**ZN AS A VITAL MICRONUTRIENT IN PLANTS**

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**ABSTRACT**

Macro and micronutrients are vital for the growth and productivity of the plants. Zinc (Zn) is considered to be one of the essential micronutrients for the growth and development of cereals as well as fodder crops. It is also a regulatory cofactor for all those enzymes which are required for the synthesis of chlorophyll, proteins and carbohydrates. The functioning of these enzymes is affected significantly due to Zn deficiency and there will be a retarded growth and productivity of plants. Deficiency of Zn is a universal problem among cereal crops. The concentration of Zn varies from 6-1.2 mg/kg in various soils, whereas its concentration reaches 20-300 ppm in plants. Zn deficiency leads to chlorosis in the leaves of plants. Various reasons affect the availability of Zn in the plants, which include soil type, pH of the soil and availability of other nutrients that work antagonistically for the absorption of Zn. Zn applied as the fertilizer gets converted into unavailable form by making insoluble complexes and thus not available for plants. Hence the best alternative to this issue is the use of Zn solubilising bacteria (ZSB). These ZSB will accumulate in the rhizosphere zone of the plants and will reduce the requirement of the applied Zn fertilizer. It will prevent Zn toxicity in the soil and will enhance the uptake of other macronutrients like phosphorus to the plants.

**Keywords:** Bio fertilizer, Zn, PGPR, Zn Solubilising Bacteria

**INTRODUCTION**

Appropriate nutrition for plants is one of the vital factors for improving the quality and quantity of product obtained from them. Macronutrients and micronutrients are the essential elements needed by the plants from the germination of the seed up to the fully developed plant. Elements that are required in large quantity are categorized as macronutrients, and those that needed in trace amount are micronutrients (Sharma *et al*., 2013). Micronutrients are very much essential for the growth of plants and their metabolism. The tremendous use of micronutrients in the soil for agricultural purposes cannot be denied. Nutrients like boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and Zinc (Zn) are classified as micronutrients. These metals are considered very significant for plants even if they are taken up in comparatively lesser amounts by the plants (Grusak *et al*., 1999). These micronutrients protect plants from various biotic and abiotic stresses by engaging in numerous roles. However, micronutrient deficiency may lead to multiple diseases in plants, which adversely affects the quantity and quality of the plant’s products (Huber and Wilhelm, 1988).

Zn (Zn) plays a phenomenal role as a micronutrient in plants. Even if it is required in small quantities by most of the plants, the correct amount of Zn is necessary for the proper functioning of several plant physiological pathways that plays a salient role in their growth and development (Mousavi *et al*., 2011; Yosefi *et al*., 2011; Cabot *et al*., 2019). Zn is necessary for various biochemical processes such as synthesis of cytochromes, nucleic acid and activation of enzymes. It is an integral factor of different enzymes playing a significant role in carbohydrates metabolism, oxidation reactions in plants, and various revival mechanisms (Alloway, 2001). Zn is an essential cofactor for enzymes required for specific protein biosynthesis and prominent in chlorophyll formation. Zn is necessary for the activity of the enzyme rubisco. Therefore, it has a significant role in regulating the photosynthesis rate in higher plants (Das *et al*., 2018). Zn is necessary to maintain membrane activity and maturation of seed and stalk of plants with an increased rate. Zn also has an intrinsic role in auxin formation, which assists the growth of plants (Barman *et al*., 2018). Hence Zn has an indispensable role in plant development and its productivity.

Cakmak, (2000) note that around 50% of the land utilized for growing crops across the world has a low concentration of accessible Zn, which is needed to support crops’ yields and hence increase the nutritional value of the grains. Zn deficit in the soils as well as in the plants has become an issue in most of the countries. The main reasons responsible for the occurrence of this widespread deficiency of Zn are Zn’s solubility in the soils is very low despite enough availability of the total amount of Zn. The deficiency of Zn in plants is a significant widespread deficiency compared to other micronutrients, which affects the rate of photosynthesis in various cereal crops (Barman *et al*., 2018). Zn deficiency leads to discoloration of leaf, and this condition is termed chlorosis (Sharma *et al*., 2013). Zn becomes a prooxidant by generating reactive oxygen species when it is in excess or even deficient in plant cells, leading to damaging the plant cell (Cabot *et al*., 2019).

**Figure 1** Application of Zn in Human, Plants, Animals and Microbes

**Uptake of zinc chemical fertilizer**

Plants uptake Zn as a divalent cation (Zn$^{2+}$); however, only a very minute amount of Zn is readily available as soluble Zn for plant uptake (Marschner, 1993; Gao *et al*., 2009). When soluble forms of zinc are applied to the soil in chemical fertilizer, it gets converted into insoluble complexes. It becomes unavailable to the plants leading to the deficiency of Zn in plants. This results in Zn deficiency, and there are various methods to assuage this critical problem. The application of Zn fertilizer is one way to combat Zn deficiency (Kamran *et al*., 2017). Zn fertilizers are generally used to facilitate crop yields in regions of the Zn deficit. The utilization of fertilizers containing Zn increases the productivity of crops (Efe and Yarpuz, 2011). However, the extensive use of fertilizers can cause a myriad of problems like acidification of the soil. When...
Zn is applied as zinc sulphate, it gets converted to insoluble complexes within seven days. Overuse of zinc sulphate leads to the accumulation of insoluble Zn in the soil. It causes Zn toxicity which later inhibits the absorption of other macronutrients, thus behaving antagonically the absorption of other required nutrients. Under such conditions, Zn is toxic and results in reducing the availability of Zn in the soil in a chelated state. When the concentration of total Zn increases from 100-1000 ppm in soil, it leads to Zn toxicity. Zn toxicity then becomes a significant issue its deficiency (Marten et al., 2013). Hence the overdose of chemical Zn fertilizer will generate the problem of Zn toxicity in plants.

