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Research Paper

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR MECHANISMS OF ZINC UPTAKE, TOXICITY AND TOLERANCE IN PLANTS

Gagan Preet Singh Sidhu

Department of Environment Studies,
Panjab University, Chandigarh 160014,
India.

Abstract

Zinc (Zn), a vital trace element is indispensable for the living biota including both plants and animals for their growth and physiological processes. It is the 23rd most abundant metal found on earth with a stable oxidation state of +2 (Zn II). Zinc uptake in plants occurs as bivalent cation or by forming complex with organic ligands via xylem of roots which is later translocated to shoots of the plants. Its presence activates a series of enzymes vital for growth, protein synthesis and production of growth promoting hormones like auxins in plants. However, higher Zn accumulation induces toxicity in terms of stunted growth, reduced biomass production and retardation in various metabolic activities like photosynthesis, transpiration and nitrogen metabolism. Supra-optimal concentration of Zn interferes with antioxidative response by generating toxic reactive oxygen species, thereby causing oxidation of cell organelles in plants. Indeed, higher Zn concentration may even cause death of the plants. Some plant species exhibited the potential to accumulate high concentration of Zn in their tissues without showing any toxic symptoms. Such Zn tolerant species can be engaged for phytoremediation purposes. The present work focuses to evaluate Zn availability in the environment, its uptake and molecular mechanisms of translocation in plants, its effect on physiological and biochemical status, alterations in protein and nitrogen metabolism, Zn hyperaccumulation and detoxification strategies adopted by plants.

Key words: Accumulation; Biochemical status; Plants; Toxicity; Uptake mechanisms; Zinc.

INTRODUCTION

Zinc (Zn) deficiency is the most wide-spread micronutrient deficiency problem in the world crops, causing declined crop yields and nutrient quality [1]. It has been estimated that about 30% population of world suffers from the deficiency of Zn [2]. Thus, Zn deficiency has emerged as a major and common problem for the health of both plants and humans. Rapid industrialisation and development of technology has resulted parallel increase in heavy metal contamination in the environment and is a concern for the living biota. Zinc concentration ranges between 10 to 300 mg kg^{-1} in soil with a mean range of 50 mg kg^{-1} which represents its ubiquitous nature in the environment [3]. In unpolluted soils, Zn concentration is usually found to be less than 125 ppm [4],

whereas in polluted soils it lies in between 150 to 300 mg kg^{-1} [5]. Zinc is a non-redox, active metal having low melting point and belongs to group 2B of the periodic table. It is a bluish grey, water insoluble lustrous metal having a melting and boiling point of 419.5°C and 908°C [6]. Electrolytic process is responsible for the production of Zn in which zinc oxide is combined with sulphuric acid to form a solution of zinc sulphate which is used to deposit Zn on cathode [7]. Zinc acts as an electroplating or galvanising agent for the protection of iron and other metals from corrosion and hence is primarily used in the galvanising industries. Sphalerite or Zinc blende (ZnS), Wurzite (ZnS), Hemimorphite ($Zn_4Si_2O_7$ $(OH)_2$. H_2O), Willemite or Zinc spar (Zn_2SiO_4), Smithsonite ($ZnCO_3$) and Zincite (ZnO) are some of its common minerals. About 50% of Zn is present in all these mineral ores. It is usually found in association with base metals like copper (Cu), lead (Pb), iron (Fe) and occurs abundantly throughout the world.

Zinc, an essential micronutrient is present in all the nine classes of enzymes and is indispensable for appropriate functioning of more than 300 enzymes. It plays a vital role in both structural and catalytic processes occuring in living organisms and is required for their growth and other physiological processes. Zinc presence activates a series of enzymes responsible for vital growth events and asserts in protein synthesis, production of chlorophyll and growth promoting hormones like auxins in plants [8, 9]. It has been confirmed that Zn provides resistance to plants against diseases and drought [10, 11]. It has been found that Zn acts as a cofactor for superoxide dismutase (SOD), an antioxidant enzyme and plays a crucial role in numerous enzymatic reactions involved in the metabolism of phosphates, nitrates, proteins, carbohydrates, DNA, RNA and synthesis of tryptophan which is a precursor to IAA [12, 13, 11]. Moreover, formation of DNA, RNA polymerases, transcription factors and reverse transcriptases are carried out by Zn metalloenzymes. These vital processes are regulated when appropriate amount of Zn is accumulated in the plant tissue.

Elevated Zn levels (>500 mg kg^{-1}) hinders the plant growth and becomes hazardous for them [14]. Supra-optimal concentration of Zn interferes with other essential microelements like Fe, Cu and Mn as it displaces other vital metallic ions located on the active sites of proteins thus promote leaf necrosis, chlorosis and oxidative stress in plants [9]. Zinc excess in plants results in the formation of highly reactive oxygen free radicals that affects the chlorophyll content, membrane integrity, plant growth, water relations and the activities of enzymes related to nitrogen metabolism [15, 16]. It also inhibits various enzyme activities like nitrogenase and interferes with the uptake of nutrients in plants [17, 18]. Its availability to plants depend upon the age of the plant, environmental factors, temperature, pH of the soil, plant species and combined interaction of Zn with other metals.

This review contains a summary of current knowledge regarding toxic effects of Zn on various physiological and metabolic processes in plants. In the first half of the manuscript, a brief summary regarding the availability of Zn in nature, its essentiality to both plant and animal systems and Zn uptake and translocation mechanisms in plants are discussed. However, its phytotoxic effects on plants by targeting various physiological and metabolic mechanisms, biochemical parameters, genotoxicity and use of some plants for Zn accumulation are discussed in the second half of the manuscript.

Zinc sources in the environment

Zinc is a ubiquitous essential micronutrient and it remains persistent naturally in the environment. It has an oxidation state of +2 and primarily occurs in combination with

various mineral ores like smithsonite (zinc carbonate), sphalerite (zinc sulfide) and zincite (zinc oxide) which contains about 50% of Zn. There are about 55 Zn mineral ores known to occur in nature. Zinc is transported and mobilised in the environment naturally by air, water and soil due to abrasion and weathering of rocks, soils and deposition of sediments by water. Natural calamities like volcanic eruptions, forest fires and formation of aerosol above seas (**Fig. 1**), also adds Zn to the environment. The natural circulation of Zn in air, water and soil maintains its balance in nature. But during the last few decades, various anthropogenic activities incremented its concentration at an alarming rate in the environment. Industrial activities like mining, smelting, coal and waste combustion, sludge decomposition, cement production, steel processing, waste incineration etc adds tremendous amount of Zn in the environment. Use of sludge formed in industrial areas as fertilizers in agricultural field also elevates the level of Zn in the environment (**Fig. 1**). Production of rubber, brass, bronze, alloys etc. is done in the presence of Zn, which may lead to its addition and deposition in the fresh water bodies. Zinc is used all over the world in metallurgical processes, as a galvanising agent so as to protect iron and other metals from corrosion, which also adds to its delterious effect on the environment. Large quantity of exhaust gases released during long term mining and smelting activities of Zn, adds various hazardous heavy metals in the environment. The addition of commercial fertilizers, liming materials or manures, pesticides by humans elevate the Zn content of farmland soils than that of natural soils [1].

Uses of Zn

Zinc salts have many applications. They are used in the preservation of wood, fertilizers, catalysts, nutritional or medicinal supplement, in textile, rubber and ceramic industries and as galvanising agent to prevent corrosion of metals [6]. Zinc chloride ($ZnCl_2$) is used in smoke bombs for dispersal of crowd and in fire fighting exercises. Zinc is used in various forms like chloride, sulphate, oxide, and sulphide for medical and dental purposes. Further, zinc chloride ($ZnCl_2$) and zinc sulfate ($ZnSO_4$) are used as chemical fertilizers and in the manufacturing of herbicides and pesticides [6].

