

## Nickel, Iron and Their Diverse Role in Plants: A Review, Approaches and Future Prospective

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### ABSTRACT

In plant sciences, the prodigious significance of micronutrient is unavoidable since plant relies primarily on micronutrient as it has profound influence on array of plant activities. Nickel and iron are considered as the most important essential heavy metals (HMs) for plant nutrition. However, the increased amount of HMs within the plant tissue adversely affected plant physiology, and displays direct and indirect toxic impacts. Such direct effects are the generation of oxidative stress, which further aggravates inhibition of cytoplasmic enzymes and damage to cell structures. Heavy metals (HMs) toxicity has an unavoidable threat to environment and public health due to their increasing contamination and accumulation in atmosphere that ultimately passes to the living beings by the route of food chain. Heavy metals are increasing rapidly in soil and water by weathering of rocks and anthropogenic activities and are now emerging as a major health hazard to humans and plants. Iron and nickel play a crucial role in biochemistry and are essential micronutrients for plants and humans alike, also controversial elements because of debate on its essentiality or non-essentiality in plants. Both elements are important micronutrients because many metallo-enzymes including urease, *Ni - Fe* hydrogenase, Ni-superoxide dismutase. High concentration of both elements may affects all cellular and metabolic processes and causes retardation of germination, competition with other essential metal ions, osmotic imbalance, alteration of many enzymatic activities, disruption of cell structure and wilting, reduced photosynthetic activity, oxidative stress. Plants also possess some natural and stress-induced strategies to cope up with Nickel excess and /or toxicity. While iron is complexed with chelators and distributed to sink tissues where it is used predominantly in the production of enzyme cofactors or components of electron transport chains. The processes of iron uptake, distribution and metabolism are overseen by tight regulatory mechanisms, at the transcriptional and post-transcriptional level, to avoid iron concentrations building to toxic excess. This review focuses on researches done on the morpho-biochemical alterations induced by elevated both *Ni - Fe* elements concentration in plants and as well as the strategies adapted by plants to survive and neutralize the effects of these alterations.

**Keywords:** Heavy Metals, Nickel & Iron, Deficiency and Toxicity Symptoms, Enzymatic Activities, Transcription factor, Disruption of cell structure

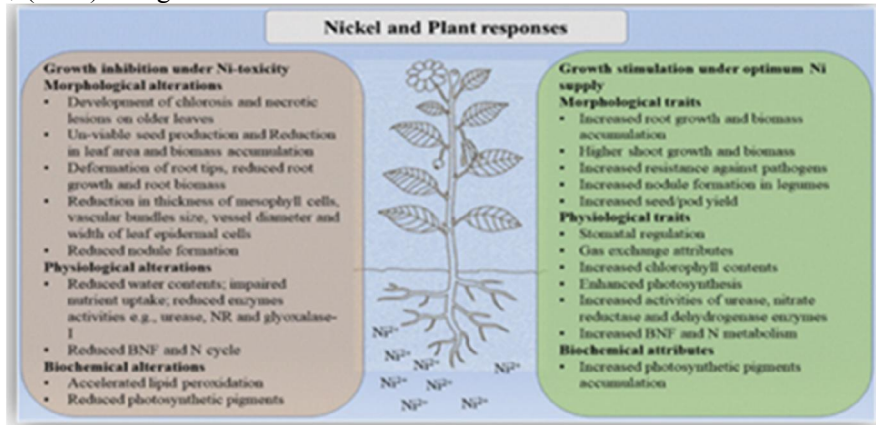
### Introduction

Based on their solubility under physiological conditions, elements may be available to living cells, and they could be significant in plant and animal communities within different ecosystems Weast, (1984). Some of the heavy metals (Fe, Cu, and Zn) are known to be essential elements for plants and animals Wintz *et al.*, (2002). Some other such as Mn, Mo, Ni, and Co are only essential micronutrients when added with the optimum concentration Reeves and Baker, (2000). Nickel is an essential micronutrient required for optimal plant growth and development; without sufficient supply of Ni, plants cannot complete their life cycle. It is vital element for the activation of several biological important processes; however, excessive Ni has certain devastating effects on plant growth and development Fig. 1, and excess uptake by plants results in toxic effects Baber *et al.*, (2018). Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage.

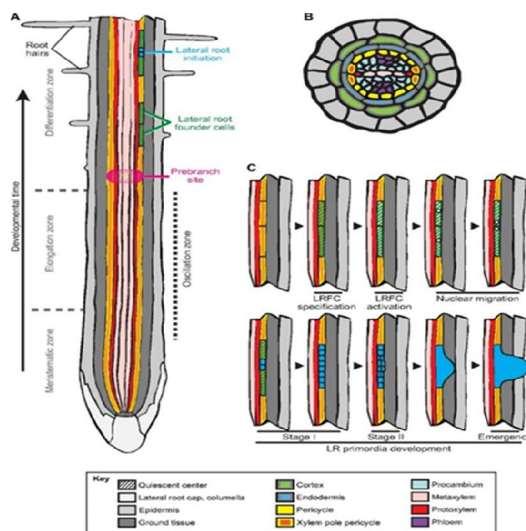
Moreover, disturbance of cellular ionic homeostasis. The specific aspects of nickel effect on plants as compared to other heavy metals; their specificity is derived from different physical and

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chemical properties. The various facets of the physiological role of nickel and its toxic activity in higher plants, the putative mechanisms of nickel hyper accumulation are considered in several representatives of angiosperm plant families. The existing evidence was used to outline the metabolic changes in plants affected by Ni as well as Fe. The comparison is used to disclose the general mechanisms that disturb plant mineral nutrition, water regime, photosynthesis, and morphogenesis as well as the common cell responses aimed at detoxification of heavy metals. The numerous nonspecific effects of heavy metals depend on their direct and indirect effect, in addition high Ni content in endoderm and pericycle cells blocks cell divisions in the pericycle and results in the inhibition of root branching as illustrated by Graca *et al.*, (2015) in Fig. 2.



**Fig. 1:** An overview of Ni essentiality and toxicity in plants. (After Babar *et al.*, 2018)

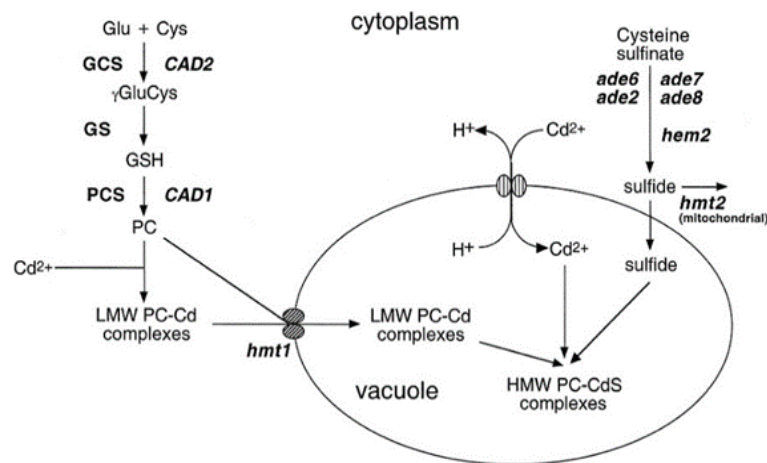


**Fig. 2:** Structure and development of the Arabidopsis root (after Graca *et al.* (2015).

(A) Median longitudinal section depicting developmental time (black arrow) in the longitudinal axis. A pre branch site (magenta) forms after an oscillation of gene expression within the oscillation zone (dotted line). Prebranch sites indicate competence to form a lateral root primordium (LRP) in the future. After competence is established, it is predicted that xylem pole pericycle (XPP) cells within a prebranch site can be specified as lateral root founder cells (LRFCs, green hatching). LRP initiate in the differentiation zone through asymmetric cell division of LRFCs, which gives rise to smaller cells (blue). (B) Transverse section. Periodic expression of DR5: GUS occurs in the protoxylem; however, because lateral root (LR) initiation occurs in the adjacent XPP cells, signaling between these cell types might be required for LRFC specification. Note that the ground tissue comprises two cell layers: the outermost cortex and the endodermis, which is immediately exterior to the pericycle. (C) Cut-away portion of the median longitudinal section focused on a region where an LR will form. XPP cells are predicted to be sequentially specified as LRFCs (green hatching), then activated to undergo cell division

(green/white hatching). LRFC activation results in the coordinated migration of nuclei (white circles) towards the common cell wall in a pair of longitudinally abutted cells. These cells then undergo asymmetric division, giving rise to smaller cells (blue), to generate a stage I LRP. The primordium grows through the outer cell layers of the primary root until it emerges from the epidermis. Drawing is not to scale

To minimize the detrimental effects of exposure to heavy metals (Cd as a model) and their accumulation, plants have evolved detoxification mechanisms that are mainly based on chelation and subcellular compartmentalization. Phytochelatins (PCs) are a principal class of heavy metal chelators in plants, which are synthesized without translation from reduced glutathione (GSH) in a transpeptidation reaction catalyzed by the enzyme Phytochelatins synthase (PCS). Therefore, the availability of glutathione is essential for PC synthesis in plants, at least during their exposure to heavy metals as explained by Yadav, (2010) in Fig 3.



