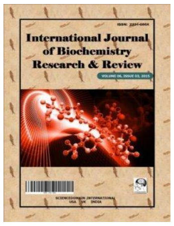

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A Review of Formation, Toxicity of Reactive Oxygen Species by Heavy Metals and Tolerance in Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors together managed the literature and wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This whole review tells about origin of ROS, Cell death, role of ROS, defense system in numerous developmental pathways.

Methodology: In plant reactive oxygen species are the main factor of cell mechanism deterioration. The stability of the reduction and regeneration is disturbed under stress environment. ROS continuously damages the main organelles of cell in plants as well as inactivate several enzymes. Break down of lipids, nucleic acid, proteins, pigments, damages in membrane which may lead to cell death.

Conclusion: ROS is diffusible measures in pathways of signal transduction in numerous developmental pathways in plants. ROS acts as a messenger. Plants protect cell from misbalancing and also damages ROS production. ROS produced in excess amount rather than required for numerous metabolic reaction. In aerobic respiration ROS is produced.

Keywords: Reactive metabolites; membrane damage; active mechanism; cell death.

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1. INTRODUCTION

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals are the greatest source of contamination in plants and other living and non-living things on the planet earth. Heavy metals such as Cd, Cu, Zn, Ni, Co, Cr, Pb and As etc are great source of contamination of agricultural soils because of the excessive use of phosphate fertilizers which are rich in amounts of phosphorous that is why these metals are also known as polluting agents [1]. There are many other factors such as sewage sludge application, dust from surroundings, and industrial waste and poor irrigation systems in agricultural land responsible for the pollution of environment. The exposure of heavy metals in plants results in the generation of reactive oxygen species which contaminates plants and this become harmful for their growth. Heavy metals in plants produce ROS through Haber-Wesis reactions (Generation of OH-, H2O2, O2-). The overproduction of ROS or oxidative stress can be a great source for heavy metal toxicity in plants. Oxidative stress can be indicated by Malondialdehyde (MDA), which is the decomposed product of polyunsaturated fatty acids [2].

2. METHODOLOGY MECHANISM OF ROS PRODUCTION IN PLANTS

The production mechanism of ROS includes factor such as the disruption of the electron transport chain in plants, their reaction with an antioxidant system or the disturbance of metabolism of essential elements. The production of lipid peroxidation (Degradation of lipids and steal the electrons from lipid on cell membrane) in plants is the most disastrous effect on plants caused by heavy metals due to which biomembrane deterioration can occur. Plants have very good potential mechanism to face the hazardous heavy metal toxicity present in the environment. In general plants have the ability to produce low molecular weight thiols which have affinity for toxic metals. Glutathione (GSH) and cysteine are the best examples of low molecular weight thiols (sulphates takes the place of oxygen in hydroxyl group) which react against the toxic metals. GSH is a thiol which contains sulphur. It is a tri-peptide thiol which contains the formula of y-glutamate-cysteine-glycine [3].

2.1 Synthesis of GSH

GSH is synthesized by the catalization of two ATP dependent enzymes y-glutamate-cysteine-glycine (GSH1) and Glutathione (GSH2). GSH is a substrate of phytochelatin synthesis. Phytochelatin (PCs) are small binding material of heavy metals. They are cysteine rich polypeptides. (y-Glu-Cys)nGly(n=2-11) is its general structure. They are found in many plants as well as in fungi and in many other living things. The enzyme known as phytochelatin synthase is catalysed during its synthesis; therefore heavy metals such as cadmium and nickel are detoxified by it. As they form complexes with toxic metal ions in the cytosol and then transport them into vacuoles so they act as best defendants of plants from heavy metals [4].

2.2 Heavy Metals and Production of Reactive Oxygen Species (ROS)

Heavy metals are the main source of production of ROS in plants. The accumulation of ROS in plants depends upon the balance between ROS production and ROS scavenging. The factors affecting the accumulation of ROS in plants depend upon the growth situations such as temperature, light intensity and presence of heavy metals [5]. In case heavy metals are present in excessive amount in plants they cause limitation of fixation of CO2 in the chloroplasts, it results in over reduction of the photosynthetic electron transport and this is the major cause of production of ROS in plants. Over reduction of electron transport chain in the mitochondria also generates ROS. Almost 1-5% of O2 consumed by mitochondria is converted into ROS. Peroxisomes produce ROS i-e hydrogen peroxide (H2O2) when glycolate is oxidized into glyoxylic acid during photorespiration [6]. Therefore due to spin inversion, ROS such as singlet oxygen, superoxide anion, H2O2, and hydroxyl radicals are produced in these organelles. The redox heavy metals such as Cu, Cd, Fe, and Zn induce ROS formation directly by participating in Haber-Weiss and Fenton reactions [7].