Two strategies are employed to overcome Zn deficiency 1) genetic biofortification 2) agronomic biofortification. Genetic biofortification involves the use of breeding practices and transgenic approaches. In inbreeding practices, the genetic morphology of plants is transformed to create suitable characters. However, the development of varieties specific for the Zn deficiency problems involves complex integrated traits. Further, it is a prolonged, labour-intensive technique that may take many years to build and perfected a biofortified variant.

Moreover, the complex laboratory procedures and the high cost involved also present challenges in the practical implementation (Hafeez et al., 2013). The transgenic approach includes augmenting biofortified crops. Abaid-Ullah et al. (2015) showed that the expression of transcription factors such as bZIP19 and bZIP23 resulted in increased bioavailability of Zn in the plants. Transport protein of plasma membranes is the main target of Zn concentration alteration in various regions of plants. In the agronomic approach, Zn fertilization is given to plants which ensure Zn translocation for a limited duration. Zn sulphate, which is transportable, can amplify the supply of Zn concentration within the plants (Kamalakannan et al., 2019).

**Rhizospheric bacteria**

The soil is the home of microscopic life forms like fungi, bacteria, algae, and protozoa, found in abundance. Some of them are pathogenic to the plants, some do not interact with them and while others are beneficial. Bacterial cells are found in abundance as compared to others. The type and concentration of bacteria depend upon the pH of the soil, type of soil, concentration of moisture in it, types of plants grown there, and the nutrients present there. Likewise, the presence and abundance of bacteria are not homogenous in the same soil. The concentration is higher around the roots of the plant. These potential bacteria present in the narrow region of soil around the bases (rhizosphere soil) of most of the plant are called rhizosphere bacteria (Vacheron et al., 2013). This narrow zone of root-soil is very high in nutrient concentration than the overall surface or subsurface soils because of the accumulation of several root exudates, sugar and various amino acids, and the primary nutrients the energy source for microorganisms. This is why the colonization of bacteria in the ecological niches of the root makes their habitat in the root zone (Abaid et al., 2015; Mumtaz et al., 2017).

The way these rhizospheric bacteria will behave depends upon many factors. They can enhance metal absorption and promote plant growth, or they may decrease the absorption of specific macro or micronutrient because of other metal nutrients. Depending upon their mode of action, bacteria can be categorized as growth-promoting or growth-inhibiting bacteria (Sirohi et al., 2011). In the former, universal growth promotion, growth-promoting bacteria or PGPR. All those bacteria that improve plant health and its productivity come under the category of PGPR; they support the development whereas works opposite in cherry trees which ensure Zn translocation for a limited duration. Zn sulphate, which is transportable, can amplify the supply of Zn concentration within the plants (Kamalakannan et al., 2019).

**Distribution and bioavailability of Zn**

The natural sources of Zn in soil include weathering of rocks into the soil and atmospheric contributions (fires, dust). In soils, the quantity of total Zn is distributed in five fractions including the Zn found in soil solutions, Zn bound to particles of soil in the form of ions, Zn complexed with organic ligands, adsorbed Zn\(^{2+}\), Zn adsorbed on to clay minerals and insoluble metal oxides (Alloway, 2001). Zn is available in the soil as ZnS (sphalerite), and sometimes in small quantities as smitsohite (ZnCO\(_3\)), ZnTe (ZnTe), zinkosite (Zn\(_2\)S\(_4\)). These are Zn- containing mineral ores (Hafeez et al., 2013). Aggregate Zn profile of soil is mainly dependent on the geographical composition of rocks. The total Zn content in earth crust is 78 ng of Zn per kg of soil (Noulas et al., 2018). Salah et al. (2015) reported that the concentration of Zn which is present in soluble form and available to plants is approximately 4 to 270 μg L\(^{-1}\) (ppb) in most of the soils. This concentration is too low when it is compared to the total Zn concentrations that range from 50 to 80 ppm. In highly acidic soils, the concentration of soluble Zn is about 7137 μg L\(^{-1}\) which indicates that solubility is strongly linked to and is inversely proportional to pH of soil. It is reported that concentration. Of Zn ranges from 10-300 μg Zn g\(^{-1}\) of soil in the majority of arable soils (Gupta et al., 2016). Although Zn is present in abundance in such soils, a large amount exists in insoluble form (zinc sulphate, zinc oxide, zinc chloride, zinc phosphate and zinc carbonate) and hence unavailable to the plant. Only the Zn that can be desorbed easily and is available in soluble fractions is used by the plants, but that fraction of available Zn is very low. Moreover, the accessibility of Zn is determined by the physicochemical properties of soils, plant root activity and presence of micro flora in the rhizosphere zone of the plant (Broadley et al., 2007). Zn interacts with chloroide, sulphate, phosphate, and nitrate ions to form soluble complexes. Out of these, nitrates, chlorides and sulphates are soluble in water and pose no hindrance in Zn availability to plants (Prasad et al., 2016). The neutral Zn phosphates (Zn\(_4\)(PO\(_4\))\(_3\)) and insoluble sulphate (ZnSO\(_4\)), species are the very crucial ones as they have been recognized as the prime ones for contributing Zn in soil solution. Addition of Zn sulphate leads to increase in the solubility of Zn\(^{2+}\) ions within the soils. Therefore, the presence of acidifying fertilizers, such as ammonium sulphate (NH\(_4\)\(_2\)SO\(_4\)), which reduces the pH of the soil and makes the soil acidic and hence increase the solubility which leads to the increased availability of Zn to the plants (Alloway, 2002). The Zn which was earlier in organically complexed form, after acidification of the soil becomes increasingly mobile. Now when the Zn exists in its soluble structure in soil then it is easily accessible to plants (Shambhavi et al., 2019).