**Fig. 3:** Illustrates genes and functions contributing to Cd detoxification in plants and fungi. The figure is a composite of different functions described in different organisms. Enzyme abbreviations are shown in bold. GS, GSH synthetase; PCS, Phytochelatins synthase. Gene loci are shown in bold italics. CAD1 and CAD2 are in Arabidopsis; hmt1, hmt2, ade2, ade6, ade7, and ade8 are in fission yeast; and hem2 is in *Candida glabrata*. The details of sulfide metabolism on the right of the figure are not well understood (After Yadav, (2010))

Diagnosis of plant nutrition disorder is often a very difficult task since there are many different causes for a given symptom, not all of which are caused by pathogenic organisms attack. Soil nutrition and texture, weather conditions, lighting and many other environmental and cultural conditions influence the overall health of a plant. Insect damage can sometimes be confused with plant diseases caused by microorganisms or abiotic factors. Knowing a complete history of the plant is essential to making an accurate diagnosis. In addition, a plant specimen should be in the early stages of disease-development when it is examined in order for an accurate diagnosis to be made. Once it has decayed, secondary organisms invade the tissue and evidence of the primary pathogen is often obscured.

For these reasons, it is difficult to construct an easy key for the diagnosis of plant problems. Even with the necessary laboratory equipment at one's disposal, it is often difficult to determine the exact cause of a plant's problem. The information provided is by no means comprehensive and other resources will be needed for many of your diagnoses. The Ortho Problem Solver is particularly useful as it contains color pictures. Since both dry weather and excess fertilizer can cause marginal leaf burn, you would want to ask the grower about recent rainfall and weather conditions in the area and fertilizer application, or, wilt can result from both dry and waterlogged soil, you would want to ask about rainfall and how well the soil drains.

Most visually appeared characteristics features of iron deficiency is chlorosis in young leaves, which caused by decreased chlorophyll biosynthesis Sharma (2007); Wang *et al.* (2012). Several reports

have shown that, those structural component that permit iron to act as an efficient catalyst and cofactor in cellular redox reactions, also have tendency to make it a potent toxin on similar structural chemical properties, when iron is taken up by plants in excess amounts of cellular needs, and as a consequence lead to overproduction of toxic oxygen radicals Olaleye *et al.* (2009); Gill and Tuteja (2010); Sharma *et al.* (2012). Moreover, elevated iron concentrations lead to enhanced oxidative stress and the excessive production of highly reactive and toxic oxygen species (ROS) Robello *et al.*(2007); Sharma *et al.* (2012). Reactive oxygen species are extremely destructive in nature because they seriously pose a threat to a variety of cellular components, including lipids, proteins, carbohydrates, and nucleic acids, thus due to their destructive activity leading to express diverse morphological, biochemical, and physiological alterations Fang *et al.* (2001); Gill and Tuteja (2010); Sharma *et al.* (2012). Stopping the uncontrolled oxidation caused by antioxidant enzymes is the only way to limit progressive oxidative damage.

Superoxide dismutase (SOD) plays a protective role against the damaging effects of ROS that requires Fe, Mn, Cu, and Zn as metal cofactors and is present in various cellular compartments, and is involved in the detoxification of  $O_2^{--}$  to  $H_2O_2$  and  $O_2$  Sinha and Saxena (2006); Sharma *et al.* (2012). In addition to SOD, CAT and peroxidases have been revealed to participate in this protective mechanism Costa *et al.* (2005); Sharma *et al.* (2012). Accelerated concentration of iron affects the uptake and accumulation of other mineral nutrients such as calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), and of iron itself Zhang *et al.* (1999). Hence, from the above-mentioned fact and recent findings, it can be concluded that the role of iron in plant metabolism is indispensable and hence efforts should be made to abolish iron (Fe) stress (deficiency or toxicity) for avoiding several nutritional disorders.

This response includes acidification of the rhizosphere by releasing protons, subsequent induction of  $Fe^{+3}$  chelate reductase activity that reduces  $Fe^{+3}$  to  $Fe^{+2}$ , and acquisition of  $Fe^{+2}$  across the plasma membrane of root epidermal cells Römheld, (1987). There are two types of causal relationships exist between the high concentration of heavy metals in soil and the expression of toxicity symptoms. Heavy metals compete with other essential mineral nutrients for uptake, thereby disturbing the mineral nutrition of plants Clarkson and Luttge, (1989). Then, after uptake by the plant, it accumulates in plant tissues and cell compartments and ultimately hampers the general metabolism of the plant Thurman and Collins, (1983); Taylor *et al.*, (1988); Turner, (1997).When plant is subjected to increased  $Fe^{+2}$  uptake and translocation by the plant, Fe toxicity appears. Moreover, Arora *et al.* (2002) stated about the elevated  $Fe^{+2}$  levels in plant induce the production of free radicals that causes membrane, DNA and proteins damages. Wu, (2016) also studied iron toxicity in rice plant.

Nickel is a component of some plant enzymes, most notably urease, which metabolizes urea nitrogen into useable ammonia within the plant. In woody ornamentals, symptoms occur in the spring in new emerging growth and may include shortened internodes (giving a rosetting appearance to the plant), weak shoot growth, death of terminal buds and eventual death of shoots and branches. In pecans, the symptoms are similar to woody ornamentals, but also include decreased expansion of the leaf blade and necrosis of the leaf tips. The leaves develop a condition called “mouse-ear” in which the leaflets are small with rounded tips vs. long and pointed Fig. (4). In plants exposed to higher amounts of nickel, leaf size and leaf area were decrease, which is also related to the accumulation of nickel in leaves. Accumulation of excess nickel plant tissues has been reported to cause leaf necrosis and chlorosis of plants Fig. (4) Chen *et al.* (1998).Chlorosis and vein necrosis appeared in newly developed leaves of water spinach after plants were treated with 0.085 to 0.255 mM (5–15 ppm Ni) for a week Rahman *et al.* (2005). Nickel at a concentration of 0.5 mM produced dark brown necrotic spots along the leaf margins resulting in wilting of outer leaves and necrosis of inner leaves in cabbage Pandey and Sharma (2002). Similarly, observed in barley grown in presence of 0.1 mM nickel for 14 days also showed chlorosis and necrosis of leaves Rahman *et al.* (2005). Such chlorosis of leaves results from decreased synthesis of chlorophyll due to deficiency of  $Fe^{+2}$  and  $Mg^{+2}$ plants Seregin and Kozhevnikova (2006).

Heavy metal accumulation in plants has multiple direct and indirect effects on plant growth, and it alters many physiological functions Woolhouse, (1983) by forming complexes with O, N, and S ligands Van Assche and Clijsters, (1990). They interfere with mineral uptake Yang *et al.*, (1998); Zhang *et al.*, (2002); Kim *et al.*, (2003); Shukla *et al.*, (2003); Drazic *et al.*, (2004); protein metabolism Tamas *et al.*, (1997), membrane function Quariti *et al.*, (1997); Azevedo *et al.*,(2005) Fig (4). In Leguminous crops, without nickel, toxic levels of urea can accumulate within the tissue forming necrotic legions on

the leaf tips (Fig. 5). In this case, nickel deficiency causes urea toxicity. Nickel is also used as a catalyst in enzymes used to help legumes fixing nitrogen. There is evidence that nickel helps with disease tolerance in plants, although it is still unclear how this happens. Minor nickel deficiency displays no visual symptoms, but can reduce growth and yield of plants. Significant nickel deficiency will display visual symptoms typically in the old leaves of the plants, as nickel is a mobile element. Deficiency symptoms in legumes are exhibited as whole leaf chlorosis along with necrotic leaf tips (caused by the accumulation of toxic levels of urea, (Fig. 5).

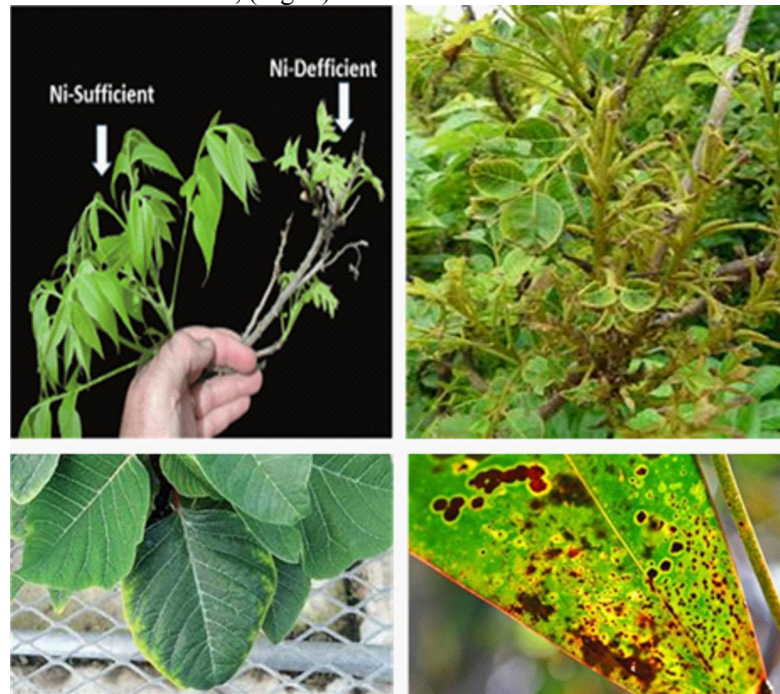


Fig. 4: Illustrates the most common sign of nickel sufficiency and deficiency (mouse-ear) in Pecan trees (After: [www.eplantscience.com](http://www.eplantscience.com) and Pandey and sharma (2002).

