

Chromium induced oxidative stress in plants and their defense mechanism via ROS homeostasis

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Abstract

In recent days, chromium (Cr) stress is one of the major problems in agricultural soil which cause lethal damages to the plants and it becomes a serious concern for public health by entering the food chain. In this review, we discussed sources of Cr toxicity, its forms and Cr uptake mechanism and its effects on plants. At high level, Cr interacts with cellular molecules which results in unnecessary and excessive generation of reactive oxygen species (ROS). Chromium toxicity caused damages to morphological, molecular and physiological processes such as inhibition of growth attributes, reduction in enzymatic and non-enzymatic antioxidants, DNA and cellular damage in plants. We critically reviewed the mechanisms plants adapt during the stress by activating antioxidant systems via ROS homeostasis.

Keywords: Antioxidants, chromium (Cr) toxicity, reactive oxygen species (Ros), ros homeostasis

Introduction

Heavy pollution of water and soil during the current stage of industrialization has drawn increased attention from experimenters around the world because the consequence can be potentially dangerous to plants and human beings via the food chain. Chromium (Cr) is extensively considered as one of the most dangerous heavy metal for agricultural soil. The high toxin of Cr has an adverse effect on the germination of seeds as well as plant's growth and development, making it gratuitous element for plants (Shahid *et al.* 2017) [36]. Plant scientists have paid lower attention to it due to its intricate electronic chemistry. Due to their potent oxidizing and mutagenizing properties, chromate oxyanions are classified as priority and carcinogenic pollutants (Shanker *et al.* 2004) [37]. Trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) are the most stable forms in the terrestrial environment, even though Cr exists in a variety of valencies (from -2 to +6) (Mortada *et al.* 2023) [16]. At high attention, however, it's extremely poisonous to both plants and animals (Shanker *et al.* 2004) [37]. A maximum permissible limit of $<2\mu\text{g/L}$ of Cr^{+6} in drinking water and 100mg/kg for soil is advised (WHO, 2020). When oxidized manganese (Mn) is present in the soil, Cr^{+3} is fluently converted to Cr^{+6} , which is further poisonous and stays in environment for a longer period (Mortada *et al.* 2023) [16]. While lower mobile and less poisonous Cr^{+3} exists in the form of hydroxides, oxides and sulphates, Cr^{+6} exists as chromate oxyanions (Cr_2O_4 and Cr_2O_7) (Mortada *et al.* 2023) [16]. Because of its great oxidizing properties and high solubility, Cr^{+6} is more poisonous than Cr^{+3} (Mortada *et al.* 2023) [16]. A lesser attention of Cr^{+6} inhibits germination, limits root and shoot growth and the accumulation of biomass that follows, results in chlorosis, reduces the product of chlorophyll and protein conflation, and eventually can cause plant death (Shahid *et al.* 2017) [36]. Several plant species such as mosses, rice, pea, and wheat have been studied oxidative stress which caused by Cr. Plants treated with Cr at the micromolar range (both Cr^{+2} and Cr^{+6}) led to change in ultrastructure of chloroplast, decrease chlorophyll content, inhibit growth and photosynthesis (Qin *et al.* 2024) [32]. In many plants, under Cr stress condition produced reactive oxygen species (ROS)

which cause lethal damage as well as cellular level of abnormalities like DNA damage in plants (Fig. 1; Table 1). Iron (Fe), sulphur (S), and phosphorus (P) some essential for plant metabolism and they are transported by carriers which present on plant cell. It was found that, Cr competes with different transporters of these essential elements. During its transportation, the concentration of P decreased with increase in concentration of Cr in different parts of the plant (Rodriguez *et al.* 2012) [33]. The Cr^{+6} can easily enter the cell membrane than Cr^{+3} because it is highly soluble in water (Rodriguez *et al.* 2012) [33]. The process of uptake and accumulation of Cr^{+6} from root to shoot may be differ in several plant species (Loprz-Bucio *et al.* 2022). Accumulation of Cr is high in plant's root and very limited amount of Cr translocate to shoot (Shahid *et al.* 2017) [36]. In earth's mantle, chromium is the 17th most abundant element. The main causes of heavy metal entry into soils are anthropogenic conditioning, urbanization, industrialization, and overuse of fungicides (Shahid *et al.* 2017) [36]. In addition to mortal exertion, some natural processes like stormy eruptions, timber fires, the creation of ocean swab aerosols, and runoff are also major contributors to the environmental impurity of heavy metals (Shanker *et al.* 2004). Multiple industrial activities including electroplating, cement plants, colour manufacturing, metal plating, leather and wood preservation, timber processing, pulp and paper product, oxidative dyeing, and filtering from unsanitary tips are the sources for the release of Cr^{+6} species into the agricultural land (Qin *et al.* 2024) [32]. Drinkable water Cr^{+6} content is increased by a variety of substances that are used to help erosion and clean water (WHO, 2020).