The properties of ROS are such that they are unstable, they are highly reactive and they generate free radicals with reaction with other macromolecules. ROS as extremely reactive in nature interact with macromolecules like DNA, pigments, proteins, lipids and many other essential molecules which depends upon the properties like chemical reactivity, redox potential, half-life and mobility leading the cellular system [8]. These all factors leads to destructive process called "Oxidative stress". OH- is the most reactive and highly damaging specie among ROS, it is short lived (1 ns) and can oxidize macromolecules within a diffusion distance. Due to this factor ROS produce reversible and irreversible modifications in lipids, proteins, and nucleic acids. As compared to all most of these ROS are damaging and irreversible. ROS act as a signaling molecule which perform the regulation of many physiological reactions such as root hair growth, stomatal movement, cell growth and cell differentiation (when finally regulated by an antioxidative defense system) [9]. ROS produced by NADPH (Nicotinamide adenine dinucleotide phosphate) oxidases during stress are channeled by the plant as it serves as a stress signal for the activation and defense mechanism, which in turn counteract oxidative stress. The fate of ROS in the cellular system depends upon the output of many complex processes that involve the ant oxidative system, Signaling cascades, redox alterations etc. Oxidative stress occurs when ROS exceeds that of the scavenging potential of antioxidants [10].

2.3 Mechanism to Keep Routinely Produced ROS at Physio-logical State

Plants have a specific mechanism to keep routinely produced ROS at physiological limit, as they prevent them from exceeding toxic threshold levels. This mechanism is known as Plant antioxidant defense system. It helps in regulation of ROS levels in the cellular system. The antioxidant system comprises of two components i-e enzymatic and non-Enzymatic. Enzymatic antioxidants are those which are superoxide dismutase (SOD), glutathione peroxide (GPX) and catalase are the key enzyamtic antioxidants of the defense system by which free radicals during metabolic reactions are released. Ascorbate and glutathione both functions as cofactors of enzymatic antioxidants [11]. These directly quench ROS and regulate the gene expression associated with biotic and abiotic stress responses. The non-enzymatic antioxidants include water soluble compounds such as ascorbate, glutathione, proline and α-tocopherol. The importance of antioxidants depends upon the increased or decreased levels are related to an enhanced or declined stress tolarance of stressed plants. Since the evolution of O2, antioxidants play an important role in sustaining ROS concentration at an appropriate level that can enhance plant developments [12].

3. RESULTS AND DISCUSSION

3.1 Uptake of Heavy Metals by the Plants

The hyper accumulators have the ability to absorb heavy metals from the soil under varying concentration. Heavy metals are taken by hyper accumulators but their uptake is affected by many factors such as pH, water content and organic substances. The uptake of heavy metals requires a suitable transporting system to enter the plant. The proton secretion by roots further acidifies rhizosphere and enhances metal dissolution. It affects the growth of metal accumulating plant species. The organic substances released from the roots affect growth in hyper accumulating plants. The organic acids influence Cd solubility by the formation of Cd complexes. The study has revealed that increased Zn uptake is due to over expression of genes belonging to ZIP (zinc regulated transporter iron regulated transporter protein) family encoding plasma membrane located transporter [13].

3.2 Toxic Effects of Various Heavy Metals in Plants

The contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence. The regulatory limit of Cd in agricultural soil is 100 mg/kg. Plants which are exposed to high levels of Cd causes reduction in photosynthesis, due to uptake of water and nutrients. Plants grow in soil which contains high levels of Cd show visible symptoms of injury infected in terms of chlorosis growth inhibition, browning of root tips leading to death [14].

3.3 Role of Zinc (ZN) in Production of ROS

Zinc and Cadmium, which have now a day's become an integral part of making the soil poisonous and are very harmful for the plants as they get most of their necessary nutrients from the soil. Thus heavy metals like these damage the growth of plants in a variety of ways. Excess amount of these heavy metals in soil is due to some reasons as semi solid materials produced by various by-products, fertilizers that are given to the plants for their rapid growth, from air, garbage, by combustion of organic substances, by waste products from mining and industries and many other human activities [15]. Zinc (Zn) is also used as an important substance for living organisms for health and body growth. If excess zinc is found in poisonous soil will cause toxic effect to plant growth. The concentration of zinc which is to be estimated as useful for living being is 150-300mg/kg and if the concentration of soil increase above this point, it cause severe damage to plant. Due to high concentration of zinc, the plants tend to lose their actual color and become yellow. This damage to plants is more prominent in small leaves as compare to large ones [16]. Presence of these metals is not only harmful to plants but also induces the presence of some other heavy metals that induce severe damages in plants. It reduces the process of photosynthesis and the leaves become yellow. In some cases, these metals cause deficiency of needed metals that resist the transfer of these metals from roots to the shoot or other parts of the plant. Another toxic effect is that it causes deficiency of phosphorus which results in the purplish red color of leaves [17].

