of metals and their transportation in the plant vacuoles. Under these conditions, the plant activates the synthesis of some stress-related proteins to prevent and cope with adverse effects of HMs (Ghori et al. 2019).

One of the most important pathways involved in plant abiotic stress is that mediated by the mitogen-activated protein kinases (MAPK) (Jonak et al. 2002). These phosphorylating cascades are also stimulated by hormones and control pathways of cell proliferation/differentiation, and expression of various stress-related genes. In plant cells, the MAPKs are diverse, and can interplay to synergetic action or inhibit the action of each other.

In the next sections, we discuss the toxic effects caused by chromium compounds and analyze the plants responses to the Cr stress.

16.8 Oxidative Stress as a Mechanism of Cr Toxicity

Chromium is used in different industries like electroplating, metallurgy, production of paints, pigments and paper, tanning, and manufacturing different chemical compounds (Ghani 2011). This large-scale application of Cr is a major cause of contamination of soil and water by its highly toxic valence state Cr^{6+} in the forms of chromate (CrO_4^{2-}), dichromate ($\text{Cr}_2\text{O}_7^{2-}$) and oxide (CrO_3). Toxic forms of Cr can generate H_2O_2 , O_2^- , $\cdot\text{OH}$, cause plant growth retardation, chlorosis, and roots destruction (Shanker et al. 2003a; Ozturk et al. 2015). The negative effects of Cr ions on plant photosynthetic systems, mineral and water metabolism have also shown (Stambulska et al. 2018).

In plant species, all toxic effects of Cr can be divided into four types. The first, Cr damages mitochondria and affects the synthesis of essential photosynthetic pigments, especially chlorophylls (Boonyapookana et al. 2002). The second mechanism is the increasing production of GSH and AsA or the increase in antioxidant enzyme activities as a direct response to Cr-induced ROS production to avoid significant oxidation of cellular components (Shanker et al. 2003a). Chromium ions can interact with iron-porphyrin in the active center of CAT, inactivating the enzyme (Sharma et al. 2003). A decrease in catalase activity may be one of the ways facilitating ROS increase in Cr-exposed plants and aggravating damaging effects of Cr. Cr can also inhibit other antioxidant enzymes such as GPX, GR, POX, and APX (Adrees et al. 2015; Ali et al. 2015b). The third, Cr induces the production of phytochelatin and

histidine as secondary metabolites. These metabolites contribute toward Cr stress tolerance (Schmfger 2001). The fourth mechanism is the DNA damage (Shanker et al. 2003b). Most heavy metals interact directly with DNA, but Cr realizes its genotoxicity via intensive ROS production (Fang et al. 2014). Cr⁶⁺ can produce inter-DNA strand cross-links and nucleotide strand breaks (Fang et al. 2014; Meng et al. 2017). Chromium-DNA adducts are considered to be the primary cause of Cr⁶⁺-induced mutagenicity (Fang et al. 2014).

Chromium toxicity involves the damage of cell membranes via the induction of lipid peroxidation, as well as the degradation of genetic material, and inactivation of enzymes, which results in the activation of programmed cell death (Zhang et al. 2016; Wakeel et al. 2019). An increase in malondialdehyde content was observed with a sharp increase in ROS upon chromium treatment (Ali et al. 2015a; Adrees et al. 2015). Increased malondialdehyde level is a marker of lipid oxidation; therefore, its accumulation leads to the deterioration of membrane permeability (Rahman et al. 2010).

High Cr concentration disturbs the photosynthetic process. Cr-induced chloroplast ultrastructural changes, alterations in auto-fluorescence of chloroplast, or binding of Cr to cytochrome groups were established (Shahid et al. 2017; Balasaraswathi et al. 2017). As a result, it disturbs CO₂ fixation and electron transport in photosystems I and 2, with inhibiting of respective enzymes (Shahid et al. 2017; Wakeel et al. 2020). Furthermore, high Cr levels mediate elevated ROS synthesis in chloroplasts, being involved in the suppression of photosynthesis (Rodriguez et al. 2011; Wakeel et al. 2020). Delta-aminolaevulinic acid dehydratase can be inhibited by the Cr accumulation in chloroplasts, which leads to a decrease in the content of chlorophyll (Yildiz et al. 2012; Paul 2016).