Phytotoxicity effects on plant

Plant growth inhibition is one illustration of chromium phytotoxicity. Due to the import between nutrients and metal, the presence of Cr in soil causes dislocations in the pattern of plant uptake of nutrients. Different Cr attention phytotoxic effects on seed germination as well as seedling growth in different crops *Vigna radiata* (L.), *Vigna angularis* (L.), *Daucus carrota* (L.), *Beta vulgaris* (L.), *Raphanus sativus* (L.), *Lathyrus odoratus* (L.), *Lablab purpureus* (L.), *Solanum melongena* (L.), *Hibiscus*

esculentus (L.) and *Triticum aestivum* (L.), were reported (Lakshmi and Sundaramoorthy 2010) ^[47]. Under Cr stress, some phytotoxic effects were observed in seed germination, seedling growth and vigour index, chlorophyll content and resistance indices (Amin *et al.* 2013) ^[4]. Elevated level of heavy metal decreased the germination of seed in comparison to less amount of heavy metal and large amount of Cr also affect elongation of root and shoot (Amin *et al.* 2013) ^[4]. Chromium caused various damages in plants such as, reduced plant growth, new leaves chlorosis, top wilting, decreased rate of photosynthesis and ultimately caused plant death (Mathur *et al.* 2016) ^[23]. In light reaction of photosynthesis various types of protein complex play major role. In this reaction, PSII act as photosynthetic protein which shows diverse type of nature, structure, and functional role (Mathur *et al.* 2016) ^[23]. In cell, mitochondria are the main cell organelle that regulate aerobic respiration. In mitochondrial matrix, organic acid oxidized CO₂ and H₂O through tricarboxylic acid cycle and this process provides energy for the respiratory electron transport chain (ETC) located in the inner mitochondrial membrane to reduce NADH equivalents to O₂. (Keunen *et al.* 2011) ^[20]. On the basis of presence of heavy metal, they caused damage to mitochondria and start signalling outside of mitochondria. It can change redox state of organelles and generate signal which transport to nucleus this process was called retrograde signalling. ROS or secondary signals caused by oxidative stress can mediate this process, which take place between the nucleus, mitochondria, and chloroplast (Keunen *et al.* 2011) ^[20].

Effects on seed germination

A plant's capability to maintain germination in a polluted land determines its capability to repel or tolerate Cr toxin (Table 1). However, great variations were observed in plants under Cr toxicity depending upon the dose and exposure time of Cr (Lopez- Luna *et al.* 2009). Studies on the forbearance of plants to Cr contaminated land showed that Cr⁺⁶ decreased germination of seed in plants *Triticum aestivum* (Lopez- Luna *et al.* 2009), *Apium graveolens* (Scoccianti *et al.* 2006), *Glycine maximum*, *Vigna radiata*, and *Vigna angularis* (Jun *et al.* 2009) ^[18], *Beta vulgaris*, *Raphanus sativus*, *Daucus carota*, *Solanum melongena*, and *Lycopersicon esculentum* (Lakshmi and Sundaramoorthy, 2010) ^[47]. According to recent reports, germination and growth were inhibited by Cr⁺³ nanoparticles (25-100 µg/ml) (Vajpayee *et al.* 2011). It was proved that, Cr⁺³ and Cr⁺⁶ disrupted the structure and function of the male

gametophyte in *Actinidia deliciosa* var. *deliciosa*, preventing pollen germination, suppressing the growth of the pollen tube, and causing changes in the shape of the pollen tube (Speranza *et al.* 2009) ^[40]. According to Speranza *et al.* (2009) ^[40], Cr changed the distribution of arabinogalactan proteins and callose deposit in pollen walls.

