

Plant-microbe interaction in improving zinc nutrition in Rice: A review

ABSTRACT

Zinc is an essential micro-nutrient that affects metabolic activities, including growth and cell proliferation in all living organisms. Zinc deficiency in agricultural soil has been increasing at an accelerated rate all over the world, leading to its deficiency in plants as well as humans. Zinc solubilising bacteria (ZSB) solubilise complex zinc in soil into plant absorbable compounds through several mechanisms such as the production of acid, chelating compounds, protons etc. further improving its bioavailability in plants and humans. Improving zinc nutrition through microbes is an effective measure to overcome its deficiency. ZSB with Plant Growth Promoting (PGP) traits can be an additional advantage as along with increasing zinc amount in plant it would also promote overall growth of plants through PGP traits and can act as a biocontrol agent against several crop pathogens. In this review we attempt to study the significance of zinc; status and deficiency of zinc in Indian soil and to understand how zinc solubilizing bacteria can prove to be an effective measure to increase zinc content in plants and overcome its deficiency.

KEYWORDS: *Zinc, Rhizosphere, Deficiency of Zn, Zinc solubilising bacteria, Soil bacteria, Plant growth promoting rhizobacteria, Zn nutrition.*

1.INTRODUCTION

Zinc is an essential micronutrient for all living things. It is an essential component of several enzymes that are required for the synthesis, degradation, and metabolism of lipids, proteins, nucleic acids, carbohydrates, and other micronutrients. The molecular structure of cellular components must be preserved by zinc in order to maintain the integrity of cells and organs[1]. Deficiency of zinc affects metabolic activities, including growth and cell proliferation in plants, humans and microorganisms [2]. Zn deficiency prevails among approximately 2 billion people in the world causing growth retardation, an impaired immune system, hair loss, diarrhoea and delayed sexual maturation. Plants uptake zinc from the soil and subsequently, it moves up the food chain to humans. Prevalence of zinc in soil is in the form of augite, biotite, hornblende, olivine, and sphalerite ores. Zinc deficiency in agricultural soil accounts for 49% of the global scale and in India, 48-50 % of such soil is deficient in zinc. This decline in soil micronutrients leads to low yield of crops including rice and also causes deficiency in human population causing several diseases. Rice being a staple food, consumed by maximum population, increasing its zinc content by fortification can be a step to overcome zinc deficiency in humans. In India, low Zn soils often cause insufficient intake of Zn in the human population causing its deficiency. In Odisha, rice variety Swarna is widely cultivated, which is also deficient in Zinc [3].

Despite the fact that the soil contains more than enough zinc to sustain crop growth, plants are unable to absorb it because of inaccessible zinc fragments. The availability of zinc in the soil is influenced by a wide range of factors, such as soil phosphorus, pH, texture, and weather. Since zinc quickly transforms into inaccessible components and accumulates in the soil, exogenous zinc use in fertilisers is also unconventional. The anticipated global population in 2050 will reach 9.7 billion people (approx.). India population is expected to increase by addition of 273 million by 2050 [4]. With the application of chemical fertiliser applications and many breeding procedures to boost the Zn content in cereal grains, researchers are looking at several strategies to reduce Zn insufficiency among human populations [5]. Zinc is supplemented to the soil through expensive chemical fertilizers in the form of Zn-Sulphate or Zn-EDTA which changes to insoluble complex structures within 7 days of application and cause environmental hazard [6]. Hence, an effective and inexpensive alternative to the chemical application is the use of Zinc Solubilizing Bacteria (ZSB).

Several methods have been employed recently to lessen host plant zinc deficiencies. Among these, applying chemical fertilisers is an expensive and unsustainable method that increases crop susceptibility to diseases and gradually lowers soil fertility. Furthermore, careless fertiliser application has contaminated the water and soil, posing a risk to plant life as well as human health. As a result, scientists are particularly interested in using affordable, environmentally friendly methods to increase nutrient availability (particularly zinc) without compromising the environment [7]. ZSB readily solubilise complex zinc present in soil into soluble compound improving its bioavailability and uptake by the crop plants, subsequently entering to human through the food [8]. Zinc solubilising bacteria solubilise complex zinc by mechanisms like acidification [9], production of siderophores [8] and proton, oxidoreductive systems on cell membranes. Biofortification of Zinc through microbes is an effective measure to overcome its deficiency [10]. ZSB with Plant Growth Promoting traits can be of additional advantage which cannot only increase availability of Zn and other elements in soil but also can enhance growth of plants through PGP traits and can perform as a biocontrol agent against several crop pathogens [11].

Therefore, an attempt has been made to review the significance of zinc; status and deficiency of zinc in Indian soil and to understand how zinc solubilizing bacteria can effectively increase zinc content in crops and overcome its deficiency.

2. SIGNIFICANCE OF ZINC:

2.1: Plants:

Zinc (Zn), an essential micronutrient, is required for healthy plant, animal, human, and microbial growth and development. It is essential for protein synthesis, glucose metabolism, enzyme activation and activities in general [12]. Zinc has a critical function in plant physiology, particularly in photosynthesis, chlorophyll synthesis, nitrogen metabolism, and stress resistance in plants. It is necessary for the development of optimal fruit size and high crop yields ([13]; [14]). Zn is required for auxin synthesis in plants. Basically, auxin governs the process of cell division and elongation, hence in Zn deficit conditions, stunted growth, small & distorted leaves, reduced pollen production and internode restrictions are seen [15]. Moreover, zinc plays a significant role in transcription factors

needed for cell differentiation and proliferation [16]. Zinc is primarily known to inhibit enzyme activities as an antioxidant and to cause high levels of oxidative stress, which may be the cause of the chloroplasts' deformation [17]. Reduced leaf photosynthetic ability as a result of such photosynthetic centre death makes leaves more vulnerable to photodegradation [18].

2.2: Animals and Human beings:

All living organisms' cellular processes depend critically on zinc, which also helps human's immune systems. Several body enzymes have zinc as a catalytic and structural component. Zinc deficiency in the body can cause a variety of health issues, such as weakened body muscles, a compromised immune system, problems with learning and physical development, memory loss, hair loss, and skin problems. Inadequate levels of Zn also lead to DNA damage and can aggravate the risk of cancer [19]. Infertility is a common threat that has been observed with zinc deficient male population. In case of pregnancy, reduced Zn intake results in stunted brain development of the fetus and also congenital diseases like acrodermatitisenteropathica ([20]; [21]; [22]; [23]; [24]; [12]).

2.3: Microbes:

Zinc plays a significant role in the regulation of microbial virulence and host immune responses. Deficiency of zinc levels in microbes can cause imbalance in zinc homeostasis and dysregulation of intracellular signalling pathways. Additionally zinc acts as a cofactor for several bacterial proteins such as DNA replication and protein synthesis, hence it is required for enzymatic reactions, responses to oxidative stress situations, DNA repair and regulatory roles in various physiological processes in bacteria [25].

3. DEMAND AND SUPPLY OF ZINC:

Soil is the primary source of Zinc to plants whereas animals including humans obtain it through the food chain. Ideally, the Zinc concentration of healthy and productive soil should be 10–300 mg/kg [26]. However, it is the most deficient micronutrient in the soil and according to estimates, the zinc content of 49% of the world's agricultural soils is insufficient. About 50% of agricultural soil in India is affected with Zn deficiency, which ranges between 30-72 mg/kg and hence, responsible for nearly 40% reduction in productivity [27]. Zinc content is considerably high, mostly present in mineral forms like smithsonite ($ZnCO_3$), Zincite (ZnO), Sphalerite (ZnS) and several other ferrous and silicon mineral forms but it is insoluble and unavailable for plant uptake leading to its deficiency [21]. Optimal zinc concentration required for plants ranges between 20-100 mg/kg. Since the soluble zinc content is low in soil, deficiency of zinc in plants has also been observed.