Chromium also affects the activities of many enzymes, since Cr is able to interact with their catalytic site, thus leading to the alteration in enzymatic activity (Yadav 2010). In plant roots, Cr inhibits such enzymes as nitrate reductase (Stambulska et al. 2018) and Fe-reductase (Barton et al. 2000). In the mitochondrial respiratory chain, Cr⁶⁺ can replace Cu and Fe ions in prosthetic groups of metal-containing proteins such as cytochrome oxidase (DalCorso 2012; Singh et al. 2013). The binding of Cr to complex IV may be the reason of the inhibition of cytochrome oxidase activity. It seems that Cr binds to cytochrome a3 (Dixit et al. 2002). Studies suggest that the plasma membrane NADPH oxidase (NOX) can produce ROS in association with Cr (Potocký et al. 2012; Weyemi and Dupuy 2012). Under these conditions, NOX, consuming cytosolic NADPH, produces O₂⁻, which is quickly converted to H₂O₂ (Pourrut et al. 2008).

Chromium detoxification in plants occurs by certain mechanisms (Zayed and Terry 2003). The first is a reduction of Cr⁶⁺–Cr³⁺ due to the formation of intermediate forms, Cr⁵⁺ and Cr⁴⁺ (Fig. 16.4). ROS can produce during this reduction and Fenton reaction. In the Fenton reaction, the catalytic power of Cr³⁺ is greater than iron, copper, or other metal ions (Shahid et al. 2017).

Cr⁴⁺ and Cr⁵⁺ ions which occur during radical oxidation of Cr³⁺ are catalytically active and can generate the hydroxyl radical (Fig. 16.4). However, the Cr involvement in this reaction is not well studied (Strile et al. 2003). Next mechanism is the Cr

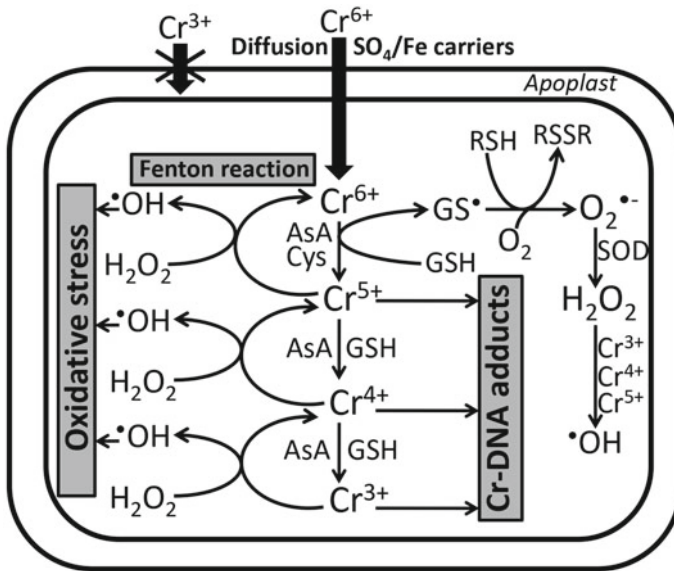


Fig. 16.4 Reactions of reduction and oxidation of chromium ions in plant cells. Cr^{6+} can actively enter the cells through channels for the transfer of the isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-} channels. In the cell, glutathione (GSH), ascorbate (AsA) or cysteine (Cys) reduce Cr^{6+} to Cr^{5+} . The glutathione-derived thiyl radical (GS^\bullet) can further react with other thiol molecules (RSH) in oxygenated tissues to give the superoxide radical ($\text{O}_2^{\bullet-}$). Cr^{5+} can also be reduced by GSH or AsA to Cr^{4+} and then to Cr^{3+} . Once formed, Cr^{5+} , Cr^{4+} or Cr^{3+} species alter the DNA conformation followed by formation of Cr-DNA adducts. However, Cr^{3+} -DNA adducts play the dominant role in the chromium mutagenicity

immobilization in the cell wall and vacuoles (Han et al. 2004). Furthermore, Cr^{3+} can form highly stable complexes with glutathione, carbohydrates, NADH, FADH_2 , and these complexes are stored in cell vacuole of plants (Lushchak 2012; Babula et al. 2008). Phytochelatins (PCs) and metallothioneins (MTs) are a very important for chromium detoxification in plants (Cobbett 2000; Cobbett and Goldsbrough 2002). However, their role in chromium detoxification in plants is not fully studied. Root exudates containing organic acids like citric, malic, aspartic, oxalic or carboxylic acids can form complexes with inorganic Cr, making them available for plant uptake (James and Bartlett 1983; Srivastava et al. 1999). However, organic acids in Cr detoxification in plants are studied not enough.