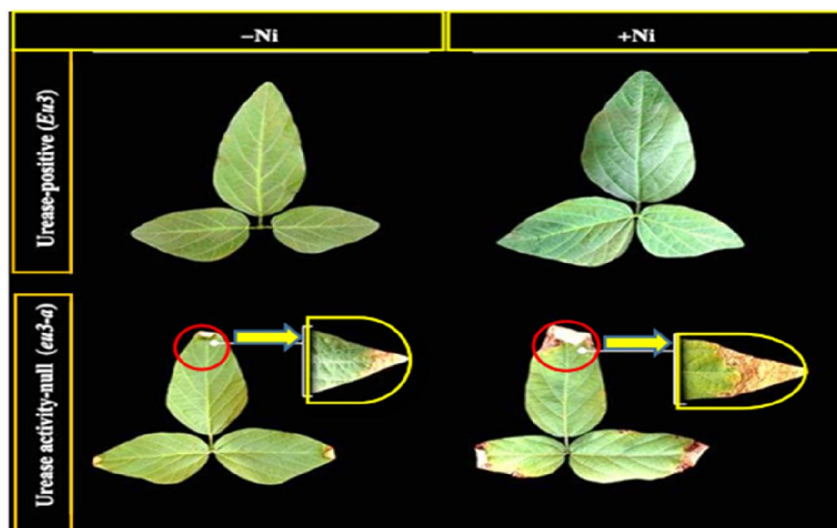


Fig. 5: Illustrates Contrast of leaves of two near-isogenic soybean lines at flowering stage, urease-positive (Eu3) and urease activity-null (eu3-a), fertilized with 0.0 and 0.5 mg Ni kg<sup>-1</sup>. Independently of Ni dose, Eu3 line developed normally while eu3-a line presented symptoms of hyponasty and initial necrosis lesions on leaflet tips. In eu3-a, these symptoms increased in the higher Ni dose due to excessive accumulation of urea (After Douglas *et al.* 2018).



Heavy metals play many significant physiological roles in plant such as the synthesis of protein, nucleic acids photosynthetic pigment, also involved in the structural and functional integrity of cell membranes particularly at low concentration Oves *et al.*, (2016). Nickel is a component of some plant enzymes, most notably urease, which metabolizes urea nitrogen into useable ammonia within the plant. Fe promotes N<sub>2</sub>-Fixation and DNA repair Møller *et al.*, (2007). While at high concentration it cause substitution of many essential functional groups, for instance, lipid peroxidation (LPO), cellular damage, generation of reactive oxygen species (ROS), disturbance in the various metabolic reaction by altering the enzymatic activity De Oliveira Jucoski *et al.*, (2013) ; Anjum *et al.*, (2015), Fig. (6).

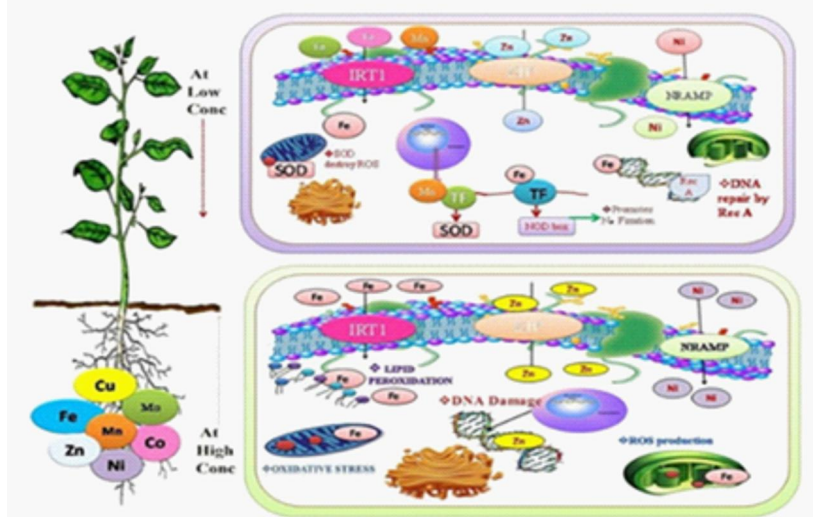


Fig. 6: Illustrates the significant roles of Heavy Metal (HMs) at low and high concentration

Because of the competition among various metals in the course of their uptake by roots, some metals are absorbed in insufficient quantities, whereas the uptake of other metals is excessive. Such situation would indirectly predetermine the effects of heavy metals on the various facets of metabolism, such as photosynthesis, respiration, electron transport inhibition, calvin cycle activation, chloroplast disorganization etc., and finally reduced crop yield, Fig. (7). It also seems important to compare the mechanisms of heavy metal accumulation, transport, toxicity, and detoxification in susceptible and tolerant plant species and populations.

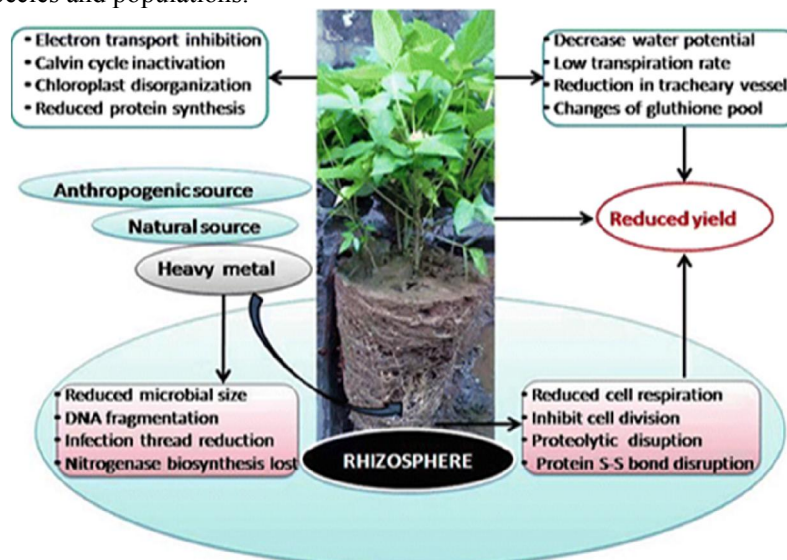
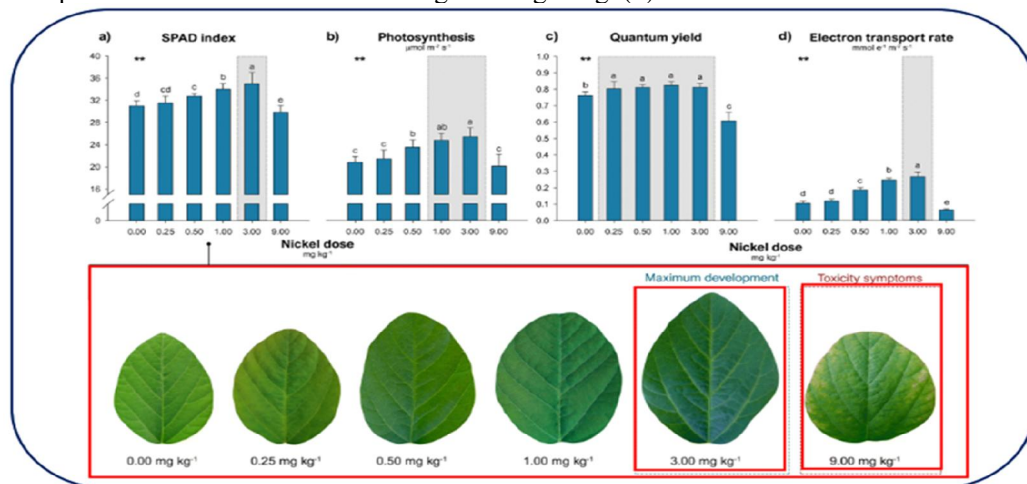


Fig. 7: Illustrates the harmful effect of heavy metals on various stages of plant metabolism (After Ahmad *et al.* (2012).

## 2- Heavy metals effects on plant growth

Nickel was as essential nutrient element for completion of plant life cycle. Its deficiency decreased the capacity of barley to develop viable seeds because of hindered embryo growth. The embryonic root developed poorly or even stayed undeveloped; in addition, several anomalies were reported in endosperm development together with declined dehydrogenase activities. The critical nickel concentration in barley tissues that reduced the yield by 15% was  $90 \pm 10$  ng/g dry wt Lal *et al.* (2010). Natural sources include weathering of rocks like metal mining, smelting, vehicle emissions, fossil fuel burning, municipal and industrial waste, electrical batteries, metallurgical and electroplating industries are anthropogenic sources. Generally, nickel is uniformly distributed through the soil profile but typically accumulates at the surface due to its mobility. The most common symptoms of nickel toxicity in plants are inhibition of growth, seed germination, photosynthesis, sugar transport Ali *et al.* (2009). and induction of chlorosis, necrosis and wilting Pandey and Sharma (2002).

Keeping in view increasing nickel toxicity to crop plants and significant importance of cereals, oilseeds, grain legumes and vegetables as source of low cost food. In plants, nickel is naturally present as an important constituent of some metalloenzymes including ureases, glyoxalases (family I), peptide deformylases, methyl Co-M reductase, hydrogenases and a few superoxide dismutases Lopez *et al.* (2011). It plays important role in various metabolic processes including urea hydrolysis, hydrogen metabolism, methane biogenesis and acetogenesis Mulrooney and Hausinger (2003). At low concentration, nickel enhances the growth and yield of plants and is essential for the biosynthesis of anthocyanin's Ragsdale (2011), Lopez *et al.* (2011). Nickel deficiency in soybean (*Glycine max* L.), leads to accumulation of toxic level of urea in their leaflet tips because of decrease in urease activity in the Eskew *et al.* (1983) as presented in Fig. (5). Douglas *et al.* (2018) stated that the most efficient N-urea metabolism was recorded in plants fertilized with  $3.0$  mg of Ni  $\text{kg}^{-1}$ . Soybean plants treated with different concentration of Ni - fertilization showed that higher urease activity following an increase in Ni doses up to  $3.0$  mg of Ni  $\text{kg}^{-1}$ , whereas a reduction in urea concentration in leaves was observed. Ammonia, a product of this pathway, had an increased concentration that was only evident in doses higher than  $0.5$  mg of Ni  $\text{kg}^{-1}$ . Metabolic changes in the biological  $\text{N}_2$  fixation process caused by Ni-fertilization, the maximum nitrogenase activity was recorded in plants treated with  $3.0$  mg of Ni  $\text{kg}^{-1}$ , with increased activity starting with the application of  $0.25$  mg of Ni  $\text{kg}^{-1}$ , and a reduction observed when plants were treated with  $9.0$  mg of Ni  $\text{kg}^{-1}$ . A red color was observed inside the nodules following nitrogenase activity. For other variables used to estimate biological  $\text{N}_2$  fixation, namely ureide concentration, and the number and dry weight of nodules, the maximum response was recorded in soybean plants treated with  $1.00$  and  $3.0$  mg of Ni  $\text{kg}^{-1}$  Fig. (8).