Low Zn soils in India often result in inadequate Zn intake in the population, which causes a deficiency. Furthermore, one third of the world's population affected with zinc deficiency. Considering that human body is unable to store zinc, it has to be consumed from zinc enriched food sources. For an adult human, the optimal dietary requirement of zinc is 15mg per day [12] necessary component for several zinc binding proteins and nucleic acid. Meat products like beef, pork, chicken and breakfast cereals such as oats, almond, peanut, walnut etc. and yogurt, cheese, milk and other dairy products are known to be rich in Zinc content [28]; [29]; [30]; [31]. Reduced cellular immunity and a later antibody response can result from insufficient Zn absorption and uptake [32] hence, it is required

to be taken up by human body in adequate amount. In case of animals, zinc supplements are found to be efficient for cattle to accelerate recovery in case of bovine rhinotracheitis [33]. Zn deficiency causes several viral infections in animals. Even though it is required in a less amount zinc is truly indispensable by all living forms.

4. CAUSE OF ZINC DEFICIENCY IN SOIL:

In soil zinc is mainly present in complex insoluble form that is unavailable to plant. Worldwide, zinc concentration of soil ranges approximately between 2-25 ppm of which a large proportion is trapped in iron and manganese oxides and other insoluble forms [34] leading to zinc deficiency and significantly affecting the crop yield. There are several factors that give rise to zinc deficiency in soil. Soil texture for instance; scalped and sandy soils are more prone to zinc deficiency than well managed soils such as silty or clayey soil. Besides, severe soil compaction leads to zinc deficit conditions. In addition to soil texture; pH, phosphorous and Iron content and weather conditions influences the zinc content of the soil. Solubility of zinc is mostly influenced by the pH of the soil; hence, alkaline conditions reduce zinc's solubility and availability. Consequently, zinc deficiency widely prevails in soil with pH above 6.5 [35]. Along with pH, zinc deficiency appears more in flooded soil and wet weather as compared to sunny weather [36]; [34]. Hence water content of the soil is an important determinant for bioavailability of zinc [37]. Furthermore, overuse of Phosphorous fertilizers may lead to zinc deficiency in some cases as phosphorous precipitates zinc in soil or at root soil interface and intervene with zinc metabolism inside the plant cells [34]. High concentration of Iron has also found to decrease the bioavailability of Zinc under anaerobic condition [38]. Therefore, in order to enhance the bioavailability of Zn, use of organic matter could be taken into account [39].

Insufficient amount of Zinc in soil severely affects the quality of production and yield of crops. It was opined by several researchers that 15-60 ppm of Zn supply is necessary for proper metabolic functioning of plant tissue and crop plant [40]; [41]. In agricultural practices expensive chemicals in form of Zn-Sulphate and Zn-EDTA are applied to the soil to supplement zinc. Nevertheless, these chemical inputs subsequently change into insoluble complex structures within 7 days of application and cause environmental hazard [6]. Besides, these fertilizers are expensive and unaffordable by the farmers. Hence, an organic, environment friendly substitute to the chemical approach is the application of Zinc Solubilizing Bacteria (ZSB) which readily solubilises complex Zinc present in soil into a soluble compound increasing its availability to plants.

5. ZINC SOLUBILIZING BACTERIA (ZSB):

A particular group of microorganisms can be used to convert the soil insoluble zinc into soluble form can overcome zinc deficiency achieving the objective of nutrient management and sustainable agriculture [42]. ZSB are the rhizospheric bacteria which can be used in the form of bio-inoculants to increase availability of native zinc for crop assimilation. Several bacterial and fungal strains are known to solubilise zinc. Some zinc solubilising bacteria are species of *Bacillus*, *Gluconacetobacter*, *Azotobacter*, *Azospirillum*, *Thiobacillusferrooxidans*, *Thiobacillusthiooxidans*, *Acinetobacter*, *Cyanobacteria*, *Serratia*, *Pseudomonas* and facultative thermophilic iron oxidizers [43]; [44]; [45].(Table.1). In case of fungal strains, arbuscular mycorrhizae and *Trichoderma* are known to exhibit

zinc solubilising traits [46]. *Bacillus* sp. are given special interest as they are spore-forming, hence they can sustain in adverse stress conditions, and can be mass cultured into easy formulation and also known to form non-specific association with several host crops [47]; [48]; [49]. Bacteria immobilize zinc metal by the process of precipitation and adsorption. As zinc is a limiting factor in crop productivity, zinc solubilization by bacteria plays an important role in zinc nutrition to plants [45].

Table. 1: Some effective zinc solubilizers isolated and characterized:

Microorganisms known to solubilise zinc	Source of organism	Source of Zinc	References
<i>Aspergillus niger</i> A. <i>omius</i> A. <i>oryzae</i>	-	ZnO, Zn ₃ (PO ₄) ₂	[50]
<i>Penicillium luteum</i>	soil	ZnO, Zn ₃ (PO ₄) ₂	[51]
<i>Pseudomonas fluorescense</i>	forrest soil	Zn ₃ (PO ₄) ₂	[52]
<i>Trichoderma harzianum</i> Rifai	soil	Granular metallic Zn	[53]
<i>Pseudomonas aeruginosa</i>	Airborne bacteria isolated from a tannery air environment	ZnO and Zn ₃ (PO ₄) ₂	[54]
<i>Bacillus</i> sp. and <i>Pseudomonas</i> sp.	zinc ore sphalerite; paddy soil	ZnO, ZnS and ZnCO ₃ .	[55]
<i>Hymenoscyphus ericae</i> and <i>Oidiodendron maius</i> (Ericoid mycorrhizae)	Heavy metal polluted sites	ZnO, Zn ₃ (PO ₄) ₂	[56]
<i>Beauveria caledonica</i>	Unpolluted soil, lawn, UK	Zn ₃ (PO ₄) ₂	[57]
<i>Gluconacetobacter diazotrophicus</i>	Rot tissue of carrot, raddish, beetroot and coffee	ZnO ZnCO ₃ or Zn ₃ (PO ₄) ₂	[58]; [43]
<i>Klebsiella</i> sp. <i>Pseudomonas</i> sp.	Soybean and Mung bean rhizosphere	ZnO, Zn ₃ (PO ₄) ₂	[21]
<i>Burkholderia</i> sp., <i>Acinetobacter</i> sp. and <i>Acinetobacter</i> sp.	soil	DTPA	[59]