3.4 Role of Copper (Cu) in the Production of ROS

Copper is count to be a very important component for production and growth of different plant parts and play a key role in adsorption and the process of taking in carbon dioxide (CO_2) and production of adenosine triphosphate by some chemical reactions. Cu is also a very important component for protein in respiratory electron transport chain but due to industrial and mining activities, copper is inducing in soil much more than its need. It is also being added in soil due to some human sources such as extraction process and the ores that contain copper. Many of the minors working in mining throw their waste products and residue in the environment [18]. When the soil has excess amount of copper, it will then be taken up by plants as the plants take their minerals from the soil and it will not only cause damage in plants but is also harmful and toxic for living beings. Such metals slow down the process of plant growth and also lead to yellowish of young leaves. When plants are exposed to large amount of copper, it will generate different type of oxygen species such as super oxides etc and the oxygen due to Cu in soil when come in contact either plants will cause damage in all the reactions taking place and all the large molecules present in them will get damaged [19].

3.5 Role of Mercury (Hg) in Production of ROS

Like other heavy metals, mercury also causes damage to plants. As we know that we get our food from plants and plants get their minerals from soil so if mercury will induce in agricultural land, it will disturb our food chain. Mercury is found in nature in different forms such as HgS, $Hg2+$ etc. and this is the reason that mercury is considered to be different from other heavy metals. However, in the agricultural environment, the predominant form of mercury is its ionic form. Mercury released in soil is mainly found in solid form. It is well known that plants in water take in mercury in ionic form in large quantity compared to that of others. Hg in large quantity is harmful mainly for the coloration of leaves. For instance, Hg in its ionic form binds with water channel protein and help in the process of opening and closing of stomata and also control the flow of water from roots to shoots and then to all parts of plant body. High level of mercury interfere in the activity of mitochondria and the reactive oxygen species are induced in them as mercury is good component to start the generation of reactive oxygen species. Hence the process of bio membrane lipids and reaction taking place in cells of plants are disrupted [20].

3.6 Role of Chromium (Cr) in the Production of ROS

Due to industrial growth, pollution into environment has increased in a dramatic manner. Chromium is considered to cause the addition of poisonous or polluting substances in soil, dregs and underground water. The industries in which soft and perishable protein of animals hides or skin are converted into durable and flexible leather is one of the main source that consume water, use it in their processes and release the contaminated water in environment that contain a very large amount of chromium from 1.07 to 7.8 mg/liters. Pollutants from living sources and discharge of chromium in fresh water is about 3550mt. Chromium is toxic as it causes very harmful effects on epithelial layer in plants and act as an irritant on epithelium. Many agencies have proven on experimental basis that several of human carcinogens are also induced by chromium [21]. Excess amount of chromium in soil causes reduces growth in plants, discoloration of young leaves and interrupted balance of nutrients. The top of plants and the roots get damaged when plants are supplied with water contaminated by chromium. The plants in terrestrial areas are exposed to discoloration of leaves due to chromium. For example: if 100µM of chromium is present then it causes about 40% of resistance in growth of plants. The process of germination, growth of stem, leaves and roots as light, water and mineral nutrition are also interrupted. Excess of chromium also generate reactive- oxygen species when it has its direct effect on enzymes [22].

3.7 Effect of ROS Due to Lead (Pb)

The Earth's soil contains many heavy metals and is abundant in lead (Pb). The toxicity in soil, due to lead, is the reason of dumping of toxic municipal sewage sludge, paints papers, pulp, gasoline and explosives rich in Pb, extraction of minerals and metals by heating and melting. It, therefore, produces the harmful effects on shape and structure, growth and essential processes of plants like photosynthesis and respiration. Increased level of Pb also stops or inhibits the original enzymatic activity at cellular levels by combining with the hydrogen and sulphur groups. Increased level of Pb also gives rise to the oxidative stress by increasing the ROS production in plants. The increased level also disturbs the balance of required normal water contents, alters the permeability across the membranes and also on other hand, disturbs the mineral nutrition [18].