**Adsortion of Zn by soil constituents**

Adsorption and precipitation are two methods which are responsible for regulating the concentration of various ions in the soil. Adsorption of Zn cations which are exchangeable with other metal cations present in soils is expressed in the simplest way as:

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\text{Zn}^{2+} + \text{X-Soil} \rightleftharpoons \text{Zn-Soil} + X^+ \]

Where X is any of the divalent cations. Cations get attracted to negatively charge solid surfaces in preference and selectivity, which varies based on the various kinds of adsorbents such as hydrous oxides, clay minerals, and sticky substances depending upon the chemical composition of the soil. The cation exchange capacity of soil will determine how efficiently the soil will supply nutrients to plant (Diatte and Koczalikowski, 1998). Speir et al. (2003) studied the adsorption of Zn on calcareous soil in which severe Zn deficiency problems in crops is being found. They found that some concentration of total Zn had been irreversibly fixed to the soil, making the reaction irreversible. According to Alloway (2002), there are two non-identical methods required for Zn adsorption by the soil and organic matter present in it. One is the cation exchange method, which is primarily applicable for acidic soil conditions and other alkaline soils, mostly involving chemisorptions and complex formation by organic ligands.

**Factors affecting the Zn availability to plants**

Many factors are responsible for affecting the Zn availability to plants, such as the total content of Zn present in the soil. The solubility of Zn in the soil is very low; hence many times, despite sufficient concentration in the soil, the Zn availability to plant remains low. As Zn is a trace element, it occurs in the soil in various chemical forms that affect its availability to the plants. It remains soluble in the soil by various mechanisms like biodegradation and application of Zn Solubilizing PGPR to promote sustainable development and reduce the use of chemical Zn fertilizer.
ranges from 10-300 ppm of the majority of agricultural soils (Gupta et al., 2016). At the same time, the total concentration of available Zn decreases by 100 folds for every unit of pH increase (Moreno Jiménez et al., 2019). Another reason affecting Zn availability to crops is the profile of the soil, geology and environment. A change in the redox conditions, soil moisture, and pH conditions of the soil, the concentration of various other micronutrients, macro-nutrients availability importantly phosphorus and climate conditions of the area control the sorption-desorption process of Zn ions in the soil and cumulatively affect the availability of Zn to plants (Rutkowska et al., 2015). Organic matter present in the soil also affects its availability. Such calcareous soils have low organic matter; hence, Zn remains in an inactivated form (Barman et al., 2018). Microbial activity present in the rhizosphere zone of the plant controls the amount of Zn that will be made available to the crop. It is also affected by various anthropological activities, such as the application of herbicides, pesticides, irrigation, and intensification near the agricultural and urban sewage discards. (Dos Santos et al., 2013). The factors cumulatively hinder or facilitate Zn availability to plants.

Uptake and translocation of Zn in plants

Assimilation of Zn micronutrient from the soil surface to the rhizosphere of a plant is the principal approach for its accretion inside the plant preceding to its transportation to seeds (Hafeez et al., 2013). Although the Zn uptake changes amid different species, it is usually calculated via the composition and concentrations of the media used for growth (Tsonev and Lindon, 2012). The assimilation of Zn varies among the grains of different crops and species (Wu et al., 2010). Zn concentrations in different cultivars of rice are found to be very different (Yang et al., 1998; Graham et al., 1999). The mechanisms through which Zn gets transferred from soil to plant are much explored and understood. The basic physiology of varied Zn uptake efficiency is the root system and its morphology (Brown et al., 1997; Cakmak et al., 2000; Gnanaman et al., 1999; Wido et al., 2010). However, the translocation of Zn and its redistribution after entering the transpiration stream is very meagre. Jiang et al. (2007) reported that Zn accumulation is related to many factors amongst which its uptake by the root system plays a crucial role. Zn status within the plant is further dictated by the redistribution and mobilization of stored Zn. Zn availability to the plant occurs when Zn complexes with organic ligands or is available in its divalent ion form. Uptake of zinc follows a linear relationship with its concentration in soil or solution of nutrients (Tsonev and Lindon, 2012). The Zn is transported from the roots to the shoot tissues through the xylem. Later, apoplast help in the translocation of Zn to xylem of roots (Broadley et al., 2007). Moreover, it is found that the phloem also had high levels of Zn, it indicates that Zn is translocated to the plants leave by both phloem and xylem tissues (Haslett et al., 2001). Zn is primarily absorbed by roots as Zn⁺ from the soil solution or as Zn(OH)2 when the pH of the soil is high. The process is found to be regulated by proteins having high affinity for Zn. Wheal et al. (1997) proposed that Zn is transported inside the plant cell towards larger negative electrical potential and its transportation capacity inside the plant cell is found to be much more when the pH of the soil is high. The process is found to be regulated by proteins having high affinity for Zn. Wheal et al. (1997) proposed that Zn is transported inside the plant cell towards larger negative electrical potential and its transportation capacity inside the plant cell is found to be much more.