**Fig. 8:** Illustrates effect of applied six Ni doses to soil on physiological parameters: (a) SPAD index, (b) photosynthesis, (c) quantum yield, and (d) electron transport rate of soybean leaves (R1–R2). An increasing intensity in the green color of the leaves was observed with the increase in Ni doses, until a maximum was reached at a dose of  $3.00$  mg of Ni  $\text{kg}^{-1}$ , after which symptoms of Ni toxicity were observed (After Douglas *et al.* (2019).

Nickel uptake from soil may be driven by high constitutive expression of poorly selective ZRT-IRT-like (ZIP) transporters responsible for Fe or Zn uptake. Chelation of Ni by an appropriate ligand (L) in the root cytosol is thought to prevent cytotoxicity. The identity and universality of the ligands used are an ongoing topic of debate, but histidine or nicotianamine perform this role in some Brassicaceae Ni hyper accumulators Fig. (9). Formation of a Ni-ligand (Ni-L) complex may also impede the vacuolar sequestration of Ni in root tissues by tonoplast localized iron- regulated/ferroportin (IREG/FPN) transporters. Whether Ni is loaded into the xylem as the free cation or as a Ni-ligand complex is unclear, and the transporter (s) involved have not been identified, but the majority of Ni is present as the free cation in xylem sap.

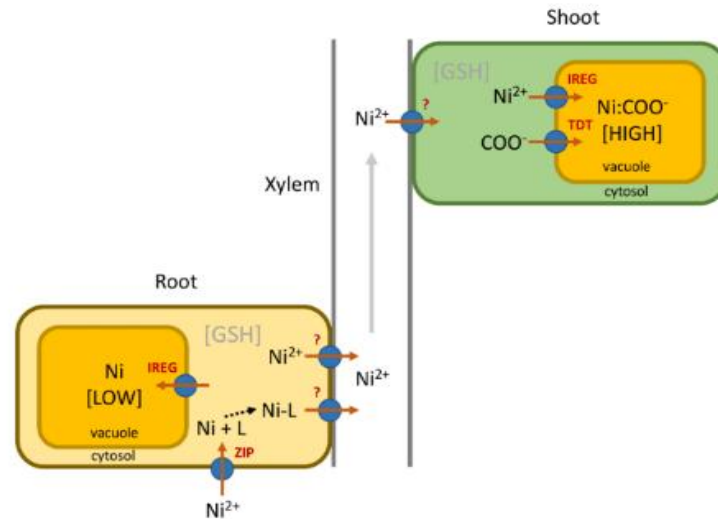


Fig. 9: Illustrates a general model for nickel hyper accumulation in plants.

The transporter(s) involved in xylem unloading into shoot cells is also unknown. Nickel is accumulated primarily in the shoot epidermis in most species, with the vacuole the major subcellular site of Nickel sequestration. Constitutively high expression of IREG/FPN transporters has been reported in Nickel hyper accumulators versus related non-accumulators across four families, and two of these transporters have been shown to drive vacuolar sequestration of Ni. In the vacuole, Ni is complexed by carboxylic acids (COO<sup>-</sup>), with Ni-citrate or Ni-malate the predominant complexes identified to date as presented in the previous.

Nickel hyper accumulating *Senecio coronatus* plants display greatly increased expression of the tonoplast dicarboxylate transporter (TDT) compared to non-accumulators. Ni hyper accumulators have enhanced capacity for the detoxification of reactive oxygen species, which may involve elevated concentrations of glutathione (GSH), flavonoids or increased activities of anti-oxidant enzymes.

Nickel deficiency is found associated with the reduced symbiotic hydrogenase activity in *Rhizobium leguminosarum* that may directly affect the symbiotic N<sub>2</sub> fixation Ureta (2005), Zobiolo *et al.*, (2009). Thus, nickel is an essential micronutrient for N<sub>2</sub> metabolism in plants. Excess of nickel adversely affects germination process and seedling growth traits of plants by hampering the activity of the enzymes such as amylase and protease as well as disrupting the hydroxylation of storage food in germinating seeds Aydinalp and Marinova (2009), Sethy and Ghosh (2013) Several studies in plants including maize Seregin *et al.* (2003) and cowpea Kopittke *et al.* (2007) have confirmed that Ni toxicity can result in inhibited lateral root formation and subsequent development. Khan and Khan. (2010) investigating the toxic effect of nickel and cobalt on chickpea (*Cicer arietinum* L.) showed that toxicity of Ni on the biomass production was more pronounced than Co and both metals led to poor germination, growth and biomass production, levels of nickel chlorophyll content and resulted in the reduced yield.

Root nodulation was suppressed and number of functional nodules appreciably decreased at higher Ni-content, Fig. (10). Al-Qurainy (2009) also demonstrated that Ni at the concentration 150



$\mu\text{g}\cdot\text{g}^{-1}$  of soil severely reduced biomass, root and shoot length, plant height and leaf area in *Phaseolus vulgaris*. The uptake of nickel in plants is carried out mainly by root system via passive diffusion and as well as active transport Seregin and Kozhevnikova (2006). However, the relative uptake mechanisms of nickel through active or passive transport differ with plant species, soil acidity, oxidation state, presence of other metals and availability of Nickel in the soil or nutrient medium Fageria *et al.* (2002), Tack (2010).

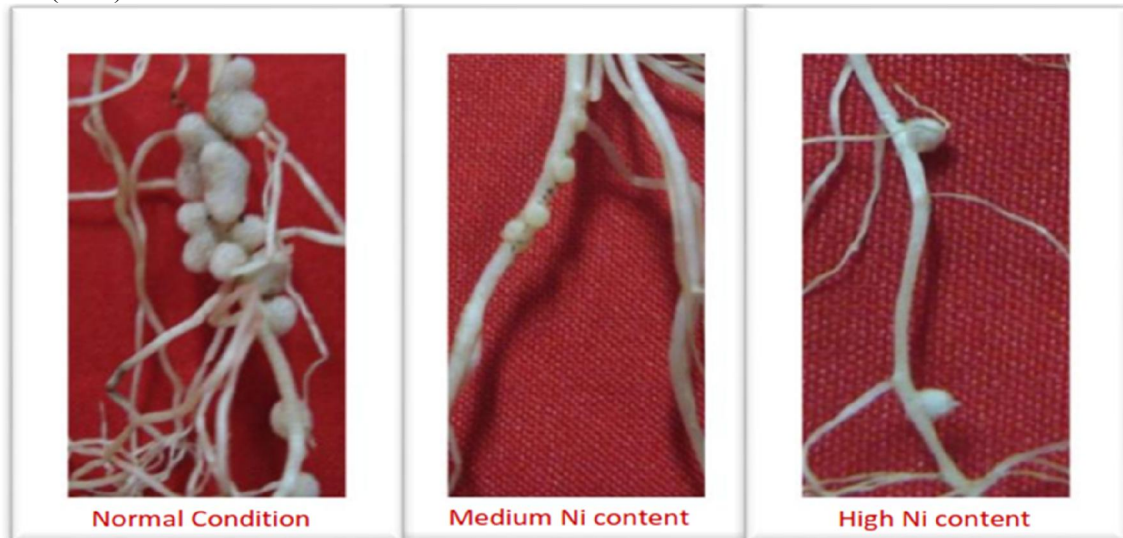


Fig. 10: Illustrates effect of Ni-content on root nodulation

For nodule dry weight, Douglas *et al.* (2018) stated that the responses of soybean plants to Ni fertilization started with the application of  $0.25\text{-mg kg}^{-1}$ , while for the other two variables, responses were only observed after the application of  $0.50\text{ mg of Ni. kg}^{-1}$ . When Ni distribution was taken into account, the nodule image overview 300 counts per second (cps) and the more detailed higher; 1500 cps revealed that Ni was concentrated in the central part of the nodules, namely the infection region, and, as the Ni doses increased, it extended to the nodule margins Fig. (11) 5a to 5f.