3.8 Effect of ROS Due to Arsenate (As)

Arsenate is competitive to phosphate as it fights and for the same carriers for uptake phenomenon in the plasma lemma of plants present in the roots. The tolerance of arsenate has been produced in several plants, like, in grass, the suppression of a high affinity phosphate or arsenate results in arsenate tolerance. The resulting suppression causes the level of toxicity to be decreased so that it can easily be recovered by the respective constitutive mechanism methods [23]. The single gene encoding for the reduced transport of arsenate gives rise to arsenate tolerance. Although this clearly makes us understand the controlling process in As uptake, but still, grass being tolerant towards As, gathers the As, however at much less rate as compared to that of the non-tolerant grass. However, the grass growing on the contaminated soil can result in a high concentration of As, e.g. 3470 mg/g As in Agrostistenuis and 500 mg/g As in Holcuslanatus grass family. The As is also transformed to several less phytotoxic As species. For example, As undergoes transformation and results in the conversion to arsenite, dimethyl arsinic acid (DMA) and mono methyl arsenic acid (MMA) in phytoplankton and macro algae. Then some As species of methyl are then, after metabolism, converted to organo phospholipids and arseno sugars. Lately, plants relating to terrestrial environment have been studied under the presence relating to arsenate and arsenite. However, a new study has also reported reduced methylated as concentrations in plants, on terrestrial environment, such as MMA and DMA (Dimethyl amine) [24].

3.9 Effect of ROS Due to Cobalt (Co)

Earth's crust normally contains cobalt in the form of cobaltite (CoAsS), erythrite ($Co_3(AsO_4)_2$)and smatter (CoAs2). The earth's soil may get increased levels of toxic cobalt due to the deposition from burning of fossil fuels, wearing of cobaltite alloys and spreading of sewage wet mud and animal wastes [19]. However, cobalt producing risks in the environment are met and managed through the establishment of checking the environment quality and meeting the standards of one's needs. Plants can, themselves, gather small amount of cobalt from the soil. The Co varies from species to species in plants and its uptake and distribution is controlled by many different mechanisms. However we know little regarding the phytotoxic effect of excess Co. But however, the harmful effects are being studied under phytotoxicity of Co in barley, tomato and oilseed rape, which shows producing effect on the growth mechanism of shoots and biomass. Along with the effect on biomass, the increased rate of Co lemmatize (group infected together) the normal required concentration of other various metals such as iron and other pigments like chlorophyll and substances such as proteins and also in leaves of cauliflower, catalase activity. On other elements like phosphorus, sulphur, manganese, zinc and copper, the increased level of Co effects the translocation from tips to roots of the cauliflower. While comparing to that of copper or chromium, Co tragically reduces the significant water potential and the rate of transpiration. While resistance and hindrance towards diffusion and osmosis increased on exposure to high content of Co [25].

3.10 Effect of ROS Due to Nickel (Ni)

Nickel is one of the transition metals which are to be found in chemical free soils in a very less quantity to be measured except for those soils which are rich in availability of minerals and metals and bases. However, many anthropogenic activities are leading to an increased level of nickel in the environmental contents. For example the extraction of different minerals from mines, removal of dumped substances, many combustion processes along with burning of oil, waste products, using of fertilizers and pesticides which are rich in phosphates and phosphate containing compounds. The polluted soil due to previously mentioned reasons may contain an increased level in nickel concentration which is more than the original required levels in the natural environment. The increased levels then causes many harmful effects and changes in the various functions and abilities of plants required in normal mechanisms [26]. Like in many plants, it causes decolouration which affects the process of photosynthesis and also causes the death of many plants cells due to an increased rate of toxicity in the plants cells, for example rice. Normal functions are disturbed in various plant species which causes the plants in reduction in normal nutrients uptake and disturbs the physiology of cell membranes in the plants. A plant Oryzasativa's shoot was studies under the increased level of toxicity produced by the nickel and it showed the adverse effects on the lipids produced within. Moreover, when wheat was exposed to a higher level of toxicity produced by nickel, it enhanced the MDA concentration. Such changes in normal metabolic processes of a living being causes the disturbances in the functions of the membranes, like the maintenance of ions, such as potassium ions, across the membranes, or also in the cytoplasm. Other adverse effects on the plants metabolism were related to the normal water uptake and hydro imbalance. As in, it produced a fall in level of the uptake of water, as it increased the uptake of nickel ions, especially the monocot and dicot plants species. The reduced level of water intake by plants is taken as a symbol for the researchers that respective plant has increased in toxicity due to relative reasons being studied on that plant [25].