Zinc as essential micronutrient for plants

Among the 17 essential micronutrients in plants, Zn is an important nutrient for growth and various other developmental and physiological processes occurring in plants. It is among the 8 essential elements required by the plants for performing various physiological and metabolic mechanisms [19]. Being a non-redox transition metal, Zn plays a vital role as a structural co-factor, due to its high affinity towards proteins [20]. In plants, it imparts a key role by catalysing the functioning of enzymes involved in synthesis of proteins, expression of genes, formation of pollen, metabolism of carbohydrates and auxin (growth regulator), maintenance of biological membranes, protection against heat stress, photo-oxidative damage, and provides resistance against the infection caused by some pathogens [21]. Moreover, Zn is an essential component of various transcription factors and regulates cell proliferation, RNA and DNA synthesis, differentiation and death of cells [22]. It performs a pivotal role in numerous catalytic processes and helps to maintain structural stability in cells [23]. Zinc is necessary in regulating gene expression which promotes tolerance mechanisms in plants against environmental stresses [24]. Besides, it also acts as an antioxidant by attributing -SH stabilisation and by suppressing reactive oxygen species (ROS) that cause oxidative

stress in plants. Its deficiency in plants retards important physiological and metabolic processes like nitrogen metabolism, photosynthesis, reduces development of flower and fruit, prolongs growth periods, decreases yield and quality. Some of the most common deficiency symptoms of Zn in plants are stunted growth, chlorosis and smaller leaves, spikelet sterility, infection by fungal diseases, increased susceptibility to injury by high light or temperature intensity, delterious effect on the uptake and transport potential of water and other nutrients to different plant parts. Tryptophan, a precursor of IAA, is synthesised in the presence of Zn, therefore, it also contributes actively in forming growth hormones like auxin, in plants [25]. Further, cell membrane integrity in plants is maintained by the participation and interaction of Zn with sulphydryl and phospholipid groups present on the membrane [26].

Zinc essentiality for living biota

Zinc plays a central role in both human and animal health. Its intake in animals is very much important for performing various biological functions. In humans, it is present in cells, tissues, organs and body fluids. Adult human dietary requirement of Zn is 15 mg per day. It acts as a co-factor for appropriate and proper functioning of around 300 enzymes present in the body of humans. Zinc performs a crucial role in growth and cell division, assist to perform the basic roles of cellular functioning in all the living organisms, promotes the growth of hair and nails, development of bones and also helps in improving immune system. Improper intake and absorption of Zn may cause its deficiency in the body. Zinc deficiency in humans may cause skin problems, hair and memory loss, weakness in body muscles, decreased immune functions and dwarfism or growth retardation. Further, Zn deficiency may also cause dull hair, brittle finger nails, white spots on the nails, delayed wound healing, dermatitis and acne, loss of fertility in men, congenital diseases like acrodermatitis enteropathica, loss of appetite, rough skin and frequent body infections [27].

Zinc uptake and translocation mechanisms in plants

Zinc mobility is very high in soils rich in organic matter and clay fractions, as they have the ability to hold Zn cations strongly at neutral or nearly alkaline pH [28]. Wong et al. [29] found that alkaline soils having pH 7.5 and high dissolved organic matter (DOM) exhibited high Zn mobility. Compared to other micronutrients, Zn solubility in soils and its mobility in plants seems to occur rapidly. Uptake of Zn in plants occur as divalent cation Zn^{2+} or by forming complex with organic ligands via xylem of the root cells that later on is translocated to upper aerial parts, which directly depend upon its concentration present in nutrient medium [30]. Other factors like moisture content, pH, temperature, organic matter content of soil and plant root distribution directly influence the availability and translocation of Zn in plants. It appears that Zn transport from external medium to plant root cell is mediated by the process of diffusion in which a potential gradient in root cell is formed and Zn^{2+} ions are transported to the negatively charged cytoplasm of root cell through plasmalemma with the help of protein transporters.

According to Reid et al. [31], Zn influx across the plasma membrane is thermodynamically favoured and it is supposed that no transport system is responsible for active transport of Zn^{2+} ions in the root cells. Elevated Zn levels in the external media results in uptake of Zn influx to xylem of root cells by apoplastic pathways or through connecting link of cytoplasm of root cells to plasmodesmata by symplastic movement [32]. There are particular cations that can be selected by specific transporters for transport through plasma membrane by symplastic movement [33]. In *Thlaspi*

caerulescens and *T. arvense*, Zn transport to xylem occurs via symplastic pathway by entering the root cells across the plasma membrane [34, 35]. The translocation of Zn from root to the shoot tissue of the plants requires its loading in the apoplastic xylem [36, 37]. Zn flux from the xylem into the shoots is mass-flow driven. Inside the xylem, Zn form chelation by low-molecular-weight ligands, thus prevent its binding to the surrounding cell walls or uptake into the cells via Zn transporters. According to Sinclair and Krämer [32] Zn uptake from the xylem is mediated across the plasma membrane of adjacent cells present in the shoots. Generally, it is believed that highest Zn level is accumulated particularly by the trichomes and the epidermal cells [38]. Nevertheless, vacuoles are thought to impart its highest contribution for storing excess Zn in the leaves. On the contrary, there exist stongly modified living cells for phloem transport pathways and these cells are interconnected with each other, both symplastically and with the help of companion cell [39]. High concentration of off-target metal-binding compounds and higher pH inside the phloem influence the formation of Zn-complexes with metals through chelation which is particularly important to move Zn ions freely inside the phloem sap. Depending upon the plant species, the translocation of compounds into the companion cells via phloem occurs either by symplastic or apoplastic pathways [40]. At the nodes, there persist the possibility of exchange of solutes between the two transport pathways, because of the close proximity of xylem and phloem [32]. However, the mechanisms regarding Zn uptake and translocation in plants remain poorly understood. Some studies reveal that Zn uptake across the roots is metabolically favoured, however, it can also be a non-metabolically favoured process.

Many studies revealed that Zn uptake and transport in plants is an active process and occurs through ion channels and by electrogenic pumps. The root exudates of plants which were found to be Zn deficient actively mobilise Zn and Fe from the soil which suggests that uptake of Zn in plants is an active process. These ion channels like carrier proteins and organic acids establish and controls voltage gradient across the cells of plasma membrane allowing the movement of ions down the concentration gradient [41]. Despite of the uptake mechanism of the Zn in plants, a small fraction of this metal has been found bounded to the metallothionines and phytochelatins in the xylem of roots which demonstrate its highly mobile nature in plants [42]. Adding to it, temperature also has a direct influence on Zn uptake in roots of the plants. Li et al. [43] observed Zn adsorption on TiO_2 at high temperature exhibited greater mobility of Zn in plant tissue.

ZIP Transporters

Various molecular techniques and advanced genetic applications have led to the identification of numerous gene families responsible for the transport of heavy metals in plants. *Arabidopsis thaliana* was identified as the first model plant involved in Zn transport gene sequences. There are 15 ZIP transporter proteins encoded in the genome of *A. thaliana* [44]. IRT 1 (Iron-Regulated Transporter 1) protein levels gets upregulated in response to excess Zn content in the growth medium [45]. IRT 1 imparts a decisive role for the influx of divalent transition metal cations like Zn^{2+} and Cd^{2+} into the root symplasm [46]. Large number of genes encoding proteins that influence detoxification of Zn and Cd excess are stimulated when the levels of IRT 1 proteins gets enhanced [47]. Another ZIP protein transporter IRT 2 is closely related to IRT 1, even though it does not play any vital function as performed by IRT 1. In roots IRT 2 is upregulated in the external cell layer of sub-apical zone and is localised to intracellular vescicles [32]. IRT 2 in yeast can assist to save the variants non-functional in cellular extraction of Zn and Fe