Effect on morphology

Chromium toxicity showed detrimental effects on morphology and growth attributes of plants (Fig. 1; Table 1). Boros-Lajszner *et al.* (2023) ^[8] reported that, *A. sativa* plants exposed to Cr⁺⁶ toxin showed suppressed growth, necrotic lesions, and inadequately developed roots. Stressed with Cr, the seedlings displayed a hard and brittle texture, hypertrophy, and brown spots on the leaves and roots. The root and shoot length were significantly decreased in *O. sativa* under Cr stress (Mishra *et al.* 2024) ^[25]. *Pisum sativum* plants showed altered morphology when exposed to Cr⁺⁶ at ≥ 1,000 mg/L. It was observed that 30 mg/L of polluted water cause necrosis in the root tips of *Genipa americana* plants (Barbosa *et al.* 2007) ^[7]. Various reports showed that membrane damage is the main cause for poisonous properties of Cr⁺⁶ (Amin *et al.* 2013) ^[4]. *Citrullus vulgaris* exposed to Cr⁺⁶ at 0.2 mM showed reduction in leaf size and number as well as altered the uptake of certain nutrients (Dube *et al.* 2003) ^[11]. In younger leaves, vein clearing, papery appearance and yellowing of *Zea mays* leaves were among the visible lesions caused by Cr⁺⁶ (Sharma *et al.* 2003) ^[38]. According to Pandey *et al.* (2005) ^[29], reduction of leaf area in the presence of Cr⁺⁶ toxin in *Brassica juncea* plant. According to Su *et al.* (2005), *Pteris vittata*, showed reduction in both fresh biomass and relative water content when exposed to Cr⁺³ and Cr⁺⁶. Many researchers examined the impact of Cr on changes in the structure and ultrastructure of different plant organs.

Physiological and molecular changes in plant

According to some reports, Cr can also affect in morphological changes and negatively affects physiological processes like photosynthesis, water relations, and mineral nutrition (Table 1; Daud, 2014) ^[9]. Chromium-induced ROS accumulation by varying antioxidant system along with cellular and molecular changes in plants (Fig. 2). Zaheer *et al.* (2020) ^[43] found that, chromium toxicity caused negative impacts on morpho-physiological traits in *Brassica napus* L. plants irrigated with different levels of tannery wastewater. The Cr-induced DNA damage, genotoxicity and caused ultrastructural changes in cells of plants (Table 1).

Table1: Effects of chromium on physiological process in plants

Plant	Cr Concentration	Observation	Reference
<i>Helianthus annuus</i>	5-19 mg/kg	Suppressed growth, biomass, gas exchange, and chlorophyll content Increase ROS, POD, EL, SOD, APX, and CAT	Farid <i>et al.</i> (2020) ^[12]
<i>Solanum lycopersicum</i>	>200ppm	Cr accumulation on root and shoot	Shoab <i>et al.</i> (2022) ^[39]
<i>Capsicum annum</i>	40 mg/L	Growth inhibition, chlorophyll degradation, increased MDA content	Mumtaz <i>et al.</i> (2022) ^[27]
<i>Hordeum valgaris</i>	0, 50, and 100 µM	Decrease gene expression of phenolic, flavonoid, ascorbic acid, and anthocyanin	Zhu <i>et al.</i> (2023) ^[44]
<i>Brassica lleracea botrutis</i> L.		Decrease growth, biomass, photosynthesis, and gas exchange parameter and increased antioxidant, catalase SOD, POD, MDA, and EL in both root and leaves	Ahmad <i>et al.</i> (2017) ^[2]
<i>Oryza sativa</i>	2.0 mg/L	DNA damage ROS induced H ₂ O ₂ O ₂ ⁻ and repressed gene expression	Kang <i>et al.</i> (2023) ^[19]

Chromium induced oxidative damage in plants

The metabolic changes brought on by Cr stress in plants described the enzymes and/or metabolites ability to produce ROS, which leads to oxidative damage and lipid peroxidation (Montes-Holguin *et al.* 2006) [26]. According to several studies, plants exposed to Cr showed oxidative damage that inhibits their capability to grow. For instance, roots and shoots of *Sorghum bicolor* showed increase in malondialdehyde (MDA) content, an index of lipid peroxidation, upon exposure to $\geq 50 \mu\text{M}$ Cr; (Shanker and Pathmanabhan, 2004) [37]. According to Pandey *et al.* (2005) [29], roots of *Brassica juncea* accumulate further MDA as compared to shoots under Cr^{+6} stress. Cr^{+6} is more poisonous than Cr^{+3} as it showed more dangerous effect on ROS product and lipid peroxidation (Scoccianti *et al.* 2008) [34]. According to Dixit *et al.* (2002) [10], Cr^{+6} causes lipid peroxidation in mitochondrial membranes and influences redox responses in *Pisum sativum* root mitochondria.