3.11 Mechanism of Plants to Cope with Heavy Metals Toxicity

We have been studying so far, the toxicity due to several heavy metals. The plants have been coped with these heavy metals by fitting in such mechanisms which gives a defensive shield to a plant towards such heavy metals. Reduced forms of oxygen are produced through several processes happening within a plant, and some species which are related to biochemical damage when an electron is accepted by a molecular oxygen from other molecules and many reactions and processes which are being taken place inside a cell reduces the molecular oxygen species, which took an electron, to other species like superoxide and hydrogen peroxide. Which are comparatively less reactive molecules and can form a hydroxyl radical which causes most of the damage in oxidative processes, mainly photosynthesis and transpiration in biological system. The reduction taking place with the one electron in the formation of molecular oxygen to the superoxide radical is not favoured regarding the stability while considering the heat changes; however, this can take place when a molecular oxygen species is made to interact with the unpaired electron from another species or the paramagnetic Centre. For example, many transition metals, like iron and copper, are electron efficient and thus can prove to be a very good catalyst towards the processes of oxygen reduction [27].

In the Haber-Weiss reaction, when iron is taken under consideration as a transition metal to catalyze the reaction it is named as Fenton reaction. The Haber-Weiss reaction producing the hydroxyl radicals oxidize many biological molecules , which, as a result, leads to necrosis, i.e. leading to several damages in and out of cell which then causes cell death. Hydroxyl radicals can also be produced near the DNA molecule by either oxidation or reduction, as referred through hydrogen, towards DNA bases or the DNA backbone, depending on the loss and gain, respectively. The changes occurring Hydroxyl-mediated DNA involves the iron to be in a complex structure with a phosphate residue or sometimes it may be indirectly related in coordination with the ringed nitrogens and, or several times being a free species. The process of Haber-Weiss reaction, metals ions also plays an important role in the oxidative modifications of free amino acids and proteins. Commonly referred sited relating to oxidation in proteins and major products are to be identified as cysteine, methionine, proline, lysine, arginine and histidine residues [12]. It has been studied and observed that only one amino acid is modified by oxidation in a given respective protein. But, it should be related to its specificity towards its target to be modified for example some of the amino acids residues are specifically related to metal binding sites. The breakdown by proteases is being a major consequence of radicals damage which is free in oxygen. Another important feature of oxidation of proteins is also taken under consideration which is the release of iron from a huge group of similar components, such as aconites. At last, the conveyance of oxygen and transition metals to lipid peroxidation is completed, and the membranes related to our natural environment which are abundant in polyunsaturated fatty acids are capable of accepting these reactions. While in the aqueous phase, the ascorbic acid then dampens down the peroxy and alkoxy radicals. Although, the ascorbate, being functioning as a pro oxidant, can reduce the iron and copper which then allows the change of one form to another of metal catalyzed lipid hydro peroxides [28].

3.12 Glutathione Biosynthesis and Its Regulation

Glutathione is an antioxidant tripeptide present in all the organisms which are bearing mitochondria including all eukaryotes including plants. It is the most abundant and has the least molecular weight among all the thiols. The plant's fluid of transportation involves the mechanism regarding the GSH. In many cellular processes which include the mechanism of defense against the ROS, forcibly taking in of heavy metals and in the removal of toxic substances such as xenobiotics [11]. The developmental processes within an organism also require the presence of GSH, such as regulation of cell division which is also referred to as cell mitosis and also included in the phenomenon of flowering. Along with all these GSH has also proved to be beneficial in the major transport system referring to that of sulphur and also helps in keeping the storage form of reduced form of sulphur. The synthesis of GSH requires the involvement of two processes which are being carried out responsibly in the presence of ATP, where y-glutamyl cyteine synthetase acts 0as a catalyst in the formation process between the carbonyl group of glutamate and the amino group of cysteine, resulting in the formation of peptide bond and results in the yield of y-glutamyl cysteine. Under the reviewed considerations of the processes relating to GSH, the first and the initial step is referred to as a control point when the GSH is under a demand of high concentrations. The next step includes the tying up of glutathione synthetase with a glycine residue takes place with y-glutamyl cysteine to form GSH. GSH1 is restricted to be present in plastids, whereas, GSH2 on the other hand, is not restricted in its presence and can be found in both, cytosol and plastids (16).