Biochemical and physiological functions of Zn in plants

The metabolic attributes of Zn are assigned to its capacity of forming complexes with N, O and S inside the plant cells. Intracellular Zn remains inactivated by forming nexus with organic ligands (Tsonev and Lindon, 2012). Zn affects the uptake of water and its transportation capacity inside the plant cells (Tsonev and Lindon, 2012). It is found to affect the negative consequences of heat and salt stress (Peck and McDonald, 2010; Tavallali et al., 2010). It is actively involved in the synthesis of auxin, which is an essential growth hormone. Zn is needed to initiate catalytic activity; therefore, metal ions are a significant part of most of the cofactors. Most of the metal ions have a close interaction with the respective enzymes; metal ions assist the interaction and enhance the catalytic activity of the respective enzyme. Hence the ions accept electrons; they stabilize the structure of enzymes and regulate the speed of biochemical reaction (Eide, 2011). Furthermore, Zn is very crucial for various plant enzyme systems, including Carbonic anhydrase, several dehydrogenases: alcohol dehydrogenase, glutamic dehydrogenase, L-lactic dehydrogenase, malic dehydrogenase, D-glyceroldehyde-3-phosphate dehydrogenase, and D-lactate dehydrogenase, Aldolase Carboxypeptidase, Alkaline phosphatase, Superoxide dismutase, RNA polymerase, Ribulose bi-phosphate carboxylase, and Phospholipase (Sharma et al., 2013). Unlike other micronutrients, Zn is present as a transition element and does not undergo a change in valency and exists only as Zn (II) in plants ((Patel et al., 2007). In plants, nearly 70 Zn containing metalloenzyme have been reported to date (Zastrow and Pecoraio, 2014). Zn is a structural, functional, and regulatory cofactor of the enzymes belonging to all the enzyme classes. Zn is omnipresent of all metallic cofactors, and more than 300 enzymes have Zn as a cofactor. As Zn exists in the Zn²⁺ form, therefore, it does not have any redox properties (McCull et al., 2000). It makes substantial nexus with free radicals of the polar groups that contain N, O, and S (Alloway, 2001; Lin et al., 2005). Atoms of Zn that are tightly bound to the apoenzyme are difficult to remove even if they are chemically treated. Zn usually bound through imidazole and cysteine rings in enzymes requiring for their activity. The binding of a water molecule to the required site of the catalytic function is required for Zn. Therefore, catalytic Zn makes the bond with one molecule of water and three protein ligands. On the other hand, in all those enzymes where Zn has a regulatory or structural role, Zn bound with four ligands (Auld and Bergman, 2008). Alcohol dehydrogenase catalyzes reverse oxidation; the enzyme has two Zn atoms. The first one is for catalytic function and the second one has a structural function. Alcohol produced under anoxic condition because of waterlogging is needed for repairing the photosynthetic apparatus, as it turns over the D1 protein that gets photo-damaged (Bailey, 2002; Hänsch and Mendel, 2009). Zn finger protein arbitrates protein-protein interactions (Cobat et al., 2019). Zn helps in modulating the functions of different molecules and has a primary role in the maintenance of cellular membrane integrity by making interaction with sulphydryl groups and phospholipids of membrane proteins. It also aids in maintaining the transportation of ions within plant cells (Disante et al., 2010; Salah et al., 2015). The plant cell membranes destabilized due to Zn deficiency could lead to leakage in the cell. The other one is needed to synthesizing nucleic acids; it also maintains the stability of nucleic acids by forming complexes (Coleman, 1992). It is also involved in the metabolism of lipids (Marschner, 1993). It is observed that Zn deficiency could also be linked to a number of undesirabley conditions due to its deficiency, which occurs due to the deformation of ribosomes. A significant reduction in the synthesis of both RNA and ribosomes in the plant occurs due to Zn deficiency. This shows that Zn is also required for protein synthesis (Brown et al., 1993). Zn is needed for carbohydrate fixation. It regulates the formation of reactive bicarbonate species from carbon dioxide, especially in C4 plants and maintains a high CA activity. Zn aids in the active functioning of PEP carboxylase by shifting the equilibrium in favour of its substrate HCO3⁻ and maintains enough supply of HCO3⁻ in guard cells where Zn also determines the K⁺ influx (Brennan, 2005; Sharma et al., 2013). Zn plays a vital role in carbonic anhydrase activity. Zn is also needed for the respiratory enzyme, quinone oxidoreductase, and altering amino acids and protein (Castillo-González et al., 2018). In addition to this, Zn also prevents damage of membrane, which is caused by Reactive Oxygen Species (ROS) and is needed for the synthesis of an antioxidant enzyme that is H2O2 scavenging ascorbate peroxidase which is synthesized in response to the oxidative stresses (Ascher et al., 1997; Cakmak, 2000). One of the primary functions of Zn is the regulation and expression of genes as Zn finger transcription factors. These are involved in the regulation mechanism of various biological processes like flowering, pathogen responses and photomorphogenesis (Castillo-González et al., 2018). Zn is also found to be vital for flowering and seed production. Brown et al. (1993) demonstrated that when Zn deficient plants are treated, it has increased the number of florescences and yield of seeds comparing with Zn treated plants. Zn affects the uptake and therefore the process of influx and efflux of HCO³⁻ in guard cells (Wu et al., 2007). The Zn is transported from the roots to the shoot tissues through the xylem. Later, apoaplast help in the translocation of Zn to xylem of roots (Broadley et al., 2007). Moreover, it is found that the phloem also had high levels of Zn, it indicates that Zn is translocated to the plants leave by both phloem and xylem tissues (Haslett et al., 2001). Zn is primarily absorbed by roots as Zn⁺ from the soil solution or as Zn(OH)2 when the pH of the soil is high. The process is found to be regulated by proteins having high affinity for Zn. Wheal et al. (1997) proposed that Zn is transported inside the plant cell towards larger negative electrical potential and this process is thermodynamically passive. It is also found that metabolic inhibitors do not have any impact on Zn⁺ uptake and therefore the process of Zn uptake is independent metabolically. Irrespective of the kinetics of Zn uptake, its mobility in plants occurs via making a bond with organic compounds in xylem fluids (Salah et al., 2015). Bowen et al. (1974) studied the impact of temperature on adsorption of Zn in Pinus radiate roots and demonstrated that Zn absorption by plant roots is inhibited by low temperatures.
metabolized by alcohol dehydrogenase. The anoxic condition leads to oxygen reduction in soil. This can result in the production of toxic substances and increase the concentration of various natural compounds like carbon dioxide, organic acids, sulphides, and hydrocarbons (gaseous). During Zn deficit, alcohol dehydrogenase activity subsides to a low limit. Moreover, this limitation is linked to reducing root binding capacity (Hafeez et al., 2013). Carbonic anhydrase, a metalloenzyme that requires a Zn cofactor, catalyzes instant change of carbon dioxide and water to bicarbonate ion (HCO₃⁻) and a proton. Zn ions activate the catalytic site of the enzyme by reacting with water and thus catalyzing carbonic anhydrase. The enzyme is responsible for numerous operations like pH regulation, photosynthetic CO₂ fixation, respiration, CO₂ transfer, ion exchange and stomata closure. Carbonic anhydrase is required in numerous physiological processes. Any alterations in Carbonic anhydrase activity promptly impact the photosynthetic fixation of carbon dioxide under the carbon dioxide limiting conditions. Carbonic anhydrase activity is thus dependent on atmospheric carbon dioxide levels. Zn deficit will eventually affect Carbonic anhydrase and influence all the processes linked to the enzyme (Castillo-Gonzalez et al., 2018).