<i>A. terreus</i> (ZSF-9)	Agricultural field soils	ZnO, ZnCO ₃ , Zn ₃ (PO ₄) ₂	[60]
<i>Enterobacter cloacae</i>	Rice rhizosphere	ZnO, ZnCO ₃ , Zn ₃ (PO ₄) ₂	[10]
<i>Bacillus cereus</i>	Zn deficit soil	ZnO, Zn ₃ (PO ₄) ₂	[61]
<i>Ralstoniapicketti, Burkholderiacepacia, and Klebsiella pneumoniae</i>	Zinc fertilizer rich rice rhizosphere	ZnO, ZnCO ₃	[62]
<i>Bacillus</i> sp., <i>Bacillus aryabhatai</i>, <i>Bacillus subtilis</i> and <i>Bacillus aryabhatai</i>	Maize Rhizosphere	ZnO	[63]
<i>Pseudomonas fragi</i>, <i>Pantoeadisversa</i> and <i>Pantoeaagglomerans</i>	soil	ZnCO ₃	[9]
<i>Acinetobacter</i> sp., <i>Serratia</i> sp.	Rice soil, Malaysia	ZnO ZnSO ₄	[64]
<i>B. aryabhatai</i>, <i>Pseudomonas taiwanensis</i>	Stone quarry Dust Powder	ZnO, Zn ₃ (PO ₄) ₂ , ZnCO ₃	[65]
<i>B. megaterium</i>	Rhizosphere of wild pepper	ZnO, Zn ₃ (PO ₄) ₂ ZnCO ₃	[66]
<i>Agrobacterium tumefaciens</i> and <i>Rhizobium</i> sp.	Rhizosphere of barley and tomato	ZnO, Zn ₃ (PO ₄) ₂ , ZnCO ₃	[67]
<i>Pseudomonas</i> sp. and <i>Bacillus</i> sp.	Chickpea Rhizosphere	(Zn ₃ (PO ₄) ₂ ·4H ₂ O)	[68]
<i>Bacillus megaterium</i>	Cow dung	ZnO, ZnCO ₃	[69]
<i>Sphingobacteriummultivorum</i>, <i>Burkholderiacenocepacia</i>, <i>Bacillus xiamenensis</i>, <i>Burkholderiaambifaria</i>, and <i>Bacillus aerius</i>	Mycorrhizal corn roots	ZnO, ZnCO ₃	[70]
<i>Acinetobacterbaumannii</i>; <i>Burkholderiacepacia</i>	-	ZnO, Zn ₃ (PO ₄) ₂ , ZnSO ₄ ·7H ₂ O	[71]
<i>Burkholderiavietnamiensis</i> and <i>Burkholderiaseminalis</i>	Rice rhizosphere	Zn ₃ (PO ₄) ₂ and ZnCO ₃	[72]
<i>Pseudomonas frederiksbergensis</i>	Jujube rhizosphere	ZnO, Zn ₃ (PO ₄) ₂	[73]
<i>Serratia</i> sp., <i>Acinetobacter</i> sp.	Wetland Rice field	ZnO, Zn ₃ (PO ₄) ₂ , ZnCO ₃	[74]

<i>Pseudomonas aeruginosa</i>, <i>Pseudomonas taiwanensis</i> and <i>Beijerinckiafluminensis</i>	Rhizospheric region	ZnO, Zn ₃ (PO ₄) ₂ , ZnCO ₃ [75]
<i>Bacillus sp.</i> & <i>Enterobacter sp.</i>	Root nodules of wild legumes	ZnCl ₂ (0.2-1.0 g conc.) [76]
<i>Priestiamegaterium</i>, <i>Priestiaaryabhatai</i>	Rhizosphere soils of peanuts, sweet potatoes, and cassava	ZnO and ZnCO ₃ [77]
<i>Bacillus altitudinis</i>, <i>B. megaterium</i>, <i>B.licheniformis</i>, <i>Brevibacillusborstelensis</i> and <i>B. xiamenensis</i>	Rhizosphere of wheat grown in the eastern parts of the Indo-Gangetic Plain of India	ZnO, Zn ₃ (PO ₄) ₂ , ZnCO ₃ [78]
<i>Acinetobacterpittii</i> and <i>Stenotrophomonasmaltophilis</i>	Soil and vegetation sample	ZnSO ₄ [79]

Although adopting suitable crop rotation can prove to be a positive agronomy approach for improving Zn phytoavailability, application of beneficial zinc solubilising rhizospheric microbes as bio-inoculants to increase tissue zinc concentration in plants and crops can be advocated to be a long-term cost-effective solution to attain sustainable agriculture [48] and to eliminate the zinc malnutrition in humans [80]. Therefore, utilization of zinc solubilising and mineralizing bacteria could be a relevant solution to zinc deficiency in crops and human.

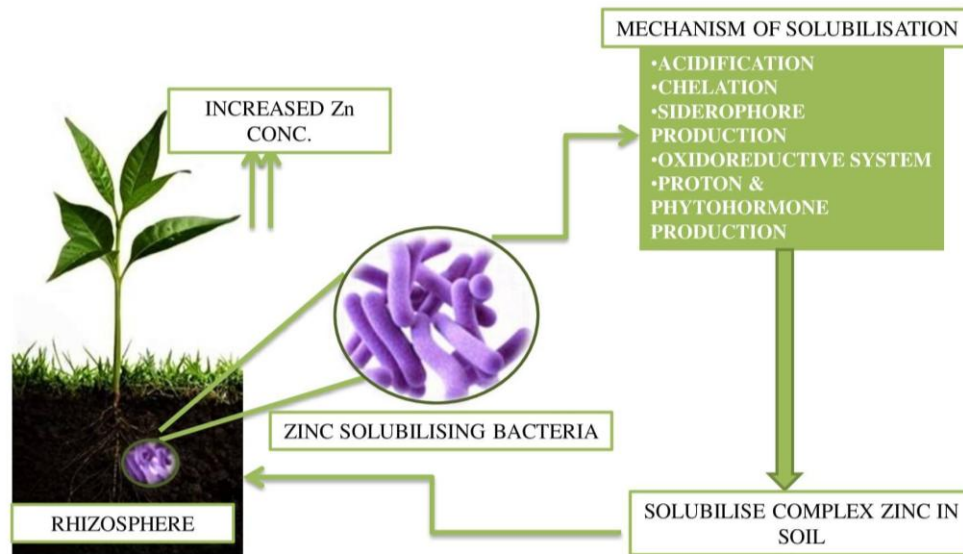


Fig.1: Zinc solubilising bacteria mechanisms

6. MECHANISMS OF ZSB:

Zinc solubilizing bacteria solubilise complex zinc through various mechanisms like production of chelating ligands, amino acids, vitamins, phytohormones, siderophores, acidification, oxido-reductive systems on the cell membrane and proton extrusion [81]; [9]; [63]. Some of the basic mechanisms exhibited by ZSBs for zinc solubilisation are:

6.1: Acidification:

Among all, the secretion of organic acids is a major zinc solubilizing process ZSB release organic acids in the soil that sequester Zinc cations while simultaneously decreasing the pH of the neighbouring soil and making it available to plants, increasing Zn content [9]. Amongst all the organic acids, 2-ketogluconic acid and gluconic acid produced by the isolates, results in solubilisation of zinc. However, the organic acid secretion by microbes varies depending upon the substrate of Zn minerals. It is also reported that some species of ZSB produce several organic acids such as oxalic, lactic, citric, malic, glycolic, malonic, tartaric, formic and succinic acids to solubilise zinc ([82]; [83]; [84]). Additionally, a change in pH with inoculation of *Pseudomonas* spp. and *Bacillus* spp. in broth culture indicate solubilisation of ZnS, ZnO and ZnCO₃[81]. Furthermore, the acid production tends to vary with respect to the Zn substrate provided in the media [84]. Several reports have suggested that ericoid and arbuscular mycorrhizal fungi lower the pH of rhizospheric soil by producing organic acids that solubilise complex zinc [56]; [85]. *Bacillus* sp. AZ6 was able to readily solubilize complex metals by secreting cinnamic, caffeic, chlorogenic, syringic, ferulic and gallic acid in a liquid medium [44].