Two forms of GSH are to be found in existence; which are i) reduced glutathione (GSH), and ii) oxidized form (GSSG). The potential of glutathione to donate an electron must be determined by the intracellular ratio, i.e. GSH/GSSG. Many factors lead to the changes and variations in the respective ratio of glutathione, which are the following; pH of the environment in which the GSH related processes takes place, total GSH concentration which is to be required in the whole phenomenon, GSH may produce complex molecules within the cellular environment and breakdown of molecules of GSH. When the study was completed it was evident from the research that only a single gene was solely responsible for the encoding of GSH1 and GSH2 [15].

While the analysis and research done regarding the transcript of GSH2 disclosed that the major part that is more than 90 percent in count, are shortened down during the processes. Truncation resulted in the loss of many metabolic mobile peptides. Hence, study shows that GSH2 is only restricted to the cytosol only and a very less part is being transported outside into the plastids. This makes the plastids automatic that is it can control its compartments itself for the GSH biosynthesis independently while others are dependent organelles. Either on $_{\gamma}$-EC or GSH which is being transported outside plastids. Further studies on several suspensions revealed that y-EC is the major transport out of the plastids. This has also been supported by several other researchers in their research work on GSH distribution in fast growing leaves [16].

The GSH representation is taken by biosynthesis in a plant cell. First enzyme of glutathione metabolic synthesis of GSH1 is vastly positioned in plastids. While, on the other hand, the second enzyme GSH2 is localized in both, cytosol and plastids, being a major portion located in cytosol. Both are the genes which are encoded by nuclear genes, having mobile transit, however, on the other hand the mobile peptide containing major portion of GSH2 is lost due to vast transcript processing and remained positioned in the cytosol. Models in researches have shown that y-EC is vastly metabolized in the chloroplast and a transported mediated efflux of y-EC, was assumed and predicted to be from plastids to the cytosol. Since both the biological catalysts are present in chloroplast therefore, GSH is also metabolized in this compartment. It is unknown the exact amount of GSH to be metabolized out of the cytosol or inside the cytosol. However, it is to be predicted that the transport processes may be regulated in the response to developmental and stress-related cues [17].

3.13 Mechanism of Glutathione-Mediated Heavy Metals Stress Tolerance in Plants

Glutathione stimulating hormone is considered to be the main part of the cell and found in almost all parts of the cell. Proteins are exposed to the cells in plants are induced due to this glutathione stimulating hormone and is major source of protein in cells. A group found in GSH is thiole group which is responsible to make it favorable in serving a large range of biological and chemical reactions in all types of living organisms. Positive charge loving nature of that group found in glutathione stimulating hormone (GSH) play a key role in forming a bond called mercaptide bond with the other metals to make a suitable reaction with the desired electron loving molecules. The reactivity of electrophilic and nucleophilic substances with respect to their stable nature and the property of solublizing water in high range to make it a good biological and chemical component to prevent the plant body from different type of stress due to which the plants get damaged. That stresses include oxidative stress that damage the plant by the help of oxygen components, by the use of heavy metals and with the help of many other organic compounds [29].

Reactive oxygen species are being introduced in plants when plants are exposed to heavy metals in a direct or indirect way and disturb the reactions taking place within the cells of plants. H2O2 present in cell is dangerous to health of plants as well as animals either its level is low or high. So the glutathione stimulating hormone used to maintain the level of H2O2 in plants. The reduction of GSH if make any alteration to oxidize it into another form which is GSSG. H2O2 in its broken form is very important in those pathways that signal the process of oxidation-reduction reaction. This ratio of GSH and GSSG is considered to be a signal to maintain the balance of oxidation and reduction of cell thus play a key role in production of oxygen specie in the cell. GSH in its reduced form can also be used as an agent which reduce the oxidation and useful in some ways that it can reduce the value of oxygen specie that are harmful for health without any need were generated in stressful conditions. Moreover, GSH plays an important role in the process of removing toxic substances from the cell of those substances that are foreign to the body and many other heavy metals [30]. The first job of GSH is to activate itself and to make a bond in reduction and oxidation process to convert itself into oxidative form. An enzyme is used in GSH which is responsible for making their links with other which is glutathione-S-transferase. The heavy metals which get into plants through soil are not directly attacking the parts of plant body but they are accumulating in the vacuole and this is the reason that plant survives more than that of animals. The heavy metals are accumulating in the cells and are not attacking the cells directly. But its first and foremost use is to detoxify heavy metals and to decrease the content of cytosolic GSH. Thus has a direct impact on GSH/GSSG potential of oxidation and reduction and this gives a signal to the cell exposed to stress. As a result, any increase in high level of GSH which has its base in the process of breaking will help in maintain the oxidation reduction of cell. But under some of such conditions, if we are going to maintain the concentration of cell might be harmful for a plant to survive anymore [31].