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide and hydrogen peroxide. Therefore, it has a role in plant defense against antioxidants. At the active site of an enzyme, Zn links with copper to form Cu-Zn SOD. During Zn deficiency, SOD activity decreases. There is a surge in oxygen production as Cu-Zn SOD is responsible for controlling the generation of toxic oxygen radicals. As toxic oxygen radicals rise, the plasma membrane permeability also surges. This elevation results in depletion in sugar, amino acids, and carbohydrates (Millán et al., 2005). During Zn deficiency, SOD is responsible for controlling the generation of toxic oxygen. It impairs the NADPH oxidation as harmful oxygen-free radicals disintegrate the polyunsaturated fatty acids and phospholipid membrane bonds. Zn is required to permeate membrane integrity as it can bond membrane phospholipid groups and configure tetrahedral groups with cysteine residues (McCracken et al., 2013).

Zn interaction with other nutrients present in the soil

Interaction with macronutrients

Interaction of Zn with other macronutrients and micronutrient is a very crucial factor in plant production. For example, in some soils, nitrogen promotes the growth of plants and changes the pH of the root area and thus affects the Zn status of crops, whereas, in many other soils, nitrogen is found to be the significant factor in inhibiting the growth and yield of crops. Thus, nitrogen and Zn fertilizers, when applied in combination, significantly improved crop yields due to the positive interactions among the two nutrients (Shri et al., 2017). At the same time, Kirk et al. (1995) reported that when nitrogen fertilizers are applied alone, they led to a deficiency of Zn. Nitrogen fertilizer enhances plant growth, but it also shows negative interaction with various other micronutrients, such as copper. Plant growth which is promoted by the application of nitrogen fertilizer, causes reduces copper concentration in the plant, which is further made worse by applying Zn fertilizer. The application of nitrogen fertilizers exacerbates Zn-Cu interaction. In solution culture experiments, many micronutrients such as magnesium, calcium, sodium and potassium are amongst the one that inhibits the Zn absorption by the plant root system. Within the soil, they affect the pH of the soil. CaSO₄ application decreased the pH of the soil, so it enhanced the mobility of Zn ions and expanded uptake by the plants. In contrast, application of the same concentration of CaCO₃ led to an increase in pH, which leads to a decrease in free ions and therefore retards the uptake of Zn. (Allen, 2002; Golubovic et al., 2012).