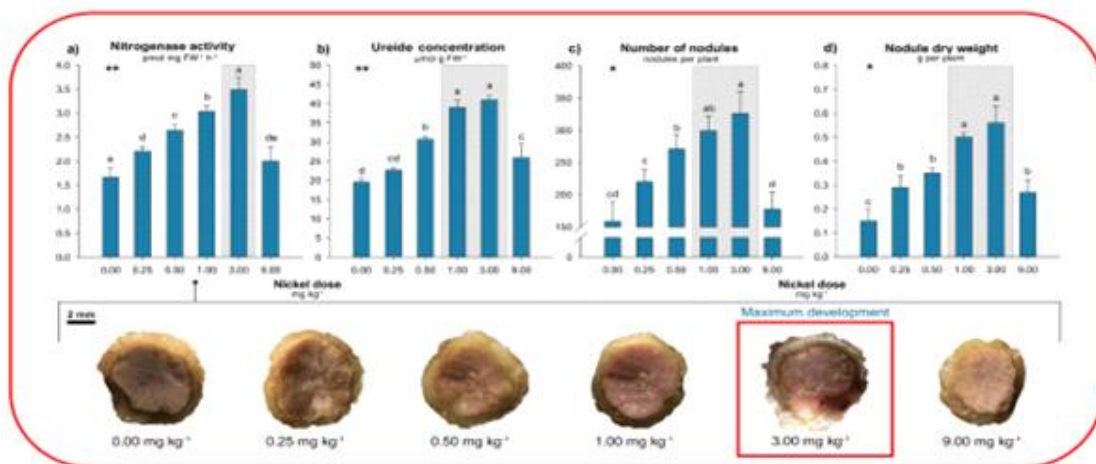
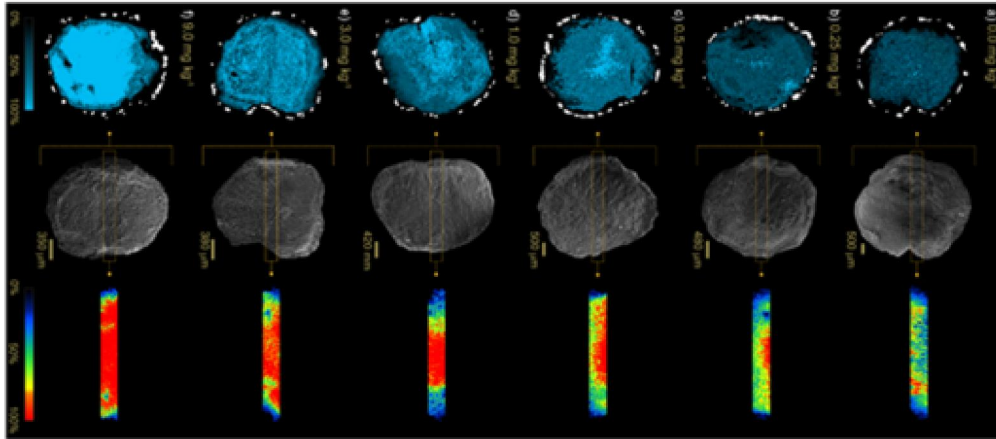


Fig. 11: Effect of applied six Ni doses to soil on biological  $\text{N}_2$  fixation parameters; (a) nitrogenase activity, (b) leaf ureide concentration, (c) number of nodules, and (d) nodule dry weight of soybean plants (R1-R2). A longitudinal section of the root nodules revealed an increasing intensity of red color due to increase in Ni doses up to  $3.00\text{ mg of Ni Kg}^{-1}$  as maximum doses, grey rectangles mark the Ni dose(s) that correspond to the most effective biological  $\text{N}_2$ -fixation (After Douglas *et al.* (2018).

Douglas *et al.* (2019) illustrated Ni-mapping on cross-sections of soybean root nodules in soil fertilized with six Ni doses (a-f) using synchrotron micro-X-ray fluorescence ( $\mu$ -SXRF) and scanning electron microscopy (SEM). (a) Shows the Ni mapping by  $\mu$ -SXRF at 300 counts per second (cps), in which the linear color scale reflects the intensity of Ni concentration and distribution, ranging from the minimum (black) to the maximum (cyan). Calcium (white) assessment was used only to limit the nodule epidermis; (b) shows the nodule structures using SEM (45 $\times$  magnification); (c) shows the high counting rate Ni mapping (1500 cps), detailing the Ni distribution on a strip across the central nodule region, ranging from the minimum (black) to the maximum (red) (Fig. 12).



**Fig. 12:** Illustrates effect of soil Ni- Fertilization on nickel mapping in cross-sections of soybean root nodules

Nickel may be delivered to roots by basipetal transport (primarily via epidermal and cortical cell layers) in the phloem Page and Feller (2005) and is then further Trans located into expanding leaves and root parts behind the meristem (growing tip) Riesen and Feller (2005). Nickel is rapidly redistributed to the youngest expanding plant parts throughout vegetative growth and the reproductive phase Page *et al.* (2006). Furthermore, the micro flora of soil may also enhance  $\text{Ni}^{2+}$  uptake by plants. In a study by Ma *et al.* (2009) nickel resistant, plant growth promoting bacteria (PGPB) *Psychrobacter* species have been reported to promote the plant growth and nickel uptake by Indian mustard *B. juncea* and Smooth-stem turnip *B. oxyrrhina* in soil contaminated with  $450 \text{ mg.kg}^{-1} \text{ Ni}^{2+}$ . Nickel availability usually declines at high pH values of the soil due to formation of less soluble complexes, in a study with *Lathyrus sativus* uptake of nickel was reported to increase up to pH 5.0 and then progressively decrease as pH reached up to 8.0 Panda *et al.* (2007). Organic acids and other dissolved organic matters, enhance the solubility of  $\text{Ni}^{2+}$  in soil and furthermore uptake by plant.

Iron is the limiting nutrient for plant growth and metabolism, due to the low solubility of the oxidized ferric form in aerobic condition Zuo and Zhang, (2011); Samaranayke *et al.*, (2012). Iron deficiency is a common nutritional disorder in many crops (Fig. 13), resulting in poor yields and reduced nutritional quality. Iron is involved in chlorophyll synthesis, and it is essential for the maintenance of chloroplast structure and function, iron is generally present at high quantities in soils; however, its bioavailability in aerobic and neutral pH environments is limited.

In aerobic soils, iron is predominantly found in the  $\text{Fe}^{+3}$  form, mainly as a constituent of oxhydroxide polymers with extremely low solubility. In most cases, this form does not sufficiently meet plant needs. The visual symptoms of inadequate iron nutrition in higher plants are interveinal chlorosis of young leaves (Fig. 13) and stunted root growth.

In waterlogged soils, the concentration of soluble iron may increase by several orders of magnitude because of low redox potential Schmidt, (1993). Under such conditions, iron may be taken up in excessive quantities. However, it is potentially toxic and can promote the formation of reactive oxygen-based radicals, which are able to damage vital cellular constituents (e.g., membranes) by lipid peroxidation. Bronzing (coalesced tissue necrosis, Fig. 14), and/or blackening of the roots as symptoms of plants exposed to above-optimal iron levels Laan *et al.*, (1991).



After Pandey and Sharma (2002).



Fig. 13: Illustrates Chlorosis of young leaves is often the first visual sign of iron deficiency



Fig. 14: Illustrates Fe-toxicity (After Rahman *et al.* (2005)

Generation of reactive oxygen species where MPO is myeloperoxidase and SOD is superoxide dismutase. In Fenton reaction ferrous iron reacts with hydrogen peroxide to produce the (A) hydroxyl radical ( $\text{HO}^\bullet$ ) or (B) lipid alkoxy ( $\text{RO}^\bullet$ ) Ferric ion from the reaction can be reduced back to ferrous iron (a) in the presence of superoxide (Haber-Weiss reaction). All the enzymes involved in these reactions are iron-dependent except glutathione peroxidase and peroxiredoxin Fig (15).

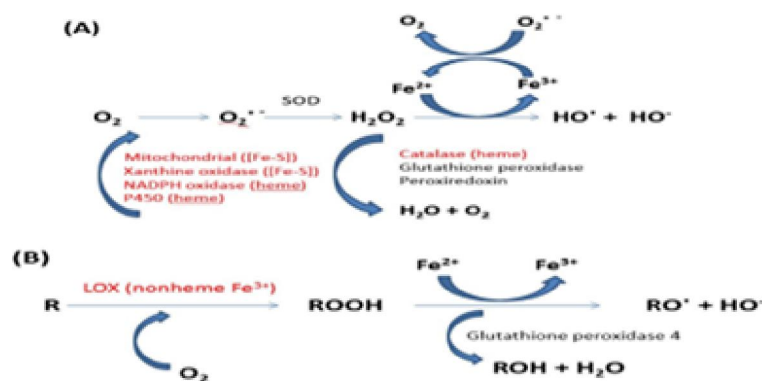
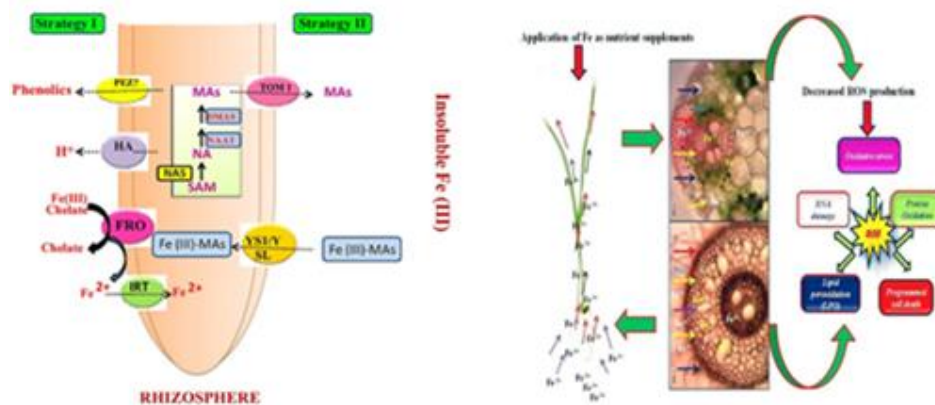


Fig. 15: Illustrates the role of iron in ROS metabolism. Adapted from Dixon and Stockwell (2014)