Reactive carbonyl groups, superoxide revolutionaries ($\text{O}_2^{\cdot-}$), and hydrogen peroxide (H_2O_2) have also been reported to be produced in response to Cr stress (Gangwar and Singh 2011) [14]. Under Cr^{+6} stress, the product of reactive carbonyl groups increased in tandem with the reduction in protein content (Gangwar and Singh 2011) [14]. The Cr^{+6} inhibited electron transport in mitochondria, which increased the product of $\text{O}_2^{\cdot-}$ (Dixit *et al.* 2002) [10]. According to Shanker *et al.* (2004) [37], there was a notable rise in $\text{O}_2^{\cdot-}$ under Cr^{+6} and Cr^{+3} stress with exposure time 5-120 hrs and 12hrs respectively. In contrast to this, H_2O_2 content in *Vigna radiata* roots didn't significantly increase 120 hours after exposure and only increased 5 and 12 hours after Cr^{+6} and Cr^{+3} treatment, independently (Shanker *et al.* 2004) [37]. The increased electrolyte leakage, which indicated membrane decomposition from Cr toxin, was another suggestion of the oxidative damage brought on by Cr^{+6} exposure (Gangwar and Singh 2011) [14].

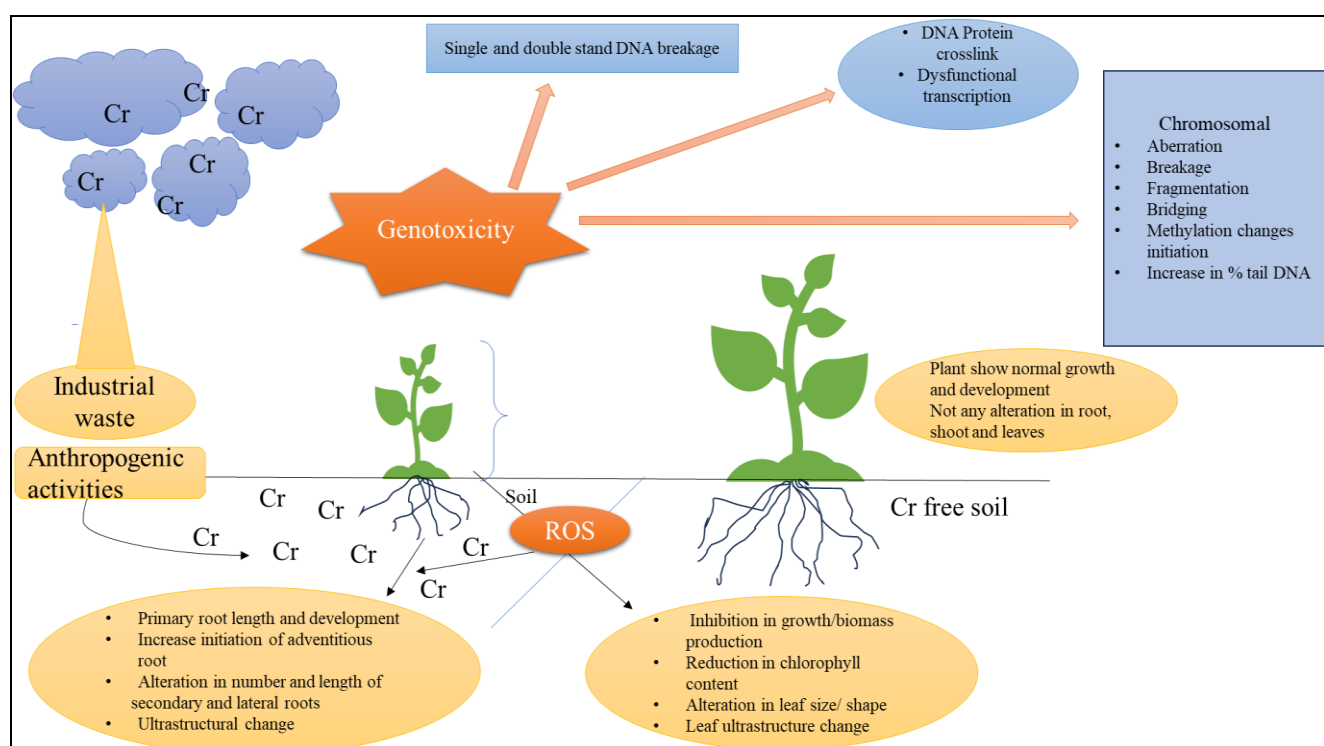


Fig 1: Showing effect of Cr toxicity on plants

Antioxidant enzymes

Plants retain a strong antioxidant system that can regulate Cr toxicity. The lower molecular weight composites known as carotenoids, flavonoids, ascorbic and phenolic acids, glutathione, etc, which act as redox buffers and modify a variety of cellular processes to impact plant growth and development, make up the nonenzymatic antioxidants (Ahmad *et al.* 2022) [3]. Through the inhibition of free revolutionaries, involvement in ascorbate- glutathione cycle, and elimination of dangerous peroxides, reduced glutathione performs antioxidant functions. The type and generation of ROS determine the position of antioxidant exertion against the poisonous attention of ROS (Ashraf *et al.* 2022) [6]. The capability of antioxidants to counterbalance the toxin of Cr in the soil can change in advanced plants (Shah *et al.* 2022) [35]. Antioxidant contents in the roots and leaves of *Spinacia oleracea* plant were significantly increased by Cr toxin in the soil (Zaheer *et al.* 2020) [43]. Consequently, the Cr