6.2: Chelating Ligands:

There are specific and non-specific transporters involved in essential metal ions uptake across the cytoplasmic membrane of bacteria driven by a chemiosmotic gradient [86]. Zinc is a highly reactive metal that is less persistent in soil which leads to its deficiency in plant. Zinc bioavailability can be

enhanced with help of Zn chelating compounds [87]. The rhizospheric microflora is known to release Zn chelating compounds that increase its availability to plant roots. Reports suggest that production of Zn chelating metallophores by *Pseudomonas monteilii*, *Microbacterium saperdae* and *Enterobacter cancerogenus* helps in generation of soluble zinc molecules in soil for plant uptake [88]. Another report suggested that biofertilizer consortia of *Pseudomonas* sp. (96-51), *Azospirillum lipoferum* (JCM-1270, ER-20) and *Agrobacterium* sp. (Ca-18) produced ethylene diamine tetra acetic acid as a chelator that attached to Zn and increases its availability for plant uptake [89].

6.3: Alteration in root structure:

Reports also indicate that change in root structures affects the availability of soluble zinc. Increase in surface area and root growth can lead to maximum uptake of Zn [85]. For instance, mycorrhizal fungus tends to increase the surface area of the roots that results in increased uptake of nutrients including zinc by plants. Arbuscular mycorrhizae are able to acquire zinc from 40mm distance of root surface [90]. With inoculation of potent ZSBs, there was accelerated growth in root weight, length, volume and zinc content of rice [89].

6.4: Others:

In some cases, cell wall modification and bio-precipitation has also been reported [91]. *Gluconacetobacter diazotrophicus* PA15 was able to solubilize ZnO by producing sugar such as glucose or sucrose as the potent carbon source [43]. Several *Bacillus* strains are known to readily solubilizes complex Zn through production of plant hormones, amino acids, proton extrusion and oxidoreductive systems of the cell membranes [92].

7. ZSB WITH PLANT GROWTH PROMOTING TRAITS:

It is evident that zinc solubilising bacterial inoculants can be used to increase bioavailability of native zinc for crop assimilation and therefore can help in reducing zinc deficiency in crops and human being. The effectiveness of zinc solubilising bacteria can be enhanced if they also possess plant growth promoting traits in addition to the zinc solubilisation. In order to achieve proper growth and enhanced productivity, bio augmentation of zinc solubilising microbial strains with PGP traits is imperative. It can result in enhanced growth, increase in yield and quality of crop produce due to the direct and indirect mechanisms. The direct mechanisms include nitrogen fixation, phosphate solubilisation, siderophore production, phytohormone production (IAA & ACC Deaminase) etc. The indirect mechanisms mainly comprise of the application of microorganisms as biocontrol agents, to control the plant diseases [93]; [94]. That includes production of HCN, Induced systemic resistance (ISR), Siderophore, antifungal activity etc. [95]. Application of Zn-solubilizing microorganisms with plant growth promoting characteristics can not only address zinc deficiencies in humans through crops, but can also be used as an alternative to chemical fertilisers and pesticides to improve crop growth and development.

Several plant growth promoting rhizobacteria have been reported earlier as they are also known to solubilise complex zinc in soil. The prime focus of agriculture has always been to improve yield through plant breeding techniques but with regards to the nutritional quality of the crops, chemical supplements or industrial fortifications have always been prioritised. Biofortification of essential

micronutrients is the process to increase availability of nutrients through microbial intervention which otherwise could be achieved through agronomic practices like conventional plant breeding and biotechnology viz. genetic engineering [96]; [97]. Although these approaches help in enhancing the nutrients availability, nevertheless are expensive, causes environmental pollution and ethical issues [12]. Hence to overcome this issue, the application of potent PGPR agents with ability to solubilise complex nutrients can prove to be a highly effective solution. Therefore, biofortification of crops with application of PGPRs can be an efficient supplementary method to improved nutrition and yield.

8. ZINC BIOFORTIFICATION APPROACHES:

The deficit of zinc in humans can only be eradicated if its concentration is enhanced in the parts of plants that are consumed on a daily basis. Hence, the popularity of biofortification of such essential micronutrients in cereal crops countries has come to existence as they are the staple food in many developing countries [13]; [96]. The main approaches to biofortification include plant breeding, agronomic strategies and use of biotechnology. The major intent behind biofortification is to produce varieties that have maximum uptake and accumulation of Zn in the edible portion of the crops. Agronomical strategies predominantly include zinc fertilizers which is a short term and trouble-free task. Different kinds of zinc fertilizers that are used for this process but predominantly zinc sulphate ($ZnSO_4$) is applied to increase zinc concentration in crops. Although these approaches are effective, relentless use of chemical fertilizers in agriculture leads to pollution of soil and environment. These chemicals are highly persistent and turn into complex insoluble forms in soil.

Although plant breeding strategies appears to be cost effective and sustainable, it is time consuming and complicated. Hence, there is necessity of developing a sustainable, effective and environment friendly approach to biofortification of zinc in grains. Transgenic approaches have considerable effects on biofortification process and the crop varieties with increased zinc concentration in grains [12]. The main targets for enhancing zinc concentration are the expression of ZIP family (transcription factors) and transport proteins which are responsible for zinc uptake and accumulation. However, overexpression of genes responsible for transport protein can lead to enhanced zinc uptake in the root regions [97].

9. ROLE OF ZIP FAMILY IN ZINC SOLUBILISATION:

Zinc loading in grains is determined not only by the availability of zinc in the soil, but also by zinc uptake and translocation into the grains. Considering rice being the staple food, zinc fortification of rice grains could prove to be a propitious approach to diminish the zinc deficiency in humans. The expression of Zn transporting genes can be affected by ZSB inoculation in rice [10]. ZSB can regulate the uptake and translocation of zinc in plants. The zinc uptake can take place by transport mechanisms such as phytosiderophore production and ZIP family of transporters which as Zn-regulated transporters and Fe-regulated transporter like protein family [98]. The ZIP proteins are the Zn-regulated, Fe-regulated transporter like proteins that help in cellular uptake of Zn, translocation, intracellular trafficking and plant detoxification [99]. The ZIP family of transporters are responsible for the transportation of four essential micronutrients such as Zn, Fe, Cu and Mn [100]; [101]. ZIP family of proteins were first reported to be present in *Saccharomyces cerevisiae* and *Arabidopsis*

thaliana[102]; [103]. However, about 15 members of ZIP protein family are found in *Arabidopsis*[101], 17 members in case of Rice (*Oryza sativa*), 12 members reported in Barley, 14 in wheat (*Triticum aestivum*) and 23 members of ZIP family reported in case of common bean [104]; [105]. ZIP proteins are comprised of 309 to 470 amino acids having 8 transmembrane domains. The amino and carboxyl ends of the proteins are located on the outer surface region of the plasma membrane [98].

Moreover, different plant ZIP family members have different functions when it comes to uptake and translocation. The expression of zip genes are known to vary from root to shoot to grains translocation. Particularly in Rice (*Oryza sativa*) several zip genes were reported such as: OsIRT1, OsIRT2, OsZIP1, OsZIP3, OsZIP4, OsZIP5, OsZIP6, OsZIP7 and OsZIP8 which regulate the zinc uptake from soil to root to shoot and also the storage in rice grains [106]; [107]; [10].