Iron predominantly exists, as  $\text{Fe}^{+3}$ chelate forms in the soil and plants ultimately cannot absorb it under some physiological conditions such as high soil pH in alkaline soils. Thus, plants growing in high-pH soils are not efficient in developing and stabilizing chlorophyll, resulting in the yellowing of



leaves, poor growth, and reduced yield. However, some plants have developed sophisticated mechanisms to be able to uptake small amounts of soluble iron. Non-graminaceous plants release protons, secrete phenolics to reduce  $\text{Fe}^{+3}$  and take it up Römheld and Marschner, (1983); Marschner, (1995a); Jeong and Guerinot, (2009); Cesco *et al.*, (2010) Fig (16). Once iron is solubilized,  $\text{Fe}^{+3}$  is reduced to  $\text{Fe}^{+2}$  by a membrane-bound  $\text{Fe}^{+3}$  reductase oxidase Jeong and Connolly, (2009).  $\text{Fe}^{+2}$  is transported into the root by an iron-regulated transporter (IRT1). Ishimaru *et al.* (2012) reported that graminaceous plants rely on a  $\text{Fe}^{+3}$ - chelation system during the secretion of mugineic acid (MA) family phytosiderophores. MAs are secreted to the rhizosphere through TOM1, and they then chelate  $\text{Fe}^{+3}$ . In rice, the yellow stripe family transporters (OsYSL15) transport the resulting  $\text{Fe}$ -MA complex Nozoye *et al.*, (2011). Rice plants also have the ability to uptake the iron transporter Ishimaru *et al.*, (2006). Rout *et al.* (2014) screened 51 varieties of upland and lowland rice using different levels of iron (0, 50, 100, and 200 mM) in nutrient solution to study the toxicity effect. They found that, out of the studied 51 varieties, only 16 varieties were tolerant (>200 mM Fe), 11 exhibited medium tolerance (<200 mM Fe), and 24 varieties were susceptible (<100 mM) to tested iron concentrations. Although total chlorophyll, total proline, total phenol, total protein, and total carbohydrate contents showed variation in both tolerant and susceptible varieties. The oxidative enzymes also showed variation among the tolerant and non-tolerant genotypes.



**Fig. 16:** Illustrates Different Strategies Adopted by Higher Plant for Iron Acquisition (After: Kobayshi and Nishizawa, (2012)

Typically, approximately 80% of iron is found in photosynthetic cells where it is essential for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters Briat *et al.*, (2007); Hansch and Mendel, (2009). In the photosynthetic apparatus, two or three iron atoms are found in molecules directly related to photosystem II (PS-II), 12 atoms in photosystem I (PS-I), five in the cytochrome complex, and two in the ferredoxin molecule Varotto *et al.*, (2002). Such distributions show that iron is directly involved in the photosynthetic activity of plants and, consequently, their productivity Briat *et al.*, (2007). Iron-sulphur (Fe-S) clusters have long been recognized as essential and versatile cofactors of proteins involved in catalysis, electron transport and sensing of ambient conditions (Fig. 17) The importance of Fe-S proteins for life is documented by an increasing number of diseases linked to these components and their biogenesis Rolland (2009).

Iron availability is assumed to affect the natural distribution of species, and it may limit the growth of fast-growing economically important plants Chen and Barak, (1982). Iron can also be potentially toxic at high concentrations. The ability of iron to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and they can ultimately kill the cell Crichton *et al.*, (2002). To prevent that kind of damage, life forms have evolved a biochemical protection mechanism by binding iron atoms to proteins.



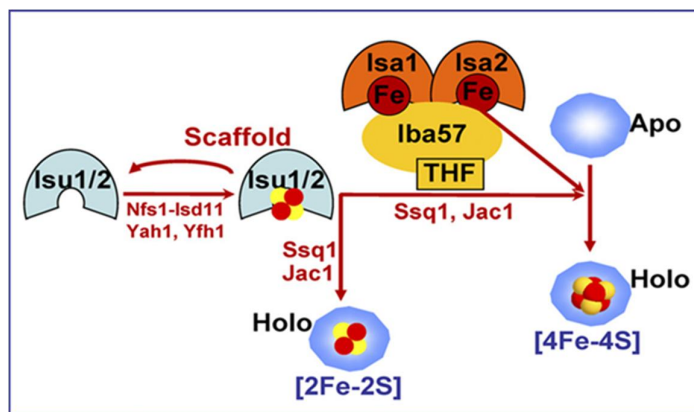


Fig. 17: Model of iron-sulfur cluster biogenesis.

### 3-Morphological and biochemical effects

High doses of nickel negatively affect plant growth and physiological processes and induce visible toxicity symptoms. Most of the morphological parameters such as root and shoot length, root nodules, leaf area, fresh weight and dry weight, chlorophylls, carotenoids, total sugar, amino acid, proline and protein contents decrease with increasing nickel chloride concentration Kaveriammal and, Subramani (2013). The reason for decrease in all these parameters could also be the reduction in cell division in meristematic cells present in region and activity of certain enzymes of cotyledon and endosperm.

In the soil, the insoluble ferric ( $\text{Fe}^{+3}$ ) form is reduced, it is converted to ferrous form ( $\text{Fe}^{+2}$ ), and is then absorbed by plants. Even though iron is hardly present in living matter ( $50\text{--}100 \mu\text{g}\cdot\text{g}^{-1}$  dry matter), it is still an essential element that is critical for plant life Guerinot and Yi, (1994), as this element is involved in plant metabolism. As a critical component of proteins and enzymes, iron plays a significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation, uptake mechanisms Kim and Rees, (1992), and DNA synthesis through the action of the rib- nucleotide reductase Reichard, (1993). It is also an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase, 1-aminocyclopropane acid-1-carboxylic oxidase Siedow, (1991), or abscisic acid (compounds that are active in many plant development pathways and their adaptive responses to fluctuating environment conditions). Iron deficiency severely affects plant development and growth, and excess iron in cells is toxic. Iron reactivity with reduced forms of oxygen produces radical elements that can cause a loss of integrity and kill the cell. Therefore, there is an optimal window for the iron concentration to ensure smooth plant development. Iron is a constituent of all redox systems in plants, and the best-known examples are enzyme systems (heme iron structure, Fig. 18), including prosthetic groups like cytochromes.



Fig. 18: Illustrates the heme iron structure with four pyrroles

Iron plays a role in the porphyrin structure of chlorophyll, and is therefore, a principal component of chloroplasts. The best-known functions of cytochrome are electron transport and the involvement of cytochrome oxidase in the terminal step in the respiration chain. Iron also interacts with non-heme proteins as an iron-sulfur protein (e.g., ferredoxin, superoxide dismutase Fig. (20)). Iron is a major component of plant redox systems. Because of its physicochemical properties, especially its affinity to active metalloprotein sites, it acts as a cofactor in redox reactions that are necessary for oxygen production and use. However, its best-known function is its structural role in the prosthetic groups of enzyme systems such as cytochromes, catalases, and peroxidases. These enzymes are also the main

components of chloroplasts and mitochondria Mengel and Kirby, (1987), Marschner, (1995b). The cytochrome essentially acts as an electron carrier in the respiratory chain.

Bashir *et al.* (2011a) explain the respiration chain and protein complexes by four complexes (Complex I to start, two electrons are carried to the first complex aboard NADH). This complex, labeled I, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B<sub>2</sub>, also called riboflavin, is one of several prosthetic groups or co-factors in the electron transport chain. A prosthetic group is a non-protein molecule required for the activity of a protein. Prosthetic groups are organic or inorganic, non-peptide molecules bound to a protein that facilitate its function; prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the inter membrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane. Q and Complex II directly receive FADH<sub>2</sub>, which does not pass through complex I. The compound connecting the first and second complexes to the third is ubiquinone (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced, (QH<sub>2</sub>), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I and the electrons derived from FADH<sub>2</sub> from complex II, including succinate dehydrogenase. This enzyme and FADH<sub>2</sub> form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the FADH<sub>2</sub> electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane, Fig 19.

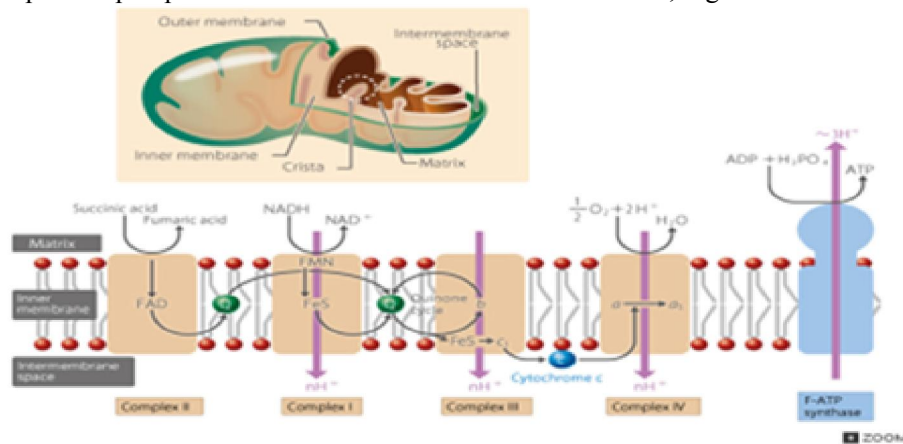


Fig. 19: Structure and function of mitochondrial membrane protein complexes. (After Bashir *et al.* (2011a)

Mitochondrion (top), and respiration chain and protein complex of ATP synthase in the inner membrane of mitochondria (bottom)Q, which indicates an electron transport component, is a ubiquinone; *c*, *c*<sub>1</sub>, *b*, *a*, and *a*<sub>3</sub> are hemes (cytochrome cofactors); and FeS is an iron-sulfur cluster cofactor. FMN is a flavin mononucleotide, and FAD is a flavin adenine dinucleotide. Reduced Q generated by Complex II also passes electrons on to Complex III via the quinone cycle. Since the mechanism of H<sup>+</sup> transport is not fully understood, the number of H<sup>+</sup> ions transported by each complex is expressed as *n*. Overall, approximately three molecules of ATP are synthesized through the complete oxidation of one NADH molecule, and the sum of *n* is approximately 9 in this case, Bashir *et al.*, (2011a).