Interaction with micronutrients

Zn interacts with micronutrients such as boron, iron, copper, and manganese and affects the concentrations of these micronutrients in plants. Interaction of Zn with these metal ions has shown varied responses in plants. Lomeragan and Webb (1993) reported that when Zn is applied with iron, it increases the supply of Zn to the plants. However, iron concentrations were found to increase in some plants, decreases in some, and even affect Fe concentrations. According to Imamzai et al. (2003), the use of Zn fertilizers had adversely affected the concentration and intake of Fe in plants. The Roots of the Zn deficient plants have shown more iron mobilization from Fe⁺⁺ hydroxides compared to the plant roots that have adequate Zn. Zn and copper, both micronutrients, share the same site on the root for absorption. Therefore, the application of Cu does affect the absorption of Zn in plants. Interaction of both Manganese and boron with Zn might have both positive and negative responses in plants (Za et al., 2005). Zn content in the plant is affected by the availability, uptake, and assignment of Zn in the plant. Zn-deficient plants absorb more boron due to decreased membrane function in the plant’s root than plants having sufficient Zn (Mousavi et al., 2012).

Role of Zn in plant defense

Zn plays a substantial role in plant growth. It has an equally important role in plants defense mechanisms in response to insects and pathogens (Yamasaki et al., 2007). According to Li et al. (2010) application of Zn fertilizers ensure this remarkable decline in disease symptoms in plants. Moreover, Helfenstein et al. (2015) observed that sometimes a particular concentration of Zn used against a particular pathogen could make that plant susceptible to other pathogens. It shows that the Zn proteins, which are involved in the plant defense, play a dual role and sometimes can support as well inhibit the pathogens combined. The response of plant and the pathogen towards the application of Zn is greatly dependent on the ability of pathogens to survive the plant defense mechanisms and whether the applied zinc is inhibiting or combating the pathogen attack. It may also depend on the conditions of the surroundings favouring either the plant or the pathogen (González et al., 2005). Cabot et al. (2019) described Zn mediated protein-based mechanisms for plant defense. The mechanism studies address effective plant defense with increased gene expression or enhanced activity of the referred protein function by enhancing Zn availability to the plant. However, it must be made clear that Zn proteins alone are not involved in these defense mechanisms; other responses related to Zn are also combined. Machado et al. (2018) documented that Zn is involved in two broad-spectrum responses in plant-pest/pathogen interactions. These include oxidative stress and regulation of Zn finger proteins. In the case of Zn deficiency, reactive oxygen species (ROS) are contemplated to be the chief factor accountable for hindrance to plant growth. The plant immune system causes oxidative damage or triggers non-oxidative mechanisms via ROS to combat pathogen attack. The oxygen radicals are triggered by plant defense mechanisms post identification of attack by pathogens on plants. The defence mechanisms mainly are hypersensitive responses and systemic acquired resistance (SAR). The Superoxide dismutase, an antioxidant enzyme that controls the activity of oxygen radicals, is increased during pathogen attack. Plant response to toxic Zn concentration is via regulating nitric oxide (NO), and systemic defence of plant response against stress is by regulating salicylic acid and jasmonates (JA) (Helfenstein et al., 2015). Response of plant and pathogen towards the application of Zn also depends on the conditions of the surroundings that can favour either the plant or the pathogen (Cabot et al., 2019).

Deficiency of zinc and appearance of symptoms in crops

Inadequate supply of Zn to crops is called Zn deficiency. Zn deficiency in crops is a severe problem that simultaneously affects food production (Alloway, 1997; Welch and Graham, 2002). Zn deficiency leads to impairment of many essential Zn dependent physiological functions and has adverse effects on plant growth (Sadeghzadeh, 2013). However, almost all Zn deficient crops positively responded to Zn application (Welch and Graham, 2002). The deficiency of Zn in plants is because of many factors like low Zn availability, low total soil Zn concentration, and high levels of nitrogen, calcium carbonate, bicarbonates, organic matter and high pH soil. Zn deficiency is very dominant in sandy soils, calcareous soils, peat soils, clayey soil and the soils that have increased silicon and phosphorus concentration (Alloway, 2001). Soil formed from gneiss and granite is also low in Zn (Sadeghzadeh, 2013). Soils rich in phosphates also might lead to Zn unavailable to the plant (Grewal, 2003). Spirit et al. (2003) also investigated the concentration of Zn in different types of soil and observed that Spodosols (28 ppm) and luvisols (35 ppm) are Zn deficient while fluvisols and cambisols showed that the Zn proteins, which are involved in the plant defence, play a dual role and sometimes can support as well inhibit the pathogens combined. The defence mechanisms mainly are hypersensitive responses and systemic acquired resistance (SAR). The Superoxide dismutase, an antioxidant enzyme that controls the activity of oxygen radicals, is increased during pathogen attack. Plant response to toxic Zn concentration is via regulating nitric oxide (NO), and systemic defence of plant response against stress is by regulating salicylic acid and jasmonates (JA) (Helfenstein et al., 2015). Response of plant and pathogen towards the application of Zn also depends on the conditions of the surroundings that can favour either the plant or the pathogen (Cabot et al., 2019).
nerves start appearing yellow in the leaves (Vitosh et al., 1998). Mousavi (2011) described that in dicots, internode distance and leaf size is shortened due to Zn deficiency. In monocots (especially corn), bands start appearing in the central nerve on both sides of monocot leaves.