16.9 Redox-Sensitive Mechanisms of Plant Adaptive Response to Cr

As mentioned above, Cr toxicity is largely due to its ability to increase ROS production and facilitate non-enzymatic oxidation of cellular components. In response to the Cr-induced increase in ROS levels, plants up-regulate a protective battery of enzymatic and non-enzymatic molecules that help to combat with redox imbalance, repair existing oxidative damage and prevent new ones under stress. It was demonstrated that high antioxidant capacity predicts high HM tolerance (Alsherif et al. 2022). Studies indicate that in plants chromium treatment induces many antioxidant and related enzymes, non-enzymatic small antioxidants (glutathione, ascorbic acid, etc.), metal-binding enzymes, and repair components (Smiri et al. 2010; Stambulska et al. 2018; Saud et al. 2022). These biochemical markers were proposed to be indicators of resistance degree of plants to complex HM contamination (Alsherif et al. 2022). Molecular mechanisms of the up-regulation of defense mechanisms under Cr stress are studied scarce, but it seems ROS are key participants in these signaling pathways.

Data suggest that molecular response to Cr is regulated at different levels—transcription of certain required genes, translation, and posttranslational modification. Posttranslational modifications ensure an immediately (“fast”) response of the cell to the stimulus to stop initiation of adverse effects. These modifications include covalent changes in protein structure (e.g., phosphorylation-dephosphorylation, oxidation-reduction, association-dissociation of subunits) that affect protein function. In parallel, transcriptional (“slow”) responses are triggered via multicomponent signaling cascades. ROS/RNS are proposed to be mediator of both “fast” and “slow” stress responses. Moreover, overlapping of two systems—“fast” and “slow” allows better adaptation of plants to stress challenges. Fast short-term signaling mechanisms are utilize the present set of proteins for defense, while a “slow” response includes the activation of expression of genes encoding defensive proteins (Lushchak 2011).

Various stresses, including heavy metals, stimulate processes that increase in different ROS levels. This increase is detected directly by redox-sensitive transcription factors (TFs) and specific sensors or act as mediators in a number of signaling pathways leading to global metabolic reprogramming (Foyer and Noctor 2013; He et al. 2018; Moore et al. 2016). Plant hormones, such as salicylic acid (SA) act through ROS-related mechanisms (Lushchak 2011; Foyer and Noctor 2013). Changes that will take place in the cell and the whole plant are determined by the type of ROS formed and the site of their subcellular generation (Foyer and Noctor 2013; Gadjev et al. 2006; Willems et al. 2016). Furthermore, elevated ROS specifically change the set of genes that are transcribed in a time-dependent manner. According to the conducted transcriptomic meta-analysis, the time of exposure to increased ROS might be a determinant in the pattern of gene expression. In a later period, the initial specific stress may cause more general changes in gene expression (Willems et al. 2016).

The direct effects of ROS, e.g. H_2O_2 , on target proteins, involve the oxidation of sulfur-containing amino acids—cysteine and methionine. Thiol-groups of cysteine are first oxidized to sulfenic acid (Cys-SOH), which reacts rapidly with other thio-containing molecules, especially GSH. Such alterations protect protein cysteines from further irreversible oxidation to sulfuric (SO_2H) or sulfonic (SO_3H) acid (He et al. 2018; Martí et al. 2020). Among proteins, which undergo Cys-modification, are metabolic enzymes (e.g., chloroplastic glyceraldehyde-3-phosphate dehydrogenase), antioxidant oxidoreductases (thioredoxins, glutaredoxins), signaling proteins, such as protein kinases, phosphatases, and transcription factors (Lushchak 2012; He et al. 2018; Martí et al. 2020).

Under non-stressful conditions, many transcriptional factors (TFs) are in an inactive form in the cytoplasm, but oxidative stress activates them followed by their translocation to the nucleus. Here, we will review the effects of redox perturbations on gene expression in nucleus with a focus on two plant stress responses that are regulated by the redox-sensitive proteins NPR1/TGA and Rap2.4a.