Complex III The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin (Fig. 18), but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced

and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe<sup>+2</sup> (reduced) and Fe<sup>+3</sup> (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes (cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time), Fig. 19, Bashir *et al.*, (2011a).

Complex IV The fourth complex is composed of cytochrome proteins c, a, and a<sub>3</sub>. This complex contains two heme groups (one in each of the two cytochromes, a, and a<sub>3</sub>) and three copper ions (a pair of CuA and one CuB in cytochrome a<sub>3</sub>). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water (H<sub>2</sub>O). The removal of the hydrogen ions from the system contributes to the ion gradient used in the process of chemiosmosis, Fig. 19, Bashir *et al.*, (2011a).

Iron also interacts with non-heminic proteins such as those in the iron sulfide group (e.g., ferredoxin, superoxide dismutase). Moreover, iron is also active in protein synthesis Bennet, (1945; Perur *et al.*, (1961). Bashir *et al.* (2011b) identified and characterized the mitochondrial iron transporter (MIT) in rice after screening 3993 mutant lines. The identification of MIT is a significant advance in the field of plant iron nutrition, and it should facilitate the cloning of paralogs from other plant species. Iron deficiency-induced chlorosis is a major problem in plants, and it affects both yield and crop quality. Plants have evolved multifaceted strategies, such as reductase activity, protons, and specialized storage proteins to mobilize iron from the environment and to distribute it within the plant. The activation of iron uptake reactions requires an overall adaptation of the primary metabolism because these activities need a constant supply of energetic substrates (i.e., NADPH and ATP). Vigani (2012) reported that mitochondria are the energetic centers of the root cell, and they are strongly affected by iron deficiency. They observed that they display a high level of functional flexibility, which allows them to maintain the cell viability. Mitochondria contain a large amount of tall proteins that require iron to carry out their function Bertini and Rosato, (2007). In fact, several enzymes belonging to both the respiratory chain and to the tricarboxylic acid cycle are iron-containing proteins. Moreover, crucial steps of the Fe-S cluster assembly for the entire cell take place in the mitochondria, suggesting an important role for this compartment in iron handling by the cell. Iron deficiency and mitochondria are strongly linked because this stress greatly induces Strategy I activities that demand considerable energy, thereby requiring the participation of mitochondria. Mitochondria represent a crucial target of studies on plant homeostasis, and it might be of interest to concentrate future research on understanding how mitochondria orchestrate the reprogramming of root cell metabolism under iron deficiency. Overall, root mitochondria are strongly affected by iron starvation, because they require at least forty iron atoms per respiratory unit to function, Vigani *et al.*, (2009). A schematic cross-section of a mitochondrion is shown in Fig. (20) , with the proteins and protein complexes that are involved in various cellular pathways and processes. Complexes I-IV are the complexes of the respiratory chain. AIF, apoptosis-inducing factor; Cyt c, cytochrome c; i-AAA and m-AAA, proteases of the AAA family in the intermembrane space and matrix, respectively; ISC, iron-sulfur cluster assembly complex; Mfn, mitofusin; TIM, translocase of the inner membrane; TOB, topogenesis of mitochondrial outer membrane  $\beta$ -barrel proteins; TOM, translocase of the outer membrane; VDAC, voltage-dependent anion channel; Opa1, Optic Atrophy 1, Dimmer and Raport, (2008). The ABC transporter STA1/AtATM3 has been characterized in Arabidopsis and implicated in the export of Fe-S clusters Kushnir *et al.*, (2001). In addition, ferric-chelate reductases, encoded by FRO genes in plants, might be involved in mitochondrial iron transport because AtFRO3 and AtFRO8, mainly located in root and shoot veins, respectively, contain mitochondria targeting sequences Mukherjee *et al.*, (2006). This suggests that a reduction-based uptake could also occur in the invagilated inner membrane of mitochondria, which consist of four compartments: outer membrane (OM), inter membrane space (IMS), inner membrane (IM) and matrix. A large variety of functions have been assigned to mitochondrial proteins and protein complexes and are indicated , energy metabolism with respiration and synthesis of ATP; metabolism of amino acids, lipids and nucleotides; biosynthesis of iron-sulfur (Fe-S) clusters and cofactors; expression of the mitochondrial genome; quality control and degradation processes including mitophagy and apoptosis; signalling and redox processes; membrane architecture

and dynamics; and the import and processing of precursor proteins that are synthesized on cytosolic ribosomes. AAA, ATP-dependent proteases of the inner membrane; E3, ubiquitin-protein ligase; ER, endoplasmic reticulum; mt DNA, mitochondrial DNA; TCA, tricarboxylic acid; Ub, ubiquitin.

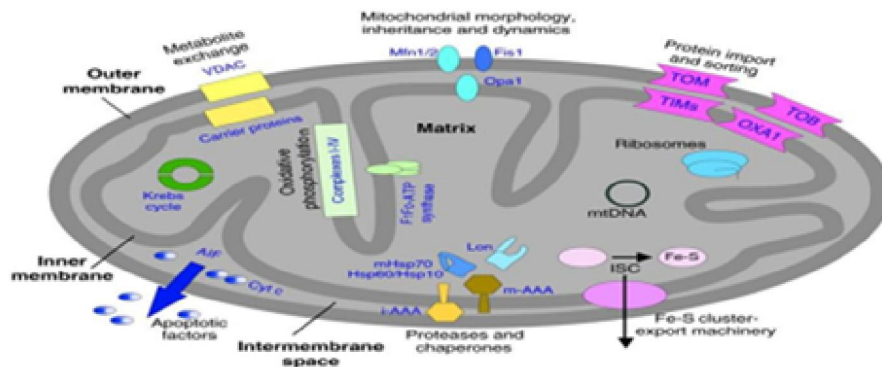


Fig. 20: Overview of mitochondria-related processes. After: Dimmer & Raport, (2008)

Moreover, the oxidizing conditions found in the Mitochondrial intermembrane space (IMS) are indicative of the need for a reduction-based mechanism Hu *et al.*, (2008); Abadía *et al.*, (2011). Because of iron's redox properties and its ability to form complexes with diverse ligands, this element is a constituent of many electron carriers and enzymes; therefore, it plays an important role in plant metabolism.

Although, low solubility of inorganic iron at physiological pH levels and its high reactivity in the presence of oxygen, which generates toxic hydroxyl radicals; represent a severe difficulty Hell and Stephan, (2003). Soil conditions that result in insufficient or excess iron uptake are widespread in nature Snowden and Wheeler, (1993); Schmidt and Fuhner, (1998); De Dorlodot *et al.* (2005). Chlorosis of young leaves is often the first visual sign of iron deficiency. It is not only associated with the loss of chlorophyll, as several steps of its biosynthesis depend on iron, but also with changes in the expression and assembly of other components of the photosynthetic apparatus Conte and El Sbeth (2011).

In recent year's chlorophyll fluorescence analyses have been applied as rapid non-destructive tools to obtain information about the state of photosynthetic apparatuses under iron deficiency or toxicity, and this is especially true regarding photosystem II (PS II). It has been demonstrated that photo protection through excessive light dissipation as well as reversible photosynthesis might lead to down-regulation and sustained photo inhibitory damage Mori and Nishizawa (2004), Kobayashi *et al.* (2010); Gill and Tuteja (2010); Donnini *et al.*, (2010), Nozoye *et al.* (2013). They also found that in pea plants, the excess iron resulted in increased pigment concentrations of up to 28%. Some changes in the pigment ratios were also observed, suggesting that their increased content could not only be attributed to concentration effects due to inhibited growth. Singh *et al.* (2016) also found evidence of photo inhibition in pea plants. The tendency of iron to form chelates and to undergo changes ( $Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$ ) are the two important characteristics underlying its numerous physiological effects. The best-known function of iron is in the enzyme systems in which heme or hemin function as prosthetic groups Mengel and Kirkby, (1987). Here, iron plays a somewhat similar role to magnesium in the porphyrin structure of chlorophyll. These heme enzyme systems include catalase, peroxidase, cytochrome oxidase, and various cytochromes, but the role of these enzymes in plant metabolism is not completely understood. Research has also provided information about the function of cytochromes in electron transport and the involvement of cytochrome oxidase in the terminal step of the respiration chain. Catalase brings about the breakdown of  $H_2O_2$  to water and  $\frac{1}{2} O_2$  Fig. (15). This enzyme also plays an important role in chloroplasts (along with the enzyme superoxide dismutase), photorespiration, and the glycolytic pathway. Cell wall-bound peroxidases appear to catalyze reactions during the polymerization of phenol to lignin, and peroxidase activity seems to be particularly depressed in iron deficient roots. Cell wall formation and lignification were impaired, and phenolics accumulated in the rhizodermis of iron-deficient sunflower roots Romheld and Marschner, (1981). Phenolics can be released into the external solution, and, in the case of caffeic acid, may bring about chelation and reduction of inorganic  $Fe^{3+}$  Olsen *et al.*, (1981), Romheld and Marschner (1985), Sharma *et al.* (2013).