Reactive oxygen species have an electron in them which is unpaired and come in contact with anything. In some cases it is harmful while in some cases it may be useful. Under that condition in which the plants are growing, the plants are in the control of the system in which they survive pn dead beings such as GSH. ROS are in plants are harmful so it should be removed from there and if ROS does not remove from the plants at time, it will leads to a stressful condition known as oxidative stress. Excess amount of ROS found in the cell can stimulate the addition of oxygen and can introduce oxygen also there where it is not being needed. Moreover, it can modify the processes of amino acids in the cell, and all the other components. This causes disruption in plants and seriously affects the process taking place in plants. This all damages the growth in plants. ROS and GSH start to do their work at the stage when the plant is just exposed to both of these. The insertion of transfer deoxyribonucleic acid in GSH and enzyme is found in them which encode a gene called gama-glutamyl cysteine synthetase. If this enzyme in not present according to its need then it will lead to severe unsuitable diseases. Addition to this, GSH also plays a role in glutaredoxin reactions to control the oxidation reduction reaction of proteins in many cells. Many kinds of different types of hormones are kept in hydrogenated form with the help of NADPH, glutathione reductase and GSH. Grx also play an important role in the redox- mediated development processes, as it is seen in flowering [32].

3.14 Plants Antioxidant Defence System

Antioxidant is a term used to define the class of that useful compounds that are used in protecting the plants from severe damage that are caused when the plants are exposed to some heavy metals that induced reactive oxygen species in them. The network that is formed when the plants are exposed to heavy metals is responsible to remove the agents which are necessary for neutralization. SOD is a useful enzyme that is involved in changing the superoxide radicals produced due to introducing oxygen to molecular oxygen into H2O2 and O2 in the entire compartment found in a cell. H2O2 which is produced by the processing of the enzyme SOD is little bit dangerous because it can very easily pass through the membrane of the cell and embed into the other compartments where there is no need of this causing damages in that compartments and as a result many of the other hormones or enzymes are being helpful to locate H2O2. Three main SODs are known to be located in plants on the basis that they contain the chloroplast Cu-Zn SOD, mitochondrial Mn SOD and the Chloroplastic Fe SOD [33].

A protein which is commonly used in chloroplast and cytosol is mainly considered as APX which is mainly used in the process of breakdown of H2O2. The substance which is found to activate the process of enzyme that increases the speed of reaction then is ascorbate and the material gain at the end of the reaction which is in the form of rad ical is mainly reduced to hydrogenated ascorbate with the help of the enzyme MDHAR when a donor of electron is present which is NADPH. CAT is another very necessary enzyme that is used in breaking of large molecules into smaller ones and hence is used in the breaking of H2O2 molecule into two smaller molecules such as H2O2 and O2 it is mostly considered to be present in plants and in plants, large entity of this enzyme is found to be peroxisomes. CAT used in the detoxification process of peroxides (reactive oxygen species) that are formed during the process of photosynthesis and respiration (process of formation of oxygen and supply it to environment and process of taking in carbon dioxide from the environment). APX and CAT both are enzymes used to speed up the reaction as well as they are used in the detoxification process, different kinds of these enzymes use to signal for the modification of H2O2 when the condition is stressful [34].

In the above paragraph, all the enzyme about which the details has been given are the components that play a very important role in making the heavy metals less severe during stressful conditions. It has been studied and on continuous experimental basis, it has come to know that if we do any treatment to reduce the action of heavy metals on plants it will eventually generate the class of reactive oxygen species, as due to increase in oxidizing agent, an increase in APX, CAT and SOD is also observed [35]. It has been studied that when plants are exposed to Pb, there is a visible rise in the concentration of SOD, oxygen species, APX and CAT activities is observed. These activities will rise in concentration of Pb from 50-100 μM [36]. Similar to this, another scientist claimed that the concentration of SOD and CAT can also be raise when plant is exposed to Pb either in 5-50μM [37] and from all these things it has been resulted that there should be co-operation in the action of oxygen reducing components to detoxify the action of metals under stressful conditions.