[48]. IRT 3 is an important ZIP protein encoded from *Arabidopsis*. IRT 3 is highly expressed during Zn deficiency and is found abundantly in the roots of Zn hyperaccumulators like *Arabidopsis halleri* and *Thlaspi caerulescens* [49, 50]. In yeast, IRT 3 expression can influence the transport of Zn and Fe whereas, in transgenic plants its upregulation allows the accumulation of excess Zn in the shoots [51]. Also, AtIRT 3 and AhIRT 3 were found to be located on the plasma membrane. Therefore, it is believed that IRT 3 ZIP transporter protein levels regulate the movement and transport of Zn across the plasma membrane into the cell. Another ZIP protein like ZIP1, ZIP2, ZIP3 and ZIP4 are also expressed in the genome of *Arabidopsis* and thus exhibited a strong cellular Zn uptake across the plasma membrane [52]. It was proposed that ZIP protein gene sequences at molecular level have played a crucial role in transport of Zn to the plants [53]. In Zn hyperaccumulator plants like *A. halleri* and *T. caerulescens*, an enhanced expression of ZIP4, ZIP6, ZIP9 and ZIP10 were reported compared to the non-hyperaccumulator plants [54]. Grotz et al. [55] and Guerinot [56] have proposed that zrt1 and zrt2 gene encodes the transporters responsible for Zn transport in plants (**Fig. 2**), whereas soil deficient in Zn, expressed ZIP1 and ZIP3 proteins in roots showing the evidence of Zn transport in plants from soil while expression of ZIP4 in both root and shoot suggested intracellular transport of Zn in plant tissue. Several reports observed that ZIP proteins are very effective in the transport of Zn in *Oryza sativa* [57, 58, 59]. Deficiency of Zn in *O. sativa* plants induced the formation of large number of ZIP transporter genes like OsIRT1, OsIRT2, OsZIP1, OsZIP3 and OsZIP4 thus, enabling the transport of Zn in plants [60]. They further found that transporter genes like OsZIP1, OsZIP3 and OsZIP4 were expressed in the vascular bundles of both roots and shoots and epidermal cells of roots in rice plants. The studies proved that enhanced accumulation of OsZIP4 mRNA in both roots and shoots of plants (**Fig. 2**), in media deficient of Zn concentration, signifies transport of Zn in plants [60]. Further, the expression of OsZIP4 accelerates Zn uptake in plasma membrane of yeast growing in Zn deficient media [61]. In *Medicago truncatula,* a Zn transporter, MtZIP2 similar to ZIP2 which encodes a gene on plasma membrane was reported [62].

Role of CDFs in Zn transport

Apart from the family of ZIP transporters, CDF (Cation Diffusion Facilitator) family members are also involved in transportation of metals in plants either by efflux of metal to the extracellular environment or their transport into organelles present intracellular from cytoplasm [63, 64]. There are 12 MTPs encoded from *Arabidopsis* genome having different phylogenetic groups [65]. Among CDF family of transporters, MTP1 and MTP3 for Zn transport in plants has been well characterised [66]. The genotypes of *Arabidopsis* having decreased MTP1 and MTP3 expression are hypersensitive to Zn [47]. MTP1 expression is found to be highest in young leaves and in the root vascular tissues of young seedlings, whereas declined with the increment in the age of the plant seedlings [67]. On the contrary, MTP3 activity is unnoticiable in shoots [67] and decreases under normal growth conditions, but strongly elevated especially in epidermal and cortex cells of root hair zone under the influence of Zn excess [47]. Nevertheless, both MTP1 and MTP3 protein levels have distinct roles and functions in Zn accumulation. MTP1 enhances Zn accumulation in leaves, whereas MTP3 decreases Zn levels [47]. MTP1 especially influences the quenching of Zn in the young dividing tissues and thus enable Zn accumulation in the shoots [68]. In contrast, MTP3 functions to abolish excess Zn under high Zn influx into roots via root to shoot transport pathways [47]. Over expression of MTP1 mRNA induces resistance in plants for Zn toxicity. It is

proposed that MTP1 localisation to the vacuolar membrane (**Fig. 2**) maintains Zn balance during its transport into the vacuole due to the high sensitivity of plants towards the increasing Zn concentrations [47]. Zinc is transported into proteoliposomes in plants and is induced by expression of MTP1 transporter protein [69]. The over expression of MTP1 in a bacterium *Ralstonia metallidurans* demonstrated its function in Zn efflux [69]. Moreover, MTP1 protein levels were highly expressed in Zn hyperaccumulators like *A. halleri*, *T. caerulescens*, *T. goensingense* [68, 70]. Hall and Williams [71] suggested that vacuolar uptake and tolerance of Zn is enhanced by a plant transporter gene ZAT (**Fig. 2**).

Role of the HMAs in Zn transport

HMAs are transmembrane protein that transport metal and play a major role during metal homeostasis in plants. They are found to be similar to subclass of type 1B P-type ATPases. The P_{1B}-ATPases (commonly known as Heavy Metal ATPases) are involved in Zn transport in plants [72]. On the basis of their different metal binding and transport specificity, P_{1B}-ATPases are classified into six subgroups (P_{1B1-6}) [73]. Eight P_{1B}-ATPases are reported in *A. thaliana* and out of them four play vital role in Zn transport. HMA1, present in the inner membrane of chloroplast (**Fig. 2**), detoxify the effect of Zn^{+2} by reducing its content in plastids [74]. AtHMA1 load Cu into the stroma and also supply it to Cu/Zn SOD in chloroplast [75, 76], and transport Ca [77]. AtHMA2 and AtHMA4 help in translocation of Zn from root to shoot as double mutant *hma2hma4* exhibited a strong Zn nutritional deficiency [76, 78]. They also play important role in transferring Cd to the shoot [79]. HMA2 was found localised in the plasma membrane of pericycle cells whereas *in situ* experiments conducted in *A. halleri* revealed the expression of HMA4 in plasma membrane and xylem parenchyma cells of *A. halleri* and *A. thalliana* [37]. According to Sinclair and Krämer [32], the stimulation of HMA2 and HMA4 transcript levels influence the pumping out of Zn into the adjacent root cells and functions in Zn loading into the xylem. Plants with higher expression of HMA2 were found to be more susceptible to tolerate and accumulate Zn and Cd than wild plants on the basis of elemental analysis of whole plants [80]. AtHMA4 is expressed in many tissues of plants but its expression is highest in roots, and it was observed that higher level of Zn and Mn resulted in the up-regulation of AtHMA4 in roots [81]. Further, Hussain et al. [36] confirmed that HMA4 is a divalent cation transporter, as with the expression of AtHMA4 in *E.coli,* Zn tolerance was restored in a Zn-sensitive *znt4* mutant. It was observed that Zn and Cd accumulates more in roots than leaves in *hma4* mutant plants [82] hence confirming the increased sensitivity of *hma4* plants towards both Cd and Zn [83]. It has been found that over expression of HMA4 increases the tolerance of both Zn and Cd in plants [82, 84]. As HMA4 are localised to specific sites on plasma membrane (**Fig.2**) and the accumulation of radio-labeled Cd and Zn decreased with the expression of HMA4 in wild yeast, which shows its role in mediating effluxing of Zn across the plasma membrane [83]. Overexpression of HMA3 in shoots of Zn and Cd hyperaccumulators like *A. halleri*, *A. thalliana* and *T. caerulescens* strongly pointed the role of HMA3 in Zn/Cd hypertolerance by storing these metals in the vacuole [85, 86]. In another study, Morel et al. [87] opined that AtHMA3 function in detoxification by sequestering Zn, Co, Cd and Pb in the vacuoles. In monocot plant like rice, nine genes corresponding to HMA have been found in genome sequence (OsHMA1-9) [76]. Of the nine HMA genes, OsHMA9 was the first to be characterised [88, 76]. Phylogenetically, it was observed that OsHMA9 forms clusters with AtHMA5-8 [89] whereas rice

phenotypic analysis of *oshma9* mutants suggested sensitivity of mutants to higher levels of Zn, Cd, Cu, and Pb [88].