These ZIP genes are also known to be induced by zinc deficiency [18]; [108]. The upregulation of ZIP genes are known to occur due to expression of Leu-zipper transcription factors (bZIP19 AND bZIP23)[109]. Under zinc deficit situation, expression of OsZIP1 in roots was higher as compared to shoots [110];[18]. The rice plasma membrane zinc transporters mainly include OsZIP1, OsZIP3, OsZIP4, OsZIP5 and OsZIP8 which are also induced by zinc deficit conditions [111]. Furthermore, overexpression of OsZIP4 and OsZIP5 leads to increased zinc accumulation in roots but decreased accumulation in shoots [106]. From germination to grain filling, expression level of all these rice ZIP genes was differed. Even though OsZIP1 and OsZIP3 are constitutively present and expressed, the expression of OsZIP3 is generally localised in the area of nodes, therefore it is known to be responsible for translocation of zinc and its distribution to other developing tissues of the plant [112]. A recent report opined that the Zn transporter OsZIP9 is responsible for the uptake of Zn in zinc deficit and hydroponic conditions [105].

Role of ZIP genes in translocation of Zn in Rice (Root to Grains)

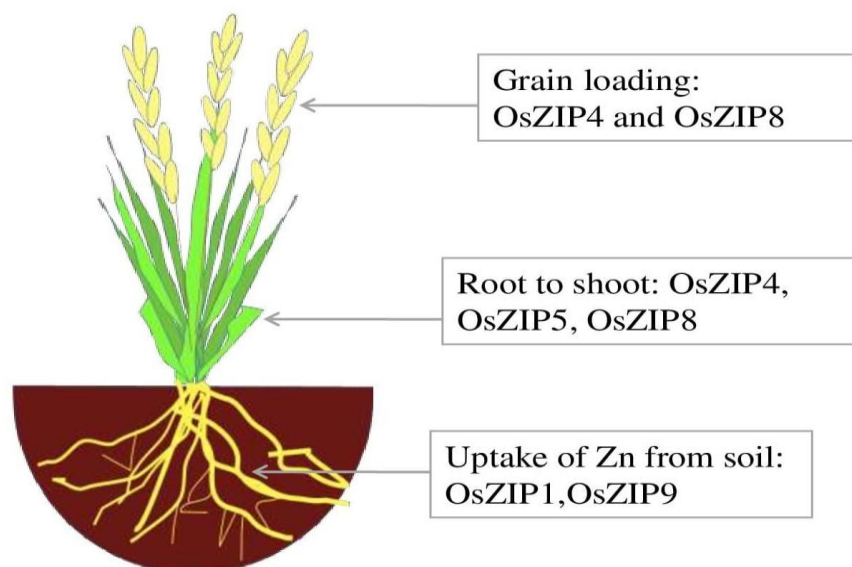


Fig.2: Role of ZIPs in Rice

Several reports suggest OsZIP1 is responsible for Zn uptake from soil [113], however the exact physiological functions of these ZIP genes and their role in Zinc uptake by plants remains poorly understood. Although the ZSBs are known to produce organic acids in their rhizospheric region to solubilise zinc and facilitate their uptake by the roots. **ZSB can confer resistance on plants against specific abiotic stressors; consequently, enhancing ZSB density in the plant rhizosphere may serve as a viable substitute strategy to boost host plant growth and productivity[7].** However, to understand their role in regulation of expression of ZIP genes is still remains undefined. To elucidate the interaction between the zinc solubilizers and Zn transporters genes can help in eliminating zinc deficiency and improving zinc absorption by plants. ZSB inoculation of rice seedlings may regulate the Zn transporters protein expression through zinc assimilation. However, more research in this field is highly essential to understand the interactions and how it is helpful for zinc fortification.

10. CONCLUSION:

The Zn deficiency is prominent in soil-plant system which leads to inadequate levels of zinc in humans. To improve the deficiency of zinc in soil chemical fertilizers are being supplemented, nevertheless they get converted into an insoluble complex that cannot be uptaken by the plants. In an attempt to increase the soluble or available zinc in soil, application of potent zinc solubilising bacteria can prove to be really beneficial to improve zinc nutrition in soil, plants and hence in humans. ZSBs solubilise zinc by acid production, chelation, production of amino acids, vitamins, proton extrusion etc. ZSBs are known to influence the expression of zinc transporters proteins belonging to ZIP family that help in its uptake and translocation. ZSBs possessing plant growth promoting traits such as nitrogen fixation, phosphate solubilisation etc. can potentially be an organic, environmentally friendly and cost-effective approach to Zinc fertilizers, that can not only increase the zinc availability in soil but also can improve the nutrition and help in the growth of plants.

References

1. (FAO) Food and Agriculture Organization of the United Nation, World Health Organization: Food & Nutrition Division, 2001. <https://www.fao.org/3/y2809e/y2809e.pdf>
2. Alloway BJ. Zinc in Soils and Crop Nutrition. 2nd Edition, IZA and IFA, Brussels, Belgium and Paris, France, 2008.
3. Das A, Patel DP, Ramkrushna GI, Munda GC, Ngachan SV, Choudhury BU, Mohapatra KP, Rajkhowa DJ, Kumar R, Panwar AS. Improved rice production technology—for resource conservation and climate resilience (Farmers' Guide). Ext Bull No. 78. ICAR Research Complex for NEH region, Umiam, Meghalaya.2012:pp 29.
4. United Nations, Department of Economic and Social Affairs, World Population Prospects 2019. <https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html>

5. Frossard E, Bucher M, Machler F, Mozafar A, Hurrell R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants of human nutrition. *J Sci Food Agri*. 2000; 80: 861-879.
6. Karak T, Singh UK, Das S, Das DK, Kuzyakov Y. Comparative efficacy of ZnSO₄ and Zn-EDTA application for fertilization of rice (*Oryza sativa* L.). *Arch Agron Soil Sci*. 2005; 51: 253-264. <https://doi.org/10.1080/03650340400026701>
7. Singh S, Chhabra R, Sharma A, Bisht A. Harnessing the Power of Zinc-Solubilizing Bacteria: A Catalyst for a Sustainable Agrosystem. *Bacteria*. 2024; 3(1):15-29. <https://doi.org/10.3390/bacteria3010002>
8. Saravanan VS, Rohini Kumar M, Sa TM. Microbial zinc solubilization and their role on plants. *Bacteria in agrobiolgy: plant nutrient management*. 2011: pp. 47-63. https://doi.org/10.1007/978-3-642-21061-7_3
9. Kamran S, Shahid I, Baig DN, Rizwan M, Malik KA, Mehnaz S. Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. *Front Microbiol*. 2017; 8: 2593–2593. doi: <https://doi.org/10.3389/fmicb.2017.02593>
10. Krithika S, Balachandar D. Expression of zinc transporter genes in rice as influenced by zinc-solubilizing *Enterobacter cloacae* strain ZSB14. *Front Plant Sci*. 2016; 7. doi: <https://doi.org/10.3389/fpls.2016.00446>
11. Pahari A, Mishra BB. Antibiosis of Siderophore Producing Bacterial Isolates against Phytopathogens and Their Effect on Growth of Okra. *Int J Curr Microbiol App Sci*. 2017; 6:1925-1929. doi: <https://doi.org/10.20546/ijcmas.2017.608.227>
12. Hussain A, Zahir ZA, Asghar HN, Ahmad M, Jamil M, Naveed M, Fakhar M, et al., Zinc solubilizing bacteria for zinc biofortification in cereals: a step toward sustainable nutritional security. *Role of Rhizospheric Microbes in Soil: Volume 2: Nutrient Management and Crop Improvement*. 2018: 203-227. https://doi.org/10.1007/978-981-13-0044-8_7
13. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification, *Plant Soil*. 2008; 302: 1–17
14. Efe L, Yarpuz E. The effect of zinc application methods on seed cotton yield, lint and seed quality of cotton (*Gossypium hirsutum* L.) in east Mediterranean region of Turkey. *Afr J Biotechnol*. 2013; 10: 8782–8789.
15. Irshad M, Gill MA, Aziz T, Ahmed I. Growth response of cotton cultivars to zinc deficiency stress in chelator-buffered nutrient solution. *Pak J Bot*. 2004; 36: 373–380.
16. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology, *Physiol Rev*. 1993; 73: 79–118.
17. Chen W, He Z, Yang X, Feng Y. Zinc efficiency is correlated with root morphology, ultrastructure, and antioxidative enzymes in rice. *J Plant Nutr*. 2009; 32: 287–3.