contents in the soil caused change in antioxidant contents against Cr stress (Adhikari *et al.* 2020) [1].

ROS homeostasis by organelles under Cr stress in plant Chloroplast ROS regulation

Production of ROS in both unstressed and stressed condition in chloroplast (Fig. 2). Pandey *et al.* (2009) [30] reported that Cr^{+6} reduced chlorophyll (Chl) content, Fv/Fm ratio of Chl fluorescence, electron transport rate in *Pisum sativum* plants. The production of ROS occurs in the chloroplast by inhibiting CO_2 through photosystems (PS) I and II. Normally, electrons released from the excited PS centers flow to NADP, which is then reduced to NADPH, which subsequently enters the calvin cycle and lowers CO_2 ; the last electron acceptor. When the ETC is overloaded, electrons from ferredoxin leak to O_2 , reducing it to $\text{O}_2^{\cdot-}$. This occurs because of a reduced NADP supply brought on by stressful circumstances. A direct electron transfer to

molecular oxygen occurs through the PS-I Mehler reaction under exposure of high light intensity with little CO₂ intake because of stomatal closure (Asada, 2006) ^[15]. A membrane-bound Cu/Zn-SOD converts these superoxide radicals to H₂O₂, which is then transformed into water by a thylakoid-bound (Miller *et al.* 2010) ^[24].

Mitochondrial ROS regulation

Mitochondria produce ROS in different sites of ETC (Fig. 2). As mitochondria is the site of photorespiration, it causes reduction of oxygen to O₂^{•−} in the complex I of the respiratory chain (Turrens *et al.* 2003). In contrast to the chloroplast, less ROS are produced in the mitochondria (Janku *et al.* 2019) ^[17]. Reverse electron transfer from complex II to complex I, can happen when NAD⁺-linked substrates for complex I are restricted. This procedure has been shown to enhance the generation of ROS at complex I and is controlled by ATP hydrolysis (Turrens *et al.* 2003). When UQ is completely reduced, one electron is given to cytochrome C₁. This produces a very unstable radical complex that leads to O₂ and electron leakage construction (Janku *et al.* 2019) ^[17]. Aconitase is one of the several ROS-producing sources found in the mitochondria (Janku *et al.* 2019) ^[17].