Role of MAs in Zn transport

Welch and Shuman [90] suggested the role of mugineic acid (MAs) in Zn acquisition and other metal nutrients by plants belonging to family Gramineae. In natural system, there occurs a characterised biosynthesis of MAs. The amino acid methionine is a precursor for the biosynthesis of MAs. Methionine gets converted to SAM by an enzyme S-adenosyl-L-methionine (SAM) synthetase. Afterwards, three molecules of SAM combine to form nicotianamine (NA) using enzyme nicotianamine synthase. Enzyme nicotianamine amino transferase convert NA to 3″-keto acid and later enzyme DMA synthase (DMAS) leads to synthesis of 2′-deoxymugineic acid (DMA) [91]. Zinc is absorbed from the rhizosphere by the complex formed between Zn cations and phytosiderophores as MAs. According to Widodo et al. [92] tolerance to the deficiency of Zn in rice plants induce the efflux of MAs in the rhizosphere but no secretion of MAs in rhizosphere was observed in roots of barley and wheat [93, 94]. TOM1, a gene coding protein induces the secretion of synthesised MAs to the rhizosphere [60]. In barley and other plants of family Gramineae, synthesis of dioxygenases like IDS2 and IDS3 was induced by the hydroxylation of DMA [95]. Studies revealed that DMA secretion is induced by two genes OsNAS1 and OsNAS2 as all the root cells expressed these genes whereas OsNAS3 gene expression is only localised to companion cells and pericycle of roots, as a result of which OsNAS3 has no role in DMA secretion [96]. In barley roots, expression of HvNAAT-A, HvNAAT-B, HvNAS1, HvDMAS1, HvIDS2 and HvIDS3 enhanced during Fe-deficiency [97][98][99][100][101] whereas in both roots and shoots of rice OsNAS1, OsNAS2, OsNAAT1 and OsDMAS1 expression was incremented due to deficiency of Fe [102][103]. It was observed that during deficiency of Zn in barley, its shoots have increased expression of NAS genes like NASHOR2 and HvNAAT-B [104], but IDS2 and IDS3 expression could not be detected in Zn deficient shoots [105]. This proves that deficiency of Zn in barley induces synthesis of DMA in shoots whereas deficiency of Zn and Fe induces secretion and biosynthesis of MAs in roots [106]. It was found that translocation of Zn in shoots of rice plants is induced by Zn-NA or Zn-MAs transporters [60].

Role of NA in Zn transport

A. halleri is a model for accumulation and tolerance of Zn and Cd and is a close relative of *A. thaliana* [107]. Metal homeostasis gene nicotianamine-synthase is highly expressed in *A. halleri*, and it catalysis the synthesis of a non-proteinogenic amino acid, nicotianamine (NA), which helps in translocation and binding of variety of transition metals. Deinlein et al. [108] observed that increased NA content in the roots of *A. halleri* plays an important role in Zn hyperaccumulation. It also showed that RNAi lines in *A. halleri* have reduced levels of NA in roots but had higher content of Zn in roots and lower content in leaves, which shows that root NA normally help in promotion of root-to-shoot transport. According to Haydon et al. [109] a major facilitator protein Zinc-Induced Facilitator 1 (ZIF1) in *A. thaliana* is localised to membrane of vacuole and induces Zn tolerance in plant [110]. Upregulation of ZIF1 implicates NA accumulation in the vacuole that further enhanced Zn accumulation in the vacuole of the plant cells **(Fig. 2)**. It was observed that over-expression of ZIF1 in root cell vacuoles results in vacuolar sequestration of NA and thus reduces the availability of Zn transport from root to shoot. Stephan and Scholz [111] proposed NA for the transport of Mn, Cu and Zn in the phloem.

Transport of Zn in phloem

In plants, there exist stongly modified living cells for phloem transport pathways and these cells are interconnected with each other, both symplastically and with the help of companion cell [39]. High concentration of off-target metal-binding compounds and higher pH inside the phloem influence the formation of Zn-complexes with metals through chelation which is particularly important to move Zn ions freely inside the phloem sap. Depending upon the plant species, the translocation of compounds into the companion cells via phloem occurs either by symplastic or apoplastic pathways [40]. In the phloem, mobility of Zn varies and depends on the status of Zn in the tissue and organs of plant [112]. Welch et al. [113] conducted an experiment wherein an inadequate supply of Zn to one part of roots blocks its transport to other parts thereby suggesting lack of Zn transport in the phloem. Although, during sufficient supply of Zn in one part of root, the uptake and transportation of Zn to the other root section was approximately 29% suggesting that with increase in supply of Zn, phloem transport increases [113]. However, in *T. aestivum* reduction in phloem transport was observed during excessive supply of Zn due to the reduction in loading of Zn in phloem but increased phloem loading of sucrose in plant [112]. Pearson et al. [114] suggested that presence of Cu inhibits the entry of Zn in phloem cells of *T. aestivum* grains as Cu competes for the empty sites localised on phloem cells and has same size as that of Zn. This suggests that plant possess preventive mechanism for excessive Zn distribution to young and developing tissues. Furthermore, in *Phaseolus vulgaris* excess Zn supply has resulted in reduced phloem loading of sucrose [115]. Moreover, increased supply of Zn has shown inhibitory effect on transport and phloem loading of other essential elements [113]. Rauser and Samarakoon [115] further observed that Zn-induces limited supply of ATP in plants which might be responsible for reduction in loading of phloem and transportation and further increased amount of Zn could be responsible for harmful effects like reduction in plant biomass production.

Zinc phytotoxicity in plants

Although, Zn is an essential micronutrient and is required by the plants for performing various physiological processes but at elevated levels Zn may prove toxic and hence cause phytotoxicity in plants. Soils having low pH have generally high Zn availability and in this acidic pH it can easily be taken up by the plants and can cause harm to them at higher levels [8]. In most parts of the world, Zn is major pollutant of both aquatic and terrestrial ecosystem [116]. The threshold of Zn toxicity depends on the type of species, growth media or levels of Zn contamination in growth media and time of exposure. In plants, Zn toxicity occurs at concentration range between 400–500 μg g^{-1} in leaves [117]. At concentration higher than 500 mg kg^{-1}, Zn interferes both cell division and elongation, inhibits plant growth, formation of chlorophyll, rate of photosynthesis and transpiration [118]. Above the permissible limit, Zn retards the activity of enzyme nitrogenase in plants [118]. Excess concentration of Zn imparts pessimistic effects on the Calvin cycle and Photo System (PS) activity [119]. The first visible sign of excess level of Zn in plants is chlorosis of young leaf [120]. An elevated level of Zn in plants promotes chlorosis because of deficiency of Fe and its interference with Ca metabolism [17], which can further lead to red colour of younger leaves due to the production of anthocynin in them [121]. Plants grown under control conditions exhibited larger leaves as compared to the plants exhibiting Zn toxicity [122]. In severe cases of Zn toxicity, plants exhibits necrotic leisons on leaves which may latter result in death of whole leaf [123].