Zn and its association with crop productivity

It is evident from the studies that Zn is necessary for the proper functioning of many important plant physiological pathways. Hence, the concentration of Zn available to plants is very critical (Alloway, 2001). As discussed earlier, Zn has a vital role in enzyme activation, protein synthesis, and carbohydrates metabolism and revival reactions. Efe and Yarpuz, (2011) reported that the performance of crop and the quality of their products increase by utilizing Zn containing fertilizers. In contrast, there is a sharp decline in photosynthesis and, therefore the productivity due to the shortage of Zn. Zn also acts as a regulatory cofactor of many different enzymes other than those associated with photosynthesis. It is a structural constituent of the proteins involved in critical biochemical pathways related to auxin metabolism, carbohydrate metabolism, protein metabolism, photosynthesis, pollen formation, sugars to starch conversion and plants defence mechanism (Alloway, 2001). Therefore plays a significant role in crop production (Graham et al., 1992). Chemical fertilizers are most used to deal with Zn deficiencies, but the cost of these fertilizers is usually too high. Therefore, there is a need for suitable alternative methods (Alloway, 2002).

PGPR for dealing with Zn deficiency

Preventing the occurrence of Zn deficiency and using appropriate management methods to regulate the Zn concentration in the soil are two ideal ways to deal with the widespread problem of its deficiency (Alloway, 2002; Mousavi et al., 2011). Due to an increased demand for animal and human food, there is a need to increase edible plants and fodder crops. Hence, farmers’ application of fertilizers in crops is the most preferred method used to increase productivity following the population’s demand. The agricultural departments of various countries focus on enhancing biofertilizers in place of chemical ones. Recently, environment-friendly methods are being implemented in agriculture which has led to applying sustainable alternative methods in place of chemical fertilizers (Sindhu et al., 2019). One such alternative used to achieve increased crop production is the inoculation of microorganisms that enhances the growth of plants and maintains the quality of the soil. These microbes are called “microbial inoculants” or plant growth-promoting rhizobacteria (PGPR) (Abaid et al., 2014).

“Plant Growth Promoting Rhizobacteria” (PGPR) play a central role in promoting sustainable agriculture. PGPR are bacteria belonging to different groups that live in the rhizospheric region of the plant in association with roots or on the root surfaces (Maheshwari et al., 2012). Bacteria translocate from the soil’s surface to the plant rhizosphere and start colonization in the rhizospheric zone of plant roots (Hafeez et al., 2013). All those bacteria that enhance the growth and development of plants fall under the category of PGPR (Hayat et al., 2010; Hafeez et al., 2013). It has been demonstrated by Noulas et al. (2018) that the enhancement in development and productivity of the PGPR inoculated plants was achieved due to the increased uptake of nutrients and the improved nutrient status in the plant. Some strains of Zn solubilizing bacteria can solubilize nutrients by producing phosphatases and organic acids, enhancing the accessibility of nutrients to the plants. PGPR enhance plant growth by various direct and indirect mechanisms. Directly they promote growth by synthesizing phytohormones and indirectly by getting involved in the accessibility of nutrients or acting as control agents (Yasmin et al., 2004). In addition to some quantitative effects and plant growth promotion, PGPR also aids in increasing the concentration of macronutrients and micronutrients (Fe, Zn, P, N and K) in tissues of plants and provides qualitative benefit to the plants (Intiaz, 2003). Therefore, this is considered the latest and efficient approach to increase Zn in various crops. Some of the Zn solubilizing PGPR includes Acinetobacter spp., Pseudomonas spp., Trabussiella spp., Bacillus spp., Aeromonas spp., Arthrobacter spp., Gluconacetobacter spp., and Exiguobacterium spp. (Sillanpaa, 1990; Intiaz, 2003). These bacteria significantly increase the growth and productivity of the plants. They also improve the overall quality of crops via the synthesis of growth-promoting hormones and various vitamins. The application of Zn solubilizing rhizobacteria as bio inoculants to enhance the yield of various crops such as barley, maize, rice and wheat has been very well documented. Tarig et al. (2007) reported a significant alleviation from Zn deficiency and a substantial increase in the total biomass, grain yield and harvest index of rice crop. It also increased the zinc concentration in the rice grains.

Table 1: Bacterial species isolated from different sources and potential bio inoculants to ameliorate the Zn status in the plants

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Zn Source used in solubilization</th>
<th>Crop/Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Zn phosphate</td>
<td>Forest soil</td>
<td>Di Simine et al., 1998</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia, Mycobacterium</td>
<td>Zn oxide and Zn phosphate</td>
<td>banana, chili, field bean, ground nut, maize, sugarcane, sorghum and tomato</td>
<td>Smithakumari et al., 2016</td>
</tr>
<tr>
<td>bronssenense, Enterobacter aerogenes, Pseudomonas</td>
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<td></td>
<td></td>
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<tr>
<td>aeruginosa and Xanthomonas retloxus.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>Zn oxide, Zn phosphate</td>
<td>Air (tannery)</td>
<td>Fasim et al., 2002</td>
</tr>
<tr>
<td>Gluconacetobacter diaziotrophicus</td>
<td>Zn Oxide, Zn carbonate</td>
<td>Maize</td>
<td>Sarathambal et al., 2010</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>Zn oxide and Zn Carbonate</td>
<td>Rice</td>
<td>Gandhi et al., 2016</td>
</tr>
<tr>
<td>Bacillus aryabhattai</td>
<td>Zn oxide, Zn Carbonate and Zn Phosphat</td>
<td>Soybean and wheat</td>
<td>Ramesh et al., 2014</td>
</tr>
<tr>
<td>Bacillus aerius, Bacillus siamensisens,</td>
<td>Zn oxide, Zn carbonate</td>
<td>Rice</td>
<td>Nepomuceno et al., 2020</td>
</tr>
<tr>
<td>Burkholderia cepacia, Burkholderia ambifaria</td>
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<td></td>
<td></td>
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<tr>
<td>and Sphingobacterium multivorum</td>
<td></td>
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</tr>
<tr>
<td>Pseudomonas fragi, Pantoea dispersa,</td>
<td>Zn Carbonate, Zn sulphate, Zn oxide, Zn phosphate</td>
<td>wheat</td>
<td>Kamran et al., 2017</td>
</tr>
<tr>
<td>Pantoea agglomerans, E. cloacae, Rhizobium sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serratia liquefaciens, S. marcescens and</td>
<td>Zn oxide, Zn Carbonate, Zn Sulphate and Zn Phosphate</td>
<td>Wheat</td>
<td>Abaid et al., 2015</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
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</tbody>
</table>