Peroxisomal ROS regulation

During stress condition, increased glycolate synthesis occurs as a result of lower CO₂ and O₂ levels in the cell (Fig. 2). Glycolate oxidase in the peroxisome subsequently converts the glycolates to H₂O₂ (Noctor *et al.* 2002) ^[28]. Production of O₂^{•−} occurs at two sites: the proximal membrane, where conversion of xanthine and hypoxanthine to uric acid and O₂^{•−} occurs by xanthine oxidases and in the peroxisomal matrix O₂ is produced by NADH and Cyt-b using it as an electron acceptor (Kosti'c *et al.* 2018). Since, the photorespiratory route is the primary mechanism of H₂O₂ generation, glycolate oxidase (GOX) in peroxisomes catalyzed 70% of the total H₂O₂ produced in photosynthetic tissues (Noctor *et al.* 2002) ^[28]. Peroxisomes, mitochondria, and chloroplasts are all involved in the photorespiratory cycle. H₂O₂ is created when the peroxisomal GOX changes glycolate that has been transported from chloroplasts into glyoxylate (Wang *et al.* 2022) ^[41]. This suggests that changes in ROS, a consequence of photorespiratory pathways, cannot be the exclusive explanation for the PCD process brought on by transcriptional level mutation of these enzymes. Leaf senescence phenotype is also caused by severe obstruction of photorespiratory processes, but at a high energy cost (Wang *et al.* 2022) ^[41]. The management of plant growth, development, and resilience to external stresses depends heavily on the accurate control of plant PCD. Plant developmental processes including trichome differentiation, and leaf senescence are influenced by dPCD,

but ePCD serves as a crucial counterbalance when plants respond to biotic and abiotic stressors. It is widely known that cellular ROS levels are elevated when the equilibrium between ROS generation and scavenging is upset by external stress or planned developmental activities (Farooq *et al.* 2019) ^[13]. Strong reactive oxygen production and scavenging abilities are possessed by peroxisomes (Farooq *et al.* 2019) ^[13].

Plant defence mechanism against Cr-induced oxidative stress

In order to scavenge ROS, plants have a sophisticated antioxidative defense mechanism made up of both enzymatic and non-enzymatic components. Plant cells include distinct methods for generating and eliminating ROS in various organelles, including peroxisomes, mitochondria, and chloroplasts. Coordinated ROS scavenging mechanisms emerge from many cellular compartments (Pang and Wang, 2008) ^[31]. Parts of the antioxidative defense system that are not enzymatic include tocopherol, carotenoids, and phenolic substances in addition to the two main non-enzymatic antioxidants glutathione (GSH), and ascorbate (AsA). These antioxidants have an impact on many different aspects of plant growth and development by direct involvement in scavenging ROS. They regulate mitosis, cell elongation, senescence, and cell death, among other processes. They also play important defensive functions and function as cofactors for enzymes (Foyer and Noctor, 2003) ^[15]. The AsA is the most prevalent low-molecular-weight antioxidant and plays a vital function in protecting the body from oxidative stress brought on by elevated ROS levels. Because of its propensity to contribute electrons to several enzymatic and non-enzymatic processes, AsA is regarded as a potent antioxidant. As an important enzyme of AsA-GSH cycle, APX used AsA which act as an electron donor for reduction of H₂O₂ by oxidizing AsA to MDHA and then MDHA to DHA via non-enzymatic reaction (Fig. 2). The GSH is an essential non-protein low molecular weight thiol that is involved in intracellular defense against oxidative damage caused by reactive oxygen species (Foyer and Noctor, 2003) ^[15]. Plant cells produce Glutamyl-cysteinyl synthetase in their cytoplasm and chloroplasts through compartment-specific isoforms of the enzyme. Glutathione synthetase one of the key elements in preserving the cellular redox state is the equilibrium between glutathione disulfide. Reducing capacity of GSH makes it an essential component of many biological processes, such as cell division and proliferation, sulfate transport control, signal transmission, metabolite conjugation, enzymatic regulation, protein synthesis, and nucleic acid production of phytochelatin for the chelation of metals, detoxification of xenobiotics, and the expression of genes sensitive to stress (Pinto *et al.* 2003) ^[46].