Zinc tends to accumulate more in roots than in leaves, and limits plant uptake of water and nutrients by retarding elongation and growth of roots [124]. In plants, Zn toxicity reduces the growth of main root and also retards the growth of lateral roots [122]. It has been found that addition of 400-1600 mM $ZnSO_4$ inhibits the growth of *Eucalyptus maculata* and *E. urophylla* after a treatment of 5 weeks [125], whereas treatment range from 1-10 mM $ZnSO_4$ retards the growth of ryegrass with complete inhibition at 50 mM $ZnSO_4$ [126]. According to Doncheva et al. [127], growth inhibition in *Pisum sativum* became apparent at 1000 µM Zn concentration. Crops grown in contaminated and acidic soils suffer Zn toxicity, as some food crops like beet and spinach can accumulate high levels of Zn in their tissues and exhibit growth retardation [17][8]. In *Populus deltoides* x *Populus nigra,* Di Baccio et al. [128] observed that application of 100 µM and 1000 µM Zn treatments exerted a significant reduction in total dry mass and foliage content of plant. Exposure of 2.5 mM and 5 mM $ZnSO_4$ concentrations for 20 days severely retards the growth of Datura species (*D. innoxia, D. sanguinea, D. metel* and *D. tatula*) [16]. Foliar Ca to Zn ratio in *Arachis hypogaea* grown in field has been found to be less than 50 which show the toxic effect of Zn on plants [118]. Khudsar et al. [129] observed reduction in root and shoot growth parameters in *Artemisia annua* at different stages of development with varying Zn concentration ranges from 100 – 400 µg g^{-1} (soil d.m.). Zinc interferes in plant growth by retarding root and shoot length in green grass, *Bacopa monniera*, *Sorghum bicolor* and *Vigna radiata* [130]. In sugarcane, elevated Zn concentrations (65 and 130 mg L^{-1}) hampered the growth, number, length and mitotic activity of roots [131]. Jordan [132] and Beyer et al. [133] observed phytotoxic effects of Zn towards mature trees and seedlings on Blue Mountain near Palmerton, Pennsylvania, USA. Beyer et al. [124] proved that Zn retards and interferes with the root growth and cause chlorosis and marginal necrosis in young leaves of seedlings growing in soils closer to Palmerton smelters. Further, higher level of Zn in soil induces scanted growth and promotes chlorosis and necrosis in leaves of *Betula pendula* and *B. pubescens* [134].

Physiological effects of Zn on plants

Effect of Zn on seed germination and growth of plants

Zinc pressence in the soil assist the development of plumule and radicle. Acording to Rout and Das [135] seeds of *Silene maritima* germinated better at different concentrations of Zn present in calcium nitrate solution. However, higher Zn exposure significantly delayed and impeded the germination of chickpea seeds [136]. In *Cicer arietinum*, lower Zn concentrations (10 and 25 µM) enhanced the seed germination, while an inhibitory effect was observed at higher levels [118]. Roots of the plant instantly respond to absorbed Zn, through reduction in growth rate and variation in branching pattern. Transmission electron microscopy revealed that Zn stress significantly affected the radicle elongation compared to that of the plumule extension [137]. Zinc treatment at 7.5 mM completely inhibits the elongation of root in *Cajanus cajan* cv. ICPL 87 after 24 hrs [135]. They further observed extensive variations in the root tip cells and damage to the root cortical cells. Zinc treatment caused significant reduction in the height of *Artemisia annua* at 50 and 100 µg g^{-1} soil d.m. and affects the root length significantly at all Zn concentrations [129]. Excess Zn imparts an inhibitory effect on plants belonging to family Leguminosae [138]. Elongation of shoot and root was retarded by Zn application in *Phaseolus mungo* [139], *Vigna radiata* and *Sorghum bicolor* [140] and *Bacopa monniera* [130]. Zinc reduced leaf growth in *Artemisia annua* and *Phaseolus vulgaris* [138]. Growth inhibition due to Zn toxicity leads to the reduction

in plant biomass. In the nutrient medium, Zn concentration at 300 µM, resulted a progressive decrease in both fresh and dry mass of shoots and roots in sugar beet [141]. Enhanced Zn levels reduce the leaf number and area, cause distortions and wrinkling of leaf margins, inward rolling of leaves and chlorosis [141]. A study revealed that *Solanum lycopersicum* showed a significant reduction in number of leaves and roots grown at 0.5, 0.75 and 1.0 mM $ZnSO_4$ concentrations, compared with the control [142]. They further observed peaked proline levels in both *S. lycopersicum* and *S. nigrum* with the increase in Zn concentrations. Excess amount of Zn prevents the growth and development of plant by disturbing the balance between mineral intake and distribution or by interfering with the antioxidant defense system and metabolic processes of the plant [143]. Chaoui et al. [139] reported that treatment of 5 µM $Cd(NO_3)_2.4H_2O$ and 100 µM $ZnSO_4.7H_2O$ caused significant reduction in growth of about 40% and 28% of roots and stems when compared with the control. *Chara aculeolata* showed a significant decrease in relative growth rate (RGR) when exposed to high concentration (5 and 10 mg/L) of Zn [144]. Zinc concentration at 250 mg kg^{-1} has led to the reduction in growth and biomass of marigold, pigeon pea, chilli and mustard which attributes to Zn toxicity at higher concentrations [145].

Effect of Zn on photosynthesis

In plants, Zn plays a vital role during photosynthesis as it acts as a co-factor of enzyme carbonic anhydrase required by the plant during photosynthesis. Deficiency of Zn resulted in 50-70% reduction in net photosynthesis depending on different plant species and the severity of deficiency. In contrast, elevated level of Zn strongly affects the photosynthesis in plants. Plants exposed to Zn ions exhibit a decrease in photosynthetic activity due to disrupted chloroplast structure, impeded synthesis of chlorophyll pigments, carotenoids, interference in the activities of enzymes related to Calvin cycle, hindered electron transport and closure of stomata by restraining CO_2 fixation. It has been reported that Zn competes with Fe or Mg and causes deficiency of these elements in plants [117, 141]. Further, the replacement of Fe by Zn due to similar ionic radii induces deficiency of Fe and hence decreases chlorophyll content in leaves. In *P. vulgaris* toxic concentrations of Zn induces retardation in photosynthesis due to inhibition of PS I and II (**Fig. 3**). In addition, Zn displaces Mn at the site where splitting of water occurs in PS II. Studies revealed that Zn treatment to thylakoids for 30 minutes with concentration 1 to 2mM exhibits retardation in PS II [146]. Meanwhile, compared with the control, Zn stress at different concentrations (15, 75, 150 µM) exhibit a significant decrease in photosynthetic efficiency in *Hydrilla verticillata*, which elicits the severe damage caused to PS II reaction centers due to Zn toxicity [147]. Beyond threshold level, Zn ions may retard the stomatal conductance, pose pessimistic effects on CO_2 fixation and impart inhibitory effect on size and number of stomatal cells. It is well known that the phenomenon of stomatal opening is regulated by an enzyme carbonic anhydrase (CA), while in CA, Zn acts as a co-factor and its concentration directly influence the activity of CA. According to Evans et al. [148] in leaf stroma, mesophyll conductance is facilitated by CA by catalysing the interconversion between CO_2 and HCO_3. Elevated Zn level influence the blockage of xylem, thus lower the water content in leaf. All these factors can disturb the stomatal balance, resulting in decreased rate of transpiration and photosynthetic efficiency [149]. Higher Zn concentration interferes with the thylakoid electron transport rates (ETRs) (**Fig. 3**). Szalontai et al. [150] reported that Zn mediated inhibition of electron transport at lower levels results in alterations in membrane integrity. Nevertheless, Zn stress in plants also affects the

activity of Rubisco responsible for driving the biochemical reactions occuring in the stroma of the chloroplasts. Presence of divalent cations like Co^{2+}, Ni^{2+} and Zn^{2+} ensures the activity of Rubisco. However, these bivalent cations compete with Mg cations present in Rubisco which affects its activity in plants. Cherif et al. [151] reported that 150 µmol L^{-1} Zn concentration severely reduce the level of Chl-*a* and Chl-*b* in *S. lycopersicum* compared to the control. In *Zea mays*, treatment with 1000 mg kg^{-1} Zn in soil induces significant reduction in chlorophyll content in plants [152][153]. Rout and Das [154] reported disintegration of cell organelles by Zn toxicity. Such disintegration justifies the reduction in the rate of photosynthesis in leaves of rye plants exposed to higher concentration of Zn (2.7 mg g^{-1}) for 15 days [155].