The NPR1 protein (the non-expressor of the pathogenesis-related gene 1) is the main transcriptional coactivator for pathogenesis-related genes (PR) and other genes induced by salicylic acid. This protein also regulates SA-mediated suppression jasmonic acid signaling (Lushchak 2011; He et al. 2018). Under physiological conditions, NPR1 is the cytosolic protein in the form of oxidized oligomer with intermolecular disulfide bridges between Cys82 and Cys216 (Fig. 16.5a) (Mou et al. 2003). Nitric oxide (NO) may cause S-nitrosylation of Cys156 facilitating the formation of disulfide bonds between NPR1 monomers to form inactive oligomers. In response to pathogens or other stressors, plant hormone SA triggers thioredoxin-dependent S–S bond repair in oligomeric NPR1 and its dissociation into NPR1 monomers, which are active form of the protein (Tada et al. 2008). The active NPR1 protein is translocated to the nucleus, where it interacts with the TGAs proteins, which are basic domain/leucine zipper TFs (Després et al. 2003). The complex NPR1/TGA binds to promoters of genes containing specific nucleotide sequence, namely TGACG sequence, leading to activation of transcription of stress-protective genes (Lushchak 2011; He et al. 2018). It should be noted that SA is able to directly bind to NPR1 leading to conformational changes in NPR1 (Wu et al. 2012). Plants with inactivated NPR1 showed poor systemic acquired resistance which is mediated by SA (Dong et al. 2001). Six TGA transcription factors were identified to cooperate with NPR1. The dominant role of TGA2, TGA3, TGA5, and TGA6 have been proved (Zhang et al. 2003; Kesarwani et al. 2007). It is proposed that HM due to H_2O_2 -induced production may stimulate SA/NPR1 signaling to increase plant tolerance (Dutta et al. 2018).

Redox-changes in the structure can modulate the affinity of transcription factors towards DNA. This mechanism is well studied in transcriptional factor Rap2.4A, which is encoded by the At1g36060 gene. The Rap2.4A protein is able to bind the promoter element of the specific genes in redox-dependent manner (Fig. 16.5b) (He et al. 2018). In particular, it was found that the activation of the 2-Cys peroxiredoxin-A gene in *A. thaliana* is controlled by Rap2.4A. The use of Rap2.4a mutant lines demonstrates that this protein regulates tolerance to stressors by increasing photosynthetic ability and defense capacity of chloroplasts (Shaikhali et al. 2008; Rudnik et al.

to target genes. It is assumed that before binding, the protein undergoes reduction by disulfide bridges, but the mechanism of this reduction is hypothetical. Only a moderate ROS increase leads to the Rap2.4a activation, when severe oxidative stress cause inactivation of this protein (Shaikhali et al. 2008). This is likely due to the fact that with increased ROS levels, there are formed aggregates of oxidized Rap2.4a protein that do not have DNA-binding activity. Thus, Rap2.4a serves as a highly sensitive and precisely controllable redox system that provides an adaptive response to small changes in the redox state, which can be a useful strategy to prevent the development of significant oxidative damage in the plant cell. It is not currently known whether Rap2.4a is involved in tolerance to heavy metals. However, its role as an inducer of antioxidant defense suggests that it may be involved in protection against metal toxicity. Detailed molecular redox mechanisms that provide chromium tolerance require further research.

16.10 Conclusions and Perspectives

Toxicity of heavy metals (HM) is the topic which is intensively studied. In this context, it is important to know how plants cope with and tolerate HMs, especially with regards to agricultural plants. Contamination of agricultural plants with HM has a direct effect on human health. This review summarizes available information on the relationship between chromium toxicity and ROS/RNS generation in plant cells. The analysis of literature data indicates that chromium causes disturbances in the redox balance through a number of mechanisms—directly inactivating protective proteins, disrupting the functioning of electron transport systems, destroying membrane integrity. In chloroplasts, Cr inhibits electron transport, reduces CO₂ fixation and glucose synthesis, causing drying, chlorosis, necrosis, and wilting of leaves. Plant cells up-regulate complex antioxidant non-enzymatic and enzymatic mechanisms that help the cells to adapt their metabolism to Cr stress. However, the mechanisms of Cr-induced toxicity at the biochemical and molecular levels still require deeper investigation. Molecular mechanism underlying Cr tolerance is still poorly understood, and this is an avenue for future research.

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