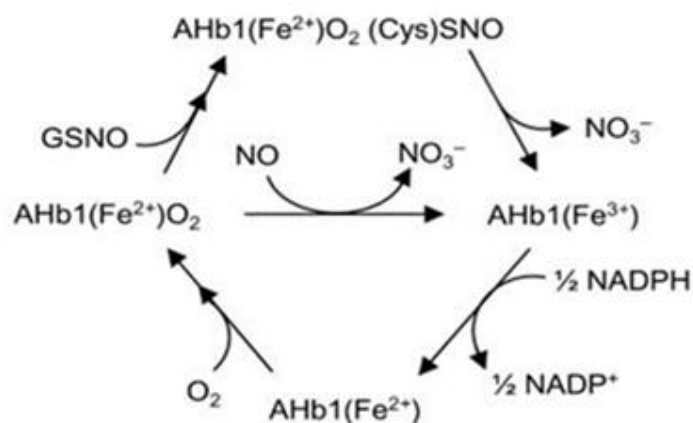


This reaction is not favored by the accumulation of phenolics, but also by an increase in the reducing capacity of the roots, which accompanies the impairment of lignin synthesis. Barton (1970) observed large quantities of phytoferritin in chloroplasts, which confirmed earlier evidence that chloroplasts are rich in iron, containing as much as 80% of the total iron in plants. Like heme iron, Fe-S proteins play a major role in oxide reduction, and, both binuclear Fe-S clusters (2Fe-2S) and tetra nuclear Fe-S clusters (4Fe-4S) occur. Four cysteine residues associated with a polypeptide chain Singh *et al.* (2016) surround each cluster.

Fig. (20) Showed that in the mitochondria, the complex of Nfs1 and Isd11 functions as a cysteine desulfurase to generate the sulfur needed for biogenesis. An Fe/S cluster is then assembled on the Isu1 scaffold, with ferrous iron transported into the organelle by Mrs3/4 and possibly delivered by Yfh1. Presumably, the electron transfer chain from NADH to the ferredoxin Yah1 serves to reduce the sulfur atom to sulfide. A chaperone system, which includes the Hsp70-like protein Ssq1, the J-type cochaperone Jac1, and the nucleotide exchange factor Mge1, transfers de novo assembled clusters from the scaffold to apo-proteins. Grx5 also participates in some capacity in this cluster delivery system. Isa1, Isa2, and Iba57 all have a specific role in the maturation of a subset of apoproteins, which includes aconitase-type proteins and radical-SAM enzymes. The ISC export machinery, comprised of the ABC transporter, Atm1, the inter membrane space sulfhydryl oxidase, Erv1, and glutathione (GSH) moves an intermediate (X) produced by the mitochondrial ISC system to the cytosol. This intermediate facilitates Fe/S protein maturation in the cytosol and nucleus, which further requires the cytosolic Fe/S protein assembly (CIA) machinery. In this pathway, a transient Fe/S cluster is initially formed on the complex of the P-loop NTPases, Cfd1 and Nbp35, which thus serve as scaffolds for Fe/S cluster assembly. Delivery of the Fe/S clusters from the scaffold complex to apo-proteins requires the hydrogenase-like protein Nar1 and the WD40 repeat protein Cia1.

Iron and chlorophyll concentrations are often well correlated in green plants. The same metabolic pathway involved in chlorophyll formation also operates during the biosynthesis of heme. Iron appears to control the rate of delta aminolevulinic acid (ALA) formation, which is the precursor of porphyrins Miller *et al.*, (2013). During iron deficiency, a decrease occurred in the condensation rate of glycine and succinyl-CoA to form ALA. In iron-deficient leaves, the photosynthetic apparatus remained intact, but the number of photosynthetic units decreased Terry, (1980). Iron is also involved in protein metabolism. During iron deficiency, the protein fraction decreases simultaneously with an increase in the level of soluble organic N compounds. Nitric oxide (NO) is a bioactive molecule that has recently emerged as a cellular messenger in numerous physiological processes in plants Neil *et al.*, (2003). Nitric oxide NO is biologically active at a concentration of  $1 \text{ nmol} \cdot \text{L}^{-1}$ , and it participates in signaling cascades that drive plant growth and developmental processes Beligni and Lamatting., (2000 a&b). It is known that NO displays a high affinity towards, and reacts with, transition metals. The three redox-related states of biochemically distinguishable NO species, nitroxyl anion ( $\text{NO}^-$ ) nitric oxide radical (NO), and nitro sodium cation ( $\text{NO}^+$ ), can react with the ferric ( $\text{Fe}^{3+}$ ) and/or ferrous ( $\text{Fe}^{2+}$ ) forms of iron Perazzolli *et al.*, (2004), Fig. 21. The finding that hexacoordinate hemoglobins are upregulated by similar conditions in both plants and animals supports the intriguing hypothesis that these proteins share a common function.

It was found that the nonsymbiotic hexacoordinate hemoglobin AHb1 from *Arabidopsis* metabolizes both NO and GSNO. The nitrate forming reactions involve an Fe(III) intermediate, which is efficiently reduced by NADPH, and the reaction of AHb1 with GSNO involves S-nitrosylation of the protein (Fig. 21). Moreover, S-nitrosylated AHb1 is endogenously produced in plants, thus indicating that AHb1 scavenges NO in vivo through production of S-nitrosohemoglobin. This article reports S-nitrosylation of a plant hemoglobin, suggesting a conserved role of hemoglobin S-nitrosothiols in processing NO and NO-donating SNO across humans, nematodes, and plants, Perazzolli *et al.*, (2004).



**Fig. 21:** Illustrates Proposed Reaction Cycle for NO Metabolism by Nonsymbiotic Hexacoordinate Hemoglobin (AHb1) from Arabidopsis plant (After: Perzzolli *et al.*, 2004)

The complexes formed between iron and NO are called iron nitrosyl complexes Stamler *et al.*, (1992); Stamler *et al.*, (1997), and these interactions are central in NO biochemistry. NO can form iron-nitrosyl complexes in vivo with Fe-S and heme protein centers that are important for the biological activity of NO Wink *et al.*, (1998). The Fe-S cluster plays an important role in iron homeostasis because it can act as an iron sensor that regulates protein expression associated with iron balance such as ferritin and the transferrin receptor in mammals Beinert *et al.*, (1997). In addition, Fe-S proteins are viewed as carrier proteins that avoid the toxicity associated with free iron and allow its delivery at lower intracellular concentrations Mansy *et al.*, (2008). The redox related NO species could have simultaneous effects on cellular iron metabolism and homeostasis via mechanisms that might involve S-nitrosylation and/or the ligation of NO to Fe-S clusters Richardson *et al.*, (1995). The uptake of iron, translocation, and regulation in higher plants has been reviewed Kobayashi and Nishizawa, (2012), and they reported that higher plants have developed two distinct strategies to acquire slightly soluble iron from the rhizosphere. Key molecular components, including transporters, enzymes, and chelators, have been clarified for both strategies, and many of these components are now thought to also function inside the plant to facilitate internal iron transport.

## Conclusion

Heavy metal stress is one of the major problems affecting agricultural productivity of crops. Natural flora show relative differences in their heavy metal tolerance capacity. Some of them grow well in a soil enriched with toxic levels of heavy metals while others could not grow. Heavy metal plays a critical role in life sustaining processes. Due to its ability to gain and lose electrons, some heavy metals such as iron works as a cofactor for enzymes involved in a wide variety of oxidation-reduction reactions (photosynthesis, respiration, hormone synthesis, DNA synthesis,). This function makes the heavy metals; Ni and iron as essential micronutrients when found in the growing media with an optimum concentration, and its deficiency and /or toxicity causes chlorosis, necrosis symptoms, which seriously constrains normal plant development. Large amounts of heavy metals especially nickel and iron will display plants to stress and adversely affected the plants through protein degradation, due to the formation of reactive oxygen radicals that are highly phytotoxic and cause protein degradation. Glutathione is involved in detoxifying ROS through ascorbate–glutathione cycle, while accumulated nickel and iron ions are detoxified by phytochelatin, which are synthesized from glutathione in plants during their exposure to nickel and iron stress. The scientific observations on some plants have indicated that glutathione is a major player determining their relative tolerance to heavy metals stress. This mechanism of heavy metal tolerance in plants has strongly suggested that glutathione should not be limiting. Therefore, attempts have been made to generate transgenic plants using several different genes regulating glutathione levels in plants. Particularly, role of glutathione, phytochelatin, cysteine synthesis and glyoxalase pathway genes have been reported in imparting heavy metal stress tolerance. Additionally, several natural heavy metal accumulator plants species have been identified. Initial

indications in such plants documented the involvement of glutathione in the mechanism of heavy metal stress tolerance. These natural heavy metal accumulators' plants could be a potential source for genetic manipulation of other important agricultural crop plants. Higher heavy metals uptake by plants may reduce protein synthesis in leaves. Ferritin is considered crucial for iron homeostasis, and it consists of a multimeric spherical protein called phytoferritin, which is able to store up to 4500 iron atoms inside its cavity in non-toxic form. Chlorosis is a widespread problem, especially for regions where the bioavailability of heavy metals, especially Ni and Fe in the soil are low. Thus, genetically improved chlorosis resistant rootstocks and new cultivar combinations offer the best solution to iron chlorosis, and it is one of the most important lines of investigation needed to prevent this nutritional problem. Therefore, there is a need for new methods to diagnose and correct this nutritional disorder. These studies expanded our knowledge of stress responses to heavy metal deficiency, and identified the filter and processes for further trials to increase our understanding of heavy metals stress, especially iron and nickel. This will contribute greatly to understanding the regulatory mechanisms, and these factors will prove beneficial for plants under the stress conditions of heavy metal and especially for nickel and iron.

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