Effect of Zn on oxidative metabolism

The major delterious phytotoxic effect of Zn is the induction of oxidative stress in different plant parts due to generation and accumulation of ROS that disturb the cellular redox status in plants. Unlike other essential elements, Zn in plants is a redox inactive metal but elevated and long term exposure of Zn may induce cytotoxicity, thus severely disrupts normal cellular metabolism. Different ROS species like singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydroxyl ion ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are accumulated in plants during normal cellular metabolism under Zn stress. The most promising and primary indicator of oxidative stress in cellular components is the peroxidation of membrane lipids. The oxidative stress caused by ROS is responsible for lipid peroxidation and membrane damage which include the increased membrane permeability, loss of membrane integrity and further impairment of membrane function. These oxygen free radicals are extremely reactive and led to toxicity or even death of plants by damaging lipid membranes, proteins and nucleic acids [156]. Tsonev and Lidon [30] found that Zn at higher levels induces oxidative stress by disturbing antioxidant defense system and photosynthetic electron transport. In plants, heavy metal stress (Zn, Pb, Cd) alter the activities of various antioxidant enzymes like CAT, GR, GPX, APX and SOD which act as first line of defense against ROS [157][158].

Zinc induces lipid peroxidation when H_2O_2 and $O_2^{\cdot-}$ produced by Fenton reaction are converted into extremely toxic $OH^{\cdot}$ radical that causes peroxidation of lipids [156]. Biochemical estimations showed that in *P. vulgaris,* treatment of both Cd and Zn with concentrations (5 µM Cd $(NO_3)_2.4H_2O$ and 100 μM $ZnSO_4.7H_2O$) in nutrient media induced oxidative stress in all organs of plant seedlings, as was evidenced by the enhanced accumulation of lipid peroxidation products [139]. **Youssef** and Azooz [159] suggested that elevated levels of lipid peroxides with increasing concentration of Zn induced oxidative stress in *Hibiscus esculentus* plants. They further opined that in shoots of *H. esculentus*, the H_2O_2 content decreased at 5 mM Zn^{2+} but incremented later on depending upon the increased concentrations of Zn, when compared with the control. Another study revealed that elevated levels of Zn in *Sedum alfredii*, a Zn hyperaccumulator promoted rapid generation of H_2O_2 in tissue parts of the plant [160]. In *S. lycopersicum*, Zn exposure to 100 µmol L^{-1} promotes toxicity and induces lipid peroxidation by evaluating the increased amount of TBARS in Zn treated plants [151]. In leaves of wheat plants, significant increase in H_2O_2 content was observed under 1 mM and 3 mM Zn treatment when compared with the control but no such significant effect was observed on lipid peroxides in the leaves of wheat seedlings. Furthermore, remarkable increase in H_2O_2 level in roots of wheat seedlings was observed when exposed to varied Zn concentrations (i.e. 0.5, 1, 3 mM) and malondialdehyde (MDA) content elevated to about 22, 17 and 28 % with the elevated Zn levels in roots of wheat

seedlings [161]. Dai et al. [162] found that exposure to varied Zn concentrations (300, 600, 900 µM) resulted an increase in MDA level in roots, stem and leaves of alfalfa plants compared to that of the control. Further, it was observed that among the four cultivars of alfalfa (Aohan, Golden Empress, Sanditi, Longxi), the MDA content was markedly high in roots of Longxi while lower content was found in stem and leaves of Aohan. Zinc exposure to alfalfa plants at 300 µM increased H_2O_2 content by 9 %, 34 % and 60 % in roots of Golden Empress, Sanditi and Longxi whereas in leaves and stem, H_2O_2 content increased in Sanditi, Longxi, Golden Empress, and Aohan under 900 µM Zn with respect to the control [162]. Similar findings were reported by Ozdener and Aydin [163] and claimed that Zn stress at 500 µg g^{-1} induces tremendous increase in MDA content in the *Eruca sativa*. Prasad et al. [164] observed that exposure of Zn at and above 5 mM in *Brassica juncea* brought over 2.5-fold enhancement in MDA content over the control. Another study has found that Zn induces an increase in MDA and H_2O_2 contents in *Lemna minor* by 46% and 170% at 0.3 mM, when compared with the control [165].

Effect of Zn on antioxidative status

In plants, Zn exposure influences the alteration in the activities of large number of enzymes involved in different metabolic pathways. High Zn levels promote the inhibition in the activities of large number of antioxidant enzymes. This hindrance in enzyme activity is caused by the interaction of Zn with -SH group of enzymes. These -SH groups are located on the active sites of enzymes and are vital to stabilise the tertiary structure of enzymes. Besides, excess Zn concentration exhibit the blockage of -COOH groups and thus restrict the enzyme activity. Additionally, displacement of essential microelements by Zn impedes the activities of antioxidant enzymes. Past studies have revealed that exposure of *B. juncea* seedlings to 10 mM Zn for 10 days caused significant and parallel increase in the activities of SOD (450%), CAT (960%), POD (920 %) and various components of the ascorbate–glutathione cycle like AsPOD (260%), MDHAR (372%), DHAR (195%) and GR (up to 158%), with respect to the control [164]. Radić et al. [165] observed that 15 day exposure of Zn to *Lemna minor* resulted a remarkable decline in CAT activity by 30% in a concentration dependent manner while SOD and APX activity increased by 104% and 52% at different levels of Zn exposure. In leaves of *Myracrodruon urundeuva,* Zn stimulated the activities of SOD, GPX and CAT at 120 and 200 mg Zn kg^{-1} but declined the APX and GR activity [166]. On the contrary, seedling of *Raphanus sativus*, Ramakrishna and Rao [167] noted that Zn toxicity caused an increase in activities of APX and MDHAR by 40.4 % and 32 % while reduction in the activities of DHAR and GR by 20.7% and 52.7% compared with the control. However, they further added that supplementation of 24-epibrassinolide EBR to Zn stressed seedlings has shown a gradual and significant increase in activity of all these enzymes. Similar study conducted by Kösesakal et al. [168] suggested that upon 5 and 10 mM Zn exposure to roots of *Raphanus sativus* result an increment in POD activity. Further, in nutrient solution, 7 days treatment of 3mM Zn result a decrease in the activities of SOD, APX and CAT in roots of both Zn -resistant (TY-167), and Zn-sensitive (FYY-326) cultivars of rice [169]. However, addition of 1.5 mM silicon in Zn rich medium enhanced the activities of SOD and CAT by 42.9% and 79.4% in both the cultivars [169]. In leaves of *E. sativa*, Zn treatment for 20 days above 1000 µg g^{-1} declined the activities of CAT, POD and SOD by 45–48% [163]. **Youssef** and Azooz [159] demonstrated that Zn exposure at toxic levels result a significant increase in the activities of CAT, SOD and APX in shoots of *H. esculentus.* In *P. vulgaris*, Zn concentration at 100 µM stimulated the activities of APX

and GPX by 44% in leaves and 90% in stem but CAT activity declined in both roots and leaves [139]. Zn stress in *Salvinia natans* induced an increment in the activities of CAT and SOD but the activity of POD decreased when the plants were exposed to 50 mg L^{-1} ZnO nano particles [170]. Cherif et al. [151] reported an increase in the activities of CAT and SOD at 50 µmol L^{-1} of Zn treatment in *S. lycopersicum* plants. They further added that 50 µmol L^{-1} Zn amended to Cd treatments enhanced the APX and GR activity upto 80-85%. However, Zn supplementation of 150 µmol L^{-1} reduced the GR activity upto 29% in *S. lycopersicum* plants. Cui and Zhao [153] reported that under Zn stress, CAT activity reduced upto 1.48 µmol H_2O_2 min^{-1} mg^{-1}protein at 1000 mg kg^{-1} Zn but POD and SOD activities were increased effectively by 41 nmol min^{-1} mg^{-1} protein in maize plants. Mukherjee et al. [171] revealed that CAT activity increased in leaves of green pea on 500 mg kg^{-1} Zn exposure. Li et al. [161] claimed an increase in APX, POD and GR activities in leaves of *T. aestivum* while SOD activity remained unaffected after 6 days under 3 mM Zn stress. On the contrary, in roots, SOD and APX activities significantly increased upto 30% and 37% while activities of GR and POD remarkably reduced to 90% and 94% in response to 3 mM Zn stress [157][161]. Recent studies have revealed that Zn exposure to 900 µM for 23 days in alfalfa induced an increment in the activities of CAT, APX and GR in all the 4 cultivars (Sanditi, Longxi, Golden Empress, and Aohan) of alfalfa [162]. Excess Zn exposure in *V. mungo* resulted in an increase in GR activity [172]. In roots of tobacco plants, increased Zn treatments stimulated a significant decrease in GR activity with respect to the control, which suggested that Zn has an inhibitory effect on GR enzyme [173]. Furthermore, sugarcane plants showed increased activity of POD with elevated Zn levels [174].