Furthermore, a notably positive impact on root weight, root length, root area, root volume, and shoot weight was determined. The inoculated one exhibited significant Zn mobility as compared to uninoculated and control. PGPR solubilized the Zn with efficiency in the liquid culture as well. The Zn mobilizing strains are isolated from the rhizosphere of various crops. The isolated strains are screened by using a plate assay method. The efficient Zn solubilizers formed clear halo zones over the media contains insoluble zinc forms. Yasmin (2004) isolated Pseudomonas sp. from the rhizospheric soil of rice crop and determined its Zn mobilizing ability. It enhanced the Zn solubilization and improved yield, and increased Zn concentrations in rice plants. Similarly, Sirohi et al. (2015) reported Zn biofortification in wheat crops using Pseudomonas strain isolated from the rhizosphere of wheat. Muntaz et al. (2017) reported the application of Bacillus sp. in maize. Bacillus aryabhattai and Bacillus subtilis significantly promoted the productivity of the maize crop. Thus, these bacteria are potential inoculants for biofortification to deal with malnutrition in populations where it is grown as a major cereal crop.
It is well-considered that PGPR present around the roots of a specific crop can also be inoculated in the different locality and for the different crop. They are found to grow well in a similar soil environment. This microbial combination of two more microbial species working together as a microbial community for promoting plant growth is called consortium. These bacterial isolates can be from the plant’s rhizosphere or can be taken from the non-rhizosphere zone to work together as a consortium (Pandey et al., 2012).

Consortium formed using bacterial and fungal species has been applied to the sugar cane crop. It increased the sugar level significantly. It also reduced the need to apply Zn sulphate fertilizer by 75% (Deshmukh D.P et al., 2019). Strains of Bacillus cereus, B. subtilis and Serratia sp. used in consortium reduced the soil-borne disease in sweet pepper. The consortium protected the plant from the pathogens, and hence via an indirect mechanism, it acted as a biocontrol agent. The consortium also acted as biofertilizers by increasing the productivity of the crop (Raklami et al., 2019). The consortium of bacterial and mycorrhizae fungi stimulates the germination of spores, root colonisation of the bacteria, and multiplies the population of other valuable bacteria in the rhizospheric soil. The synergy of bacteria and mycorrhizae enhances the nitrogenase activity in the bacteria associated with nitrogen fixation in the rhizosphere. This leads to an improvement in atmospheric nitrogen fixation. This consortium has an excellent ability to infect the plant root and enhance the shoot length, root length, biomass and overall productivity of the plants (Raklami et al., 2019). The consortium also improves soil fertility by changing the phytochemical and nutritional characteristics of the soil. Baslam et al. (2014) reported that the overall fertility of the soil had been improved after the application of consortium. Physiochemical characteristics of soil were found to be improved after the harvest of the crop grown with the consortium. The synergie has increased the organic matter and the concentration of carbon in the soil. It has also enhanced rhizospheric nitrogen and phosphorus. This improvement in the nutritional value of the soil is due to the capability of these bacteria to metabolize various organic compounds that are synthesized by the roots of the plants like various amino acids, carbohydrates and organic acids (Caravaca et al., 2003).

CONCLUSION

Microorganisms are required for many cellular and metabolic activities in plants. These nutrients are required in trace amounts but are of greater significance in plants. The adequate supply of these microelements to plants requires proper uptake, accumulation, mobilization, and storage in the plants (Hinsch and Mendel, 2009). The inadequate supply of micronutrients dramatically affects the productivity of crops throughout the world. For obtaining optimal productivity in plants, there should be a continuous supply of micronutrients in the maturation phase of plants. Zn is a vital micronutrient in higher plants and has many biochemical, physiological roles in plants. Inorganic Zn applied in the soil readily becomes unavailable after application. This conversion reduces Zn availability to plants. Zn deficiency leads to severe effects in plants and dramatically reduces their productivity.

The application of rhizobacteria is a recent approach used for increasing the availability of essential elements such as Zn and Fe in different crops. Using Zn solubilizing rhizobacteria found in rhizosphere soil has been shown to improve the quality and productivity of crops. These bacteria can also mobilize micronutrients and are excellent alternatives to Chemical fertilizers in dealing with Zn deficiency. The application of PGPR is considered the new technological approach to combat Zn deficiency problems in plants effectively by enhancing the bioavailability of Zn, Fe, and other micro and macronutrients through economic and eco-friendly aspect.

REFERENCES