18. Chen W, Feng Y, Chao Y. Genomic analysis and expression pattern of OsZIP1, OsZIP3, and OsZIP4 in two rice (*Oryza sativa* L.) genotypes with different zinc efficiency. *Russ J Plant Physiol.* 2008; 55: 400–409.
19. Ho E, Zinc deficiency, DNA damage and cancer risk. *J Nutr Biochem.* 2004; 15: 572-8. doi: <https://doi.org/10.1016/j.jnutbio.2004.07.005>
20. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. *FoodNutr Bull.* 2004; 25: 91–204.
21. Sharma A, Shankhdhar D, Shankhdhar SC. Potassium-solubilizing microorganisms: mechanism and their role in potassium solubilization and uptake. In: Meena VS, Maurya BR, Sharma, A., Patni, B., Shankhdhar, D., Shankhdhar, S.C., Zinc - an indispensable micronutrient. *PhysiolMolBiol Plants.*2016;19: 11-20. doi: <https://doi.org/10.1007/s12298-012-0139-1>
22. Verma JP, Jaiswa DK, Meena VS, Meena R. Current need of organic farming for enhancing sustainable agriculture. *J Clean Prod.* 2015
23. Meena VS, Maurya BR, Meena RS. Residual impact of well grow formulation and NPK on growth and yield of wheat (*Triticum aestivum* L.). *Bang J Bot.* 2015; 44: 143–146.
24. Shrivastava M, Srivastava PC, D'Souza SF. KSM soil diversity and mineral solubilization, in relation to crop production and molecular mechanism. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, 2016: 221–234. https://doi.org/10.1007/978-81-322-2776-2_16
25. Capdevila DA, Wang J, Giedroc DP. Bacterial Strategies to Maintain Zinc Metallostatics at the Host-Pathogen Interface. *J Biol Chem.* 2016; 30: 20858-20868. doi: <https://doi.org/10.1074/jbc.R116.742023>
26. White CL. In Zn in soils and plants (A. D. Robson ed.), Kulwer Academic Pub., Dordrecht, The Netherlands. 1993.
27. Singh MV. Micro nutritional problem in soils of India and improvement for human and animal health. *Indian J Fert.* 2009; 5: 11-16.
28. Meena VS, Maurya BR, Verma R, Meena RS, Jatav GK, Meena SK. Soil microbial population and selected enzyme activities as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi, The Bioscan. 2013; 8: 931–935.
29. Meena RS, Meena VS, Meena SK, Verma JP. Towards the plant stress mitigate the agricultural productivity: a book review. *J Clean Prod.* 2015; 102: 552–553.
30. Bahadur I, Maurya BR, Kumar A, Meena VS, Raghuvanshi R. Towards the soil sustainability and potassium-solubilizing microorganisms. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, 2016; 225–266. https://doi.org/10.1007/978-81-322-2776-2_18
31. Masood S, Bano A. Mechanism of potassium solubilization in the agricultural soils by the help of soil microorganisms, in Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, 2016; 137–147. https://doi.org/10.1007/978-81-322-2776-2_10

32. Fletcher MP, Gershwin BE, Keen CL, Hurley L. Trace element deficiencies and immune responsiveness in human and animal models. In: Chandra RK (ed) Nutrition and immunology. Alan R. Liss, Inc., New York, 1988; 215–239.
33. Chirase NK, Hutcheson DP, Thompson GB. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. *J Anim Sci.* 1991; 69: 4137–4145.
34. Schlute EE. Soil and Applied Zinc, A2528 Understanding Plant Nutrients University of Wisconsin-Extension 2004, <http://corn.agronomy.wisc.edu/Management/pdfs/a2528.pdf>
35. Rengel Z. Availability of Mn, Zn and Fe in the rhizosphere, *J Soil Sci Plant Nutr.* 2014; 15. <https://doi.org/10.4067/S0718-95162015005000036>
36. Neue HU, Lantin RS. Micronutrient toxicities and deficiencies in rice, in Yeo AR, Flowers TJ, eds, *Soil Mineral Stresses: Approaches to Crop Improvement*, Springer-Verlag, Berlin, 1994; 175–200.
37. Patnaik MC, Raju AS, Raj GB. Effect of soil moisture regimes on zinc availability in a red sandy loam soil of Andhra Pradesh. *J Ind Soc Soil Sci.* 2008; 56: 452-453.
38. Hazra GC. Zinc adsorption in soils as influenced by different soil management practices. *Soil Sci.* 1997; 162: 713–721.
39. Yoo MS, James BR. Zinc extractability as a function of pH in organic waste-amended soils. *Soil Sci.* 2002; 167: 246–259.
40. Bloodnick E. Role of Zinc in Plant Culture. 2022. <https://www.pthorticulture.com/en/training-center/role-of-zinc-in-plant-culture/>
41. Meena VS, Maurya BR, Meena SK, Meena RK, Kumar A, Verma JP, Singh NP. Can *Bacillus* species enhance nutrient availability in agricultural soils? In: Islam MT, Rahman M, Pandey P, Jha CK, Aeron A (eds) *Bacilli and agrobiotechnology*. Springer, Cham. 2017; 367–395. https://doi.org/10.1007/978-3-319-44409-3_16
42. He CQ, Tan GE, Liang X, Du W, Chen YL, ZhiGY, Zhu Y. Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. *Appl Soil Ecol.* 2010; 44: 1–5.
43. Saravanan V, Madhaiyan M, Thangaraju M. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere.* 2007; 66: 1794–1798.
44. Hussain D, Haydon MJ, Wang YE, Wong SM, Sherson J, Young J, et al. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *Plant Cell.* 2004; 16: 1327–1339.
45. Vidyashree DN, Ramaiah M, Panneerselvam P, Saritha B, Ganeshamurthy A. Isolation and Characterization of Zinc Solubilizing Bacteria from Stone Quarry Dust Powder. 2016; 3078-3081.
46. Paul ES, Clark FE. *Soil microbiology and biochemistry*. 1989, Academic, San Diego.
47. Pérez-García A, Romero D, de Vicente A. Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in