Effect of Zn on cellular non-enzymatic antioxidant activity

Various non-enzymatic antioxidants impart a crucial role in plant defense mechanism under heavy metal stress. Zn toxicity in plants strongly invokes the stimulation of cellular redox potential of reduced ascorbate and glutathione pools. A considerable increase in the amount of total and reduced ascorbate was noticed in roots of *P. vulgaris* when the plants were exposed to 50 µM Zn for 120 h in nutrient medium [175]. Similarly, an overall increase in content of both total leaf ascorbate and ratio of oxidised to reduced ascorbate levels in roots was observed in *P. vulgaris* [15]. Zinc exposure induced an increase in AsA content in *Alternanthera philoxeroides* [176]. Barrameda-Medina et al. [177] reported an increase in total and reduced AsA level in both *Latuca sativa* and *Brassica oleracea* under 0.5 mM Zn treatment. They further suggest that both DHA and GSSG content declined sharply in *B. oleracea* while an increment in their level was observed in *L. sativa* under 0.5 mM Zn treatment. Further, Michael and Krishnaswamy [138] concluded that Zn exposure to bean plants promotes AsA accumulation and thus helped to tolerate and withstand Zn toxicity.

Effect of Zn on nitrogen metabolism

Nitrogen nutritional status supports various physiological and molecular mechanisms in plants which are also essential for effective biofortification of crop plants with Fe and Zn to increase the nutritional status in crop plants [178]. Trafficking of Fe and Zn bivalent cations is supported by numerous amino acids, peptides and proteins as these essential micronutrients are required by the plants to perform various metabolic activities. Therefore, presence, abundance and activity of any of the amino acids, peptides and proteins directly influence and affect the uptake, transport and re-translocation of microelements like Fe, Zn in plants. As a vital element, Zn in plants

influence the synthesis of nitrogen containing metabolites like proline, phytochelatins etc that are helpful in providing tolerance and resistance against stress. However, excess of Zn in media or higher Zn exposure in plants may affect the various physiological and metabolic activities. Zinc is known to interact with -SH group of enzymes and cause a decrease in the activities of functional enzymes like nitrite reductase and nitrate reductase. In plants, nitrogen metabolism is considered as a crucial parameter for their response to heavy metal toxicity. Stress exerted by Zn excess causes disorganisation of chloroplasts due to lesser supply of nitrate at the site of enzyme synthesis and hence hinders the activity of nitrate reductase. Reduced activity of nitrate reductase exerted a decline in the level of free amino acids which in turn inhibits the process of photosynthesis in plants. In addition, Zn stress in plants aids nitrogen mobilisation by promoting senescence and altering the flow of nitrogen through amino acids [179]. In *Artemisia annua* plants under varied Zn concentrations (50, 100, 200, 300 and 400 μg g^{-1} soil dry matter), the nitrate reductase activity and total soluble protein content at flowering stage was found to decrease upto 62.8 % and 56.3 % at 400 μg g^{-1} Zn treatment compared with the control [129]. Further, they observed a significant decline in the activity of nitrate reductase in leaves of *A. annua* plants with the increased Zn concentrations. Exposure to 3 and 9 mg l^{-1} Zn in *Ceratophyllum demersum* caused a significant inhibition in the activity of nitrate reductase [180]. According to Ferrario et al. [181] inhibition of chlorophyll formation and CO_2 assimilation, promotes inefficient nitrogen assimilation which inhibits nitrate reductase activity. Stuiver et al. [11] observed that exposure to 5 and 10 μM Zn treatments affect the uptake and metabolism of nitrate in *Brassica pekinensis* plants. Further, they observed that the elevated Zn concentrations have reduced the total nitrogen concentration which in turn decreased the nitrate concentration in both roots and shoots of the plants. Proline, a precursor of nitrogen metabolism also plays a vital role in reducing the free radical generation from thylakoids, generated from high light intensity and also promotes adaptive role by reducing the production of ROS and hence protects the plant from oxidative damage [182][183][184]. Tripathi and Gaur [185] also notified a remarkable protective role of proline in detoxification of ROS. The accumulation of proline (stress marker) in treated plants is an indicator of primary defense mechanism adopted by plants to maintain the osmotic pressure in cells. Zinc stress promotes accumulation of proline in plants. Alia et al. [186] claimed that elevated Zn concentration induced an increase in proline and MDA content in aerial parts of *C. cajan* (Fabaceae) and *B. juncea* (Brassicaceae). However, the increase in proline content was more prominent in shoots of *C. cajan* than that of *B. juncea*. Li et al. [161] observed remarkable increase in proline content at 1 mM Zn concentration in roots of *T. aestivum* compared to the control. Comparative studies between *S. nigrum* and *S. lycopersicum* suggested that with increase in Zn concentrations (0.25, 0.50, 0.75 and 1.00 mM) significant increase in accumulation of proline was observed in tissues of *S. nigrum* than that of *S. lycopersicum* [142]. Singh et al. [187] observed an increase in proline content in treated seedlings of *Vigna unguiculata* at 250 μM and 500 μM Zn concentrations upto 15 days of Zn exposure and was highest upto 59% under 500 μM Zn stress. Further, Fatima et al. [188] reported that in *Withania somnifera* under 50–500 μM $ZnSO_4$ treatments, the proline accumulation increased with the increase concentrations in the medium while maximum accumulation of proline content 0.17 μg g^{-1} FW was observed at 500 μM $ZnSO_4$ concentration.

Effect of Zn on protein metabolism

Stress induced by heavy metals usually decreases the activity of proteins in plants [189]. Zn stress induces catabolic activities by promoting activity of hydrolytic enzymes like protease, RNAase and by reducing the content of proteins and RNA in plants. Inhibition in the activity of nitrate reductase may result in the decrease in the level of proteins. Exposure of plants to toxic Zn concentrations induces the breakdown of various mechanisms associated with the formation of proteins and also reduces the incorporation of amino acids involved in the synthesis of proteins which ultimately caused the reduction in protein levels [190]. It was observed that leaves of *A. annua* showed significant reduction in protein levels throughout the life in Zn treated plants [189]. Concentration of protein significantly reduced in roots of *P. vulgaris* by combined treatment of both Cd and Zn [139]. The effect was more severe on roots than on shoots in terms of growth. Radić et al. [165] reported decrease in soluble protein content in duckweed plants at 0.15 mM Zn concentration. In tobacco plants, Zn excess was found to cause alteration in concentration of free amino acids (glutamic acid, glutamine, aspartate, cysteine), cause variation in stomatal conductance, photosynthesis, transpiration and utilisation of nitrogen under 750 mg Zn kg^{-1} soil [191].