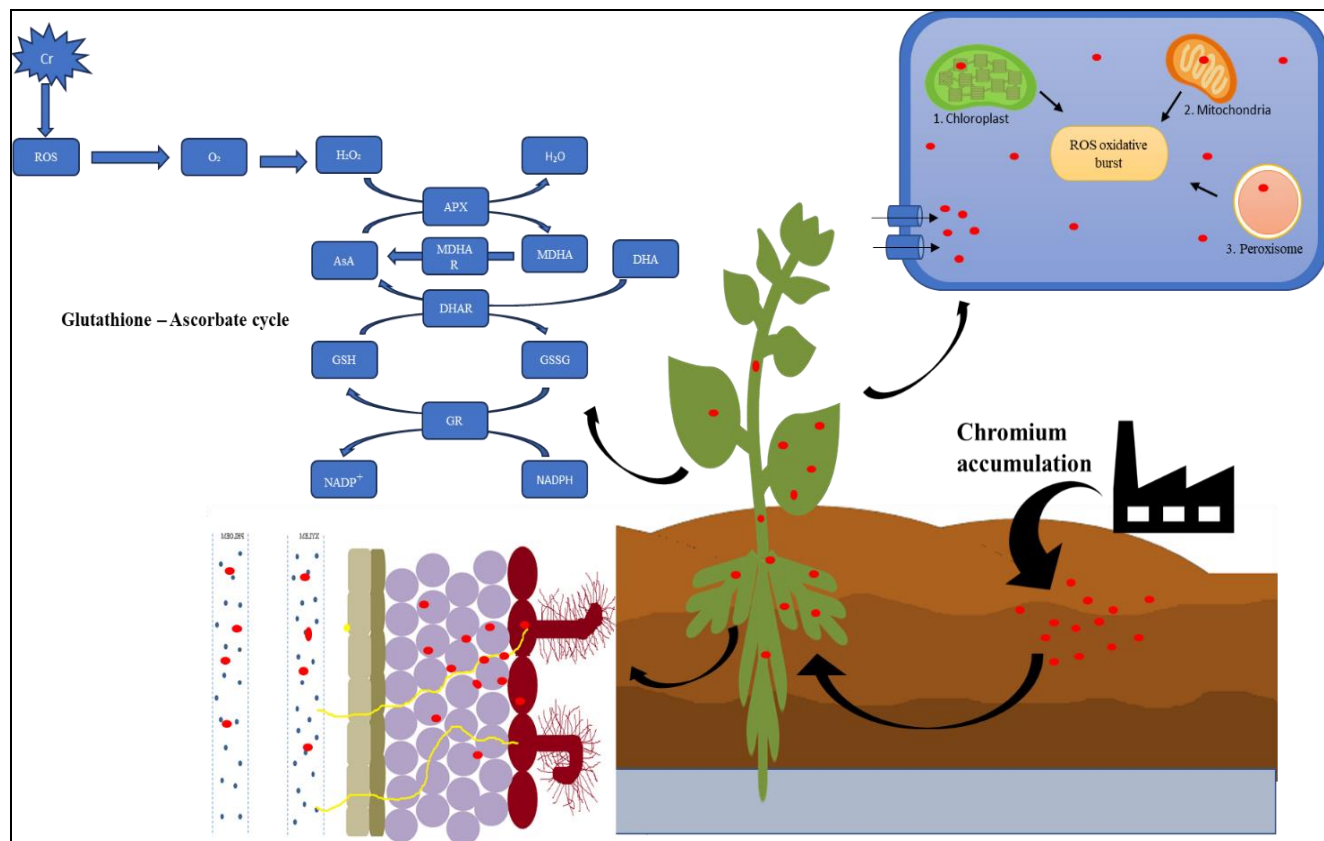


Fig 2: Showing self-defense mechanisms plants adapt against Cr stress

Conclusion

Chromium toxicity is one of the most hazardous problems in recent years which can pollute the soil profile by both natural and anthropogenic activities. Due to this, the biochemical and morphological characteristics of plants, such as root damage, photosynthetic efficiency, biomass, and seed germination, severely affected. Depending on the attention, Cr toxicity has negative impacts on the growth and development of plants. Crop yields also suffer from Cr contamination in agricultural soils. Plants under such conditions activate their antioxidant system which can scavenge excessive ROS production via self-defense mechanism. Overall, the effects of Cr on plants are varied and contingent upon the particular dosage and circumstances, underscoring the need for additional study on the toxicity of Cr and solutions for remediating soil-plant interfaces.

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