<https://doi.org/10.1016/j.copbio.2010.12.003>

48. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Appl Soil Ecol.* 2014; 73: 87–96.
49. Ramirez CA, Kloepper JW, Plant growth promotion by *Bacillus amyloliquefaciens* FZB45 depends on inoculum rate and P-related soil properties. *Biol Fertil Soils.* 2010; 46: 835-844.
50. Vachon P, Tyagi RD, Auclair JC, Wilkinson KJ. Chemical and biological leaching of aluminium from red mud. *Environ Sci Technol.* 1994; 28: 26–30. <https://doi.org/10.1021/es00050a005>
51. Sayer JA, Raggett SL, Gadd GM. Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycol Res.* 1995; 99: 987-993, [https://doi.org/10.1016/S0953-7562\(09\)80762-4](https://doi.org/10.1016/S0953-7562(09)80762-4)
52. Di Simone C, Sayer J, Gadd G. Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. *Biol Fertil Soils.* 1998; 28: 87–94. <https://doi.org/10.1007/s003740050467>
53. Altomare C, Norvell AWA, Björkman AT, Harman AGE. Solubilization of Phosphates and Micronutrients by the Plant-Growth-Promoting and Biocontrol Fungus *Trichoderma harzianum*. *J Appl Environ Microbiol.* 1999; 65: 2926-2933. doi: <https://doi.org/10.1128/AEM.65.7.2926-2933>
54. Fasim F, Ahmed N, Parsons R, Gadd GM. Solubilization of zinc salts by bacterium isolated by the air environment of tannery. *FEMS Microbiol Lett.* 2002; 213: 1–6.
55. Saravanan SV, Sudalayandy RS, Savariappan. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian J Microbiol.* 2003; 34: 121-125.
56. Martino E, Perotto S, Parsons R, Gadd GM. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biol Biochem.* 2003; 35: 133-141. [https://doi.org/10.1016/S0038-0717\(02\)00247-X](https://doi.org/10.1016/S0038-0717(02)00247-X). (<https://www.sciencedirect.com/science/article/pii/S003807170200247X>)
57. Fomina M, Alexander IJ, Hillier S, Gadd GM. Zinc Phosphate and Pyromorphite Solubilization by Soil Plant-Symbiotic Fungi. *Geomicrobiol.* 2004; 21: 351-366. doi: <https://doi.org/10.1080/01490450490462066>
58. Madhaiyan M, Saravanan VS, Jovi DBSS, Lee H, Thenmozhi R, Hari K, Sa T. Occurrence of *Gluconacetobacter diazotrophicus* in tropical and subtropical plants of Western Ghats, India. *Microbiol Res.* 2004; 159: 233-243. <https://doi.org/10.1016/j.micres.2004.04.001>.
59. Vaid SK, Kumar B, Sharma A, Shukla AK, Srivastava PC. Effect of Zn solubilizing bacteria on growth promotion and Zn nutrition of rice. *J Soil Sci Plant Nutr.* 2014; 14. <http://dx.doi.org/10.4067/S0718-95162014005000071>
60. Anitha S, Padma DSN, Sunitha KK. Isolation and identification of zinc solubilizing fungal isolates from agriculture fields. *Indian J Agric Sci.* 2015; 85: 1638–1642

61. Kumar A, Maurya BR, Raghuvanshi R, Meena VS, Islam MT. Co-inoculation with Enterobacter and Rhizobacteria on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under Indo-Gangetic Plain of India. *J Plant Growth Regul.* 2017. <https://doi.org/10.1007/s00344-016-9663-5>
62. Gontia-Mishra I, Sapre S, Tiwari S. Zinc solubilizing bacteria from rhizosphere of rice as a prospective modulator of zinc biofortification in rice. *Rhizosphere.* 2017; 3: 185–190
63. Mumtaz MZ, Maqshoof A, Jamil M, Hussain T. Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. *Microbiol Res.* 2017; 202: 51-60. <https://doi.org/10.1016/j.micres.2017.06.001>
64. Othman NMI, Othman R, MohdSaud H, Wahab PEM. Effects of root colonization by zinc-solubilizing bacteria on rice plant (*Oryza sativa* MR219) growth. *Agric Nat Res.* 2017; 51: 532–537.
65. Vidyashree DN, Muthuraju R, Panneerselvam P. Evaluation of zinc solubilizing bacterial (ZSB) strains on growth, yield and quality of tomato (*Lycopersicon esculentum*). *Int J Curr Microbiol App Sci.* 2018; 7: 1493–1502.
66. Dinesh R, Srinivasan V, Hamza S, Sarathambal C, Gowda SA, Ganeshamurthy AN, Gupta SB, Nair VA, Subila KP, Lijina A, Divya VC. Isolation and characterization of potential Zn solubilizing bacteria from soil and its effects on soil Zn release rates, soil available Zn and plant Zn content. *Geoderma.* 2018; 1(321):173-86.
67. Khanghahi MY, Ricciuti P, Allegretta I, Terzano R, Crecchio C. Solubilization of insoluble zinc compounds by zinc solubilizing bacteria (ZSB) and optimization of their growth conditions. *Environ Sci Pollut Res.* 2018; 25: 25862–25868.
68. Zaheer A, Malik A, Sher A, Qaisrani MM, Mehmood A, Khan SU, Zeenat MA, Mirza S, Karim M. Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (*Cicer arietinum* L.) growth. *Saudi J Biol Sci.* 2019; 26: 1061-1067. <https://doi.org/10.1016/j.sjbs.2019.04.004>.
(<https://www.sciencedirect.com/science/article/pii/S1319562X19300488>)
69. Bhatt K, Maheshwari DK. Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth promoting activities alleviates growth in *Capsicum annum* L. *3 Biotech.* 2020; 10. <https://doi.org/10.1007/s13205-019-2033-9>
70. Nepomuceno RA, Brown CB, Gargarino AMP, Pedro MS, Brown MB. Crop Science Society of the Philippines Growth Enhancement of Rice (*Oryza sativa* L.) by Zinc-Solubilizing Bacteria Isolated from Vesicular-Arbuscular Mycorrhizal Root Inoculant (VAMRI). *Philipp J Crop Sci.* 2020; 45: 34-40.
71. Upadhyay H, Gangola S, Sharma A, et al. Contribution of zinc solubilizing bacterial isolates on enhanced zinc uptake and growth promotion of maize (*Zea mays* L.). *Folia Microbiol.* 2021; 66: 543–553 <https://doi.org/10.1007/s12223-021-00863-3>

72. Bhakat K, Chakraborty A, Islam E. Characterization of zinc solubilization potential of arsenic tolerant *Burkholderia* spp. isolated from rice rhizospheric soil. *World J Microbiol Biotechnol.* 2021; 37. <https://doi.org/10.1007/s11274-021-03003-8>
73. Fahsi N, Mahdi I, Mesfioui A, Biskri L, Allaoui A. Plant Growth-Promoting Rhizobacteria Isolated from the Jujube (*Ziziphus lotus*) Plant Enhance Wheat Growth, Zn Uptake, and Heavy Metal Tolerance. *Agriculture.* 2021;11. <https://doi.org/10.3390/agriculture11040316>
74. Othman NMI, Othman R, Zuan AT, Shamsuddin AS, Zaman NBK, Sari NA, Panhwar QA. Isolation, Characterization, and Identification of Zinc-Solubilizing Bacteria (ZSB) from Wetland Rice Fields in Peninsular Malaysia. *Agriculture.* 2022; 12. <https://doi.org/10.3390/agriculture12111823>
75. Macwan AH, Shelat HN, Jhala YK, Shah SN. Utilization of zinc solubilizing bacteria for better growth and development of summer groundnut (*Arachis hypogaea* L.). *The Pharma Innov J.* 2022; 11: 1027-1035.
76. Desai S, Natarajan A, Naga RS. Zinc solubilizing *Bacillus* sp (SS9) and *Enterobacter* sp (SS7) promote mung bean (*Vigna radiata* L.) growth, nutrient uptake and physiological profiles, *Lett Appl Microbiol.* 2023; 76. <https://doi.org/10.1093/lambio/ovac063>
77. Srithaworn M, Jaroenthanyakorn J, Tangjitjaroenkun J, Suriyachadkun C, Chunchart O. Zinc solubilizing bacteria and their potential as bioinoculant for growth promotion of green soybean (*Glycine max* L. Merr.). *PeerJ.* 2023; 10(11):e15128.
78. Yadav RC, Sharma SK, Varma A, Singh UB, Kumar A, Bhupenandra I, Rai JP, Sharma PK, Singh HV. Zinc-solubilizing *Bacillus* spp. in conjunction with chemical fertilizers enhance growth, yield, nutrient content, and zinc biofortification in wheat crop. *Front Microbiol.* 2023; 4(14):1210938.
79. Rahman A, Ahmad MA, Mehmood S, Rauf A, Iqbal A, Ali B, Ullah M, Ali M, Mohamed HI, Uddin I. Isolation and Screening of Zn (Zn) Solubilizing Rhizosphere Bacteria from Different Vegetations for Their Ability to Improve Growth, Zn Uptake, and Expression of Zn Transporter Genes in Tomato. *Curr Microbiol.* 2024; 81(3):1-3.
80. Mader P, Kiser F, Adholeya A, Singh R, Uppal HS, Sharma AK, Srivastava R, et al. Inoculation of root microorganisms for sustainable wheat–rice and wheat–black gram rotations in India. *Soil Biol Bio Chem.* 2010; 43: 609–619.
81. Saravanan VS, Subramoniam SR, Raj SA. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian J Microbiol.* 2004;35:121-5.
82. Panhwar QA, Naher UA, Jusop S, Othman R, Latif MA, Ismail MR. Biochemical and Molecular Characterization of Potential Phosphate-Solubilizing Bacteria in Acid Sulfate Soils and Their Beneficial Effects on Rice Growth. *PLoS ONE.* 2014; 9. <https://doi.org/10.1371/journal.pone.0097241>
83. Costerousse B, Schonholzer-Mauclair L, Frossard E, Thonar C. Identification of heterotrophic zinc mobilization processes among bacterial strains isolated from wheat rhizosphere (*Triticum aestivum* L.). *Appl Environ Microbiol.* 2018; 84: e01715–e01717.