Genotoxicity of Zn

Several reports have been found regarding the phytotoxic effect of Zn, yet not much is known about its genotoxic effects on the plant cell organelles. López-Moreno et al. [192] found that the Zn ions released from nanoparticles affect the genetic stability of soyabean plants by forming highly toxic hydroxyl ions which are the primary source for DNA damage. Further, Kumari et al. [193] investigated the cytogenetic and genotoxic effect of ZnO nanoparticles on *Allium cepa* root cells *and found that there was inhibition in mitotic index due to the blockage of mitotic cycle during interphase, thus inhibiting DNA protein synthesis. They also reported that the percentage of chromosomal aberrations increased with increasing concentration of* ZnO nanoparticles*. The ZnO nanoparticles also induced several kinds of mitotic aberrations which included breakages, bridges in Allium sativum* [194]. Oladele et al. [195] reported chromosomal aberrations in Bambara groundnut in response to Zn. Further, effect of Zn on chromosomes of cowpea and maize was investigated and was observed that it resulted in formation of anaphase bridges, laggards and further the mitotic index decreased with increase in concentration of metal solutions [196]. $ZnCl_2$ and ZnO nanoparticles were also investigated to cause genotoxicity in *Vicia faba* by forming chromosome breaks (or mitotic anomalies) [197]. Tkalec et al. [173] claimed DNA damage in tobacco seedlings by combined treatments of Cd and Zn due to their interaction with DNA.

Zinc hyperaccumulating plants

Zinc as an essential micronutrient is taken up and transported in all parts of the terrestrial plants. The average Zn content in soils which are not under the influence of Zn contamination ranges from 20–135 mg kg^{-1} while in those areas where Zn contamination is prominent the concentration may reach upto 80,000 mg kg^{-1}. In unpolluted environment, the average amount of Zn in plant leaves ranges between 10 and 70 mg kg^{-1}. Higher levels of Zn i.e. more than 100 mg kg^{-1} in the environment adversely affects the plant species [198]. For a plant to be a Zn hyperaccumulator, it must accumulate more than 10,000 mg kg^{-1} Zn in its dry tissue [199]. Worldover, more than 400 plant species are known to accumulate heavy metal in their tissue and out of it about 16 plant species are known to accumulate Zn and are known as Zn hyperaccumulators [200]. Further, according to Verbruggen et al. [201] 14 plant species

belonging to 6 families have been identified as capable of accumulating ≥ 1.0% Zn. Phytoremediation is an environmentally safe, green technology in which hyperaccumulator plants have been employed to extract heavy metal ions from both soil and water bodies [158]. World over, large number of Zn hyperaccumulating plant species have been identified which have the ability to accumulate large amounts of Zn in their tissues. *T. caerulescens* a member of family Brassicacae can tolerate and accumulate up to 40,000 mg Zn kg^{-1} tissue in its aerial parts. Jiang et al. [202] observed that uptake and accumulation of Zn in aerial parts of *T. caerulescens* ranges upto 5500 mg kg^{-1} shoot dry weight when co-planted with *Lolium perenne* (**Table 1**). In *A. thaliana,* accumulation of heavy metals like Zn is primarily contributed by the uptake mechanisms prevailing in the roots. Overexpression of ZAT genes from *A. thaliana helps the plant to tolerate and accumulate increased Zn concentrations [203]*. *Gomphrena claussenii* of family Amaranthaceae grew well in Zn contaminated soils and accumulated up to 5318 µg g^{-1} of Zn and 287 µg g^{-1} of Cd in their shoots under 30 days of exposure [204]. Yang et al. [205] reported *S. alfredii* close to other Zn hyperaccumulators like *T. caerulencens* and *A. halleri* because of its ability to tolerate and accumulate Zn in its dry matter up to the levels of 1000 µM in nutrient solution and in the soil up to 2000 mg kg^{-1} without showing any reduction in dry matter. They further added that only metallicolous population i.e. hyperaccumulating ecotype (HE) of *S. alfredii*, was found to accumulate Zn upto the levels of 17,300 mg kg^{-1}, 21,945 mg kg^{-1} and 29,000 mg kg^{-1} respectively in the leaves, stem and shoot parts of the plant. Studies revealed that *A. halleri*, has the remarkable ability to accumulate Zn in its tissue. It was observed that Zn exposure to 1000 µM in *A. halleri* has caused the accumulation of Zn in the shoots upto the range of 32 000 µg g^{-1} dry weight [206]. According to Nouri et al. [207] *Reseda lutea* a native Zn hyperaccumulator plant species of Ahangaran lead–zinc mining area was found to accumulate 2,938 mg Zn $kg^{-1}$of plant dry tissue in roots whereas shoots of *Scariola orientalis* accumulated 3,538 mg Zn kg^{-1} of plant dry tissue (**Table 1**). In another study Ghaderian et al. [208] claimed significant ability in leaves of *Matthiola chenopodiifolia* to accumulate 4800 µg g^{-1} of Zn. A tree sp., *Betula pendula has also been found to accumulate high amount of Zn in its leaves upto the levels of* 3100 mg kg^{-1} [209]. Some aquatic species like *Eichhornia crassipes* also have the ability to accumulate large amount of Zn in its tissues. Under 4 days exposure to 40 mg L^{-1} Zn concentration, the roots and shoots of *E. crassipes* has been found to accumulate Zn upto the level of 9652.1 mg kg^{-1} and 1926.7 mg kg^{-1} [210]. Some grasses like *Festuca rubra* have been observed to accumulate Zn upto the range of 1500 mg kg^{-1} dry matter tissue [211]. In *Phragmites australis*, 4µg ml^{-1} Zn treatment for 3 weeks resulted in the accumulation of Zn in roots upto the range of 17,958 µg g^{-1} dry weight [212]. In *Salix viminalis*, Zn exposure under 10 µM for 20 days caused accumulation of 1810 mg kg^{-1} of Zn in shoots [213]. *Arabis paniculata*, a Zn hyperaccumulator and was found to accumulate Zn in shoots upto the level of 20,800 mg kg^{-1} dry plant tissue [214] (**Table 1**). Under Zn exposure of 300 mg l^{-1}, the roots and leaves of *Sesbania drummondii* were found to accumulate about 10,360 mg of Zn kg^{-1} and 1873 mg of Zn kg^{-1} dry weight tissue respectively [215].

CONCLUSIONS AND FUTURE PROSPECTS

Zinc is an essential micronutrient and is required by the plants for performing various physiological and metabolic functions. Elevated Zn levels in plants affects the physiological and biochemical parameters by altering various enzymatic and non-

enzymatic activities and is highly dependent on its availability and the other environmental conditions. Zinc toxicity in plants promote genotoxicity, hence interferes with various metabolic activities vital for plant growth by replacing or altering the gene sequence and other important metal ions (Fe^{2+}, Cu^{2+} etc). Transporter families play an important role in Zn homeostasis and translocation in plants, favouring genetic modification of plant species that can be effectively used in phytoremediation. Further, some above mentioned plant species have the potential to accumulate elevated levels of Zn in their tissues, thus strongly marking their role for remediation of contaminated soil and water sites. Nevertheless, future advances like purification, modification and expression of membrane proteins may assist to comprehend the interaction between transporter proteins and other components involved in cellular Zn trafficking. This may enable to understand the plant mechanisms of Zn transport in a better way. Besides, number of tools and techniques are available to locate Zn accumulation in plant tissues. The fluorophores for confocal microscopy may be used efficaciously in imaging and localisation of Zn in cell organelles of hyperaccumulator plants. Another technique like synchrotron X-ray fluorescens may be exploited in allocating Zn in tissues of both hyperaccumulator and non-hyperaccumulator plant species. Additionally, some improved and advanced spectroscopic and chromatographic methods may be employed to improve the understanding regarding Zn hyperaccumulation and tolerance in plants. Tremandous work has been done regarding the use of synthetic chelants in plant Zn homeostasis. Keeping in mind about their delterious effects on the environment, some ecofriendly amendments should be encouraged for enhancing Zn accumulation in metal tolerant plant species.

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