Fig. 1 the receptor present on cell membrane binds with incoming ligand that are heavy metals like Ni, Cd, Pb etc which starts the signalling of the production of ROS in response to ROS production occurs indicate high level of MDA in plants matrix. The rise in ROS production causes in certain antioxidants (Isoprostanes, 8O HG, Glutathione, SOD, Cat). Extreme ROS

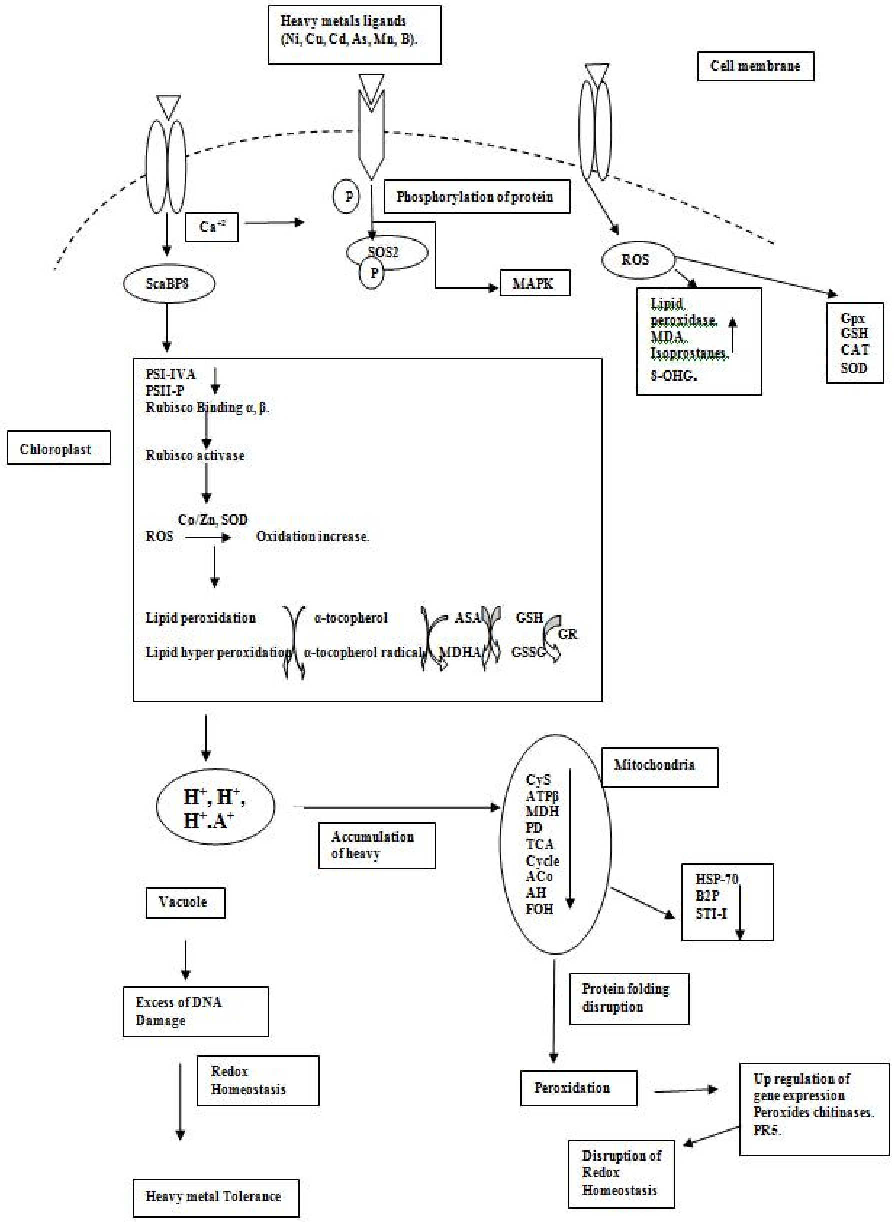

Fig. 1. Mechanism of heavy metals induced oxidative stress tolerance in plants

production disrupts the protein in mitochondria matrix i.e 8H ESP 17, SIIB2D (Heat shocked proteins) causing up regulation of gene expression peroxidase five (PR5) Chitinise and other lipid peroxidation production. This disruption redox homeostasis (coordination) cause excess DNA damage in the vacuole indicated by high level of 8OHG. However, the plant body is designed in nature in such a way that it accumulates all the heavy metals residues it is vacuole compartment preventing further oxidative damage to the plant body. This is how the plant has developed self-tolerance property of oxidative damage.

4. CONCLUSION

The exposure of plant body to heavy metals like Cd, As, Pb etc. produce reactive oxygen species which directly hit mitochondria matrix. The origin of ROS causing cell death and disturbing self-defence system of the plant body which is tolerable due to the accumulation of heavy metals produced in the vacuole which is self-phagocytes by the plant body.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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