84. Vidyashree DN, Muthuraju R, Panneerselvam P, Mitra D. Organic acids production by zinc solubilizing bacterial isolates. *Int J Curr Microbiol App Sci.* 2018; 7: 626–633.
85. Subramanian KS, Tenshia V, Jayalakshmi K, Ramachandran V. Role of arbuscular mycorrhizal fungus (*Glomus intraradices*) (fungus aided). *Agric Biotechnol Sustain Dev.* 2009; 1: 29–38.
86. Choudhury R, Srivastava S. Zinc Resistance Mechanisms in Bacteria. *Curr Sci.* 2001; 81: 768–75, <http://www.jstor.org/stable/24106396>. Accessed 21 Jan. 2023.
87. Obrador A, Novillo J, Alvarez JM. Mobility and availability to plants of two zinc sources applied to a calcareous soil. *Soil Sci Soc Am J.* 2003; 67: 564–572.
88. Whiting SN, Souza MD, Terry N. Rhizosphere bacteria mobilize Zn for hyper accumulator by *Thlaspi caerulescens*. *Environ Sci Technol.* 2001; 35: 3144–3150.
89. Tariq M, Hameed S, Malik KA, Hafeez FY. Plant root associated bacteria for zinc mobilization in rice. *Pak J Bot.* 2007; 39: 245–253.
90. Burkert B, Robson A. Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular–arbuscular mycorrhizal fungi in a root–free sandy soil. *Soil Biol Biochem.* 1994; 26: 1117–1124
91. Sunithakumari K, et al. Zinc Solubilizing Bacterial Isolates from the Agricultural Fields of Coimbatore, Tamil Nadu, India. *Curr. Sci.* 2016; 110: 196–205. <http://www.jstor.org/stable/24906745>
92. Rani N, Kaur G, Kaur S, Mutreja V, Upadhyay SK, Tripathi M. Comparison of diversity and zinc solubilizing efficiency of rhizobacteria obtained from solanaceous crops under polyhouse and open field conditions. *Biotech Gene Engineer Rev.* 2022. <https://doi.org/10.1080/02648725.2022.2157949>
93. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol.* 2009; 63: 541–56. doi: <https://doi.org/10.1146/annurev.micro.62.081307.162918>
94. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica. (Cairo)*, 2012; 19. doi: <https://doi.org/10.6064/2012/963401>
95. PANDA A, DAS L, MISHRA BB. Zinc solubilization and potash mobilization by potent plant growth promoting bacteria isolated from Odisha. *Annals of Plant and Soil Res.* 2023; 25(2):285–96.
96. Bouis H, Welch RM, Biofortification - a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci.* 2010; 50.
97. Ali M, Ahmed I, Tariq H, Abbas S, Zia MH, Mumtaz A, Sharif M. Growth improvement of wheat (*Triticum aestivum*) and zinc biofortification using potent zinc-solubilizing bacteria. *Front Plant Sci.* 2023; 12(14):1140454.
98. Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE. MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn hyper-accumulating plants. *Plant J.* 2009; 57: 1116–1127.
99. Gueriot ML. The ZIP family of metal transporters, *Biochimica et Biophysica Acta (BBA) – Biomembranes.* 2000; 1465: 190–198. [https://doi.org/10.1016/S0005-2736\(00\)00138-3](https://doi.org/10.1016/S0005-2736(00)00138-3).

100. Krishna ATP, Maharajan T, RochV, Ignacimuthu S, Ceasar SA. Structure, Function, Regulation and Phylogenetic Relationship of ZIP Family Transporters of Plants. *Front Plant Sci.* 2020; 11. <https://doi.org/10.3389/fpls.2020.00662>
101. Lin YF, Liang HM, Yang SY, Boch A, Clemens S, Chen CC, et al. *Arabidopsis* IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. *New Phytol.* 2009; 182: 392–404. doi: <https://doi.org/10.1111/j.1469-8137.2009.02766.x>
102. Milner MJ, Seamon J, Craft E, Kochian LV. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *J Exp Bot.* 2013; 64: 369–381. doi: <https://doi.org/10.1093/jxb/ers315>
103. Eide D, Broderius M, Fett J, Guerinot ML. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc Natl Acad Sci U.S.A.* 1996; 93: 5624–5628. doi: <https://doi.org/10.1073/pnas.93.11.5624>
104. Zhao H, Eide D. The ZRT2 gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae*. *J Biol Chem.* 1996; 271: 23203–23210. doi: <https://doi.org/10.1074/jbc.271.38.23203>
105. Evens NP, Buchner P, Williams LE, Hawkesford MJ. The role of ZIP transporters and group F bZIP transcription factors in the Zn-deficiency response of wheat (*Triticum aestivum*). *Plant J.* 2017; 92: 291–304. doi: <https://doi.org/10.1111/tpj.13655>
106. Huang S, Wang P, Yamaji N, Ma JF. Plant nutrition for human nutrition: Hints from Rice research and future perspectives. *Mol Plant.* 2020; 13: 825–835. <https://doi.org/10.1016/j.molp.2020.05.007>
107. Lee S, Jeong HJ, Kim SA, Lee J, Guerinot ML, An G. OsZIP5 is a plasma membrane zinc transporter in rice. *Plant Mol Biol.* 2010; 73: 507–517.
108. Lee S, Kim SA, Lee J, Guerinot ML, An G. Zinc deficiency-inducible OsZIP8 encodes a plasma membrane-localized zinc transporter in rice. *Mol Cells.* 2010; 29: 551–558.
109. Kavitha P, Kuruvilla S, Mathew M. Functional characterization of a transition metal ion transporter, OsZIP6 from rice (*Oryza sativa* L.). *Plant Physiol Biochem.* 2015; 97: 165–174. doi: <https://doi.org/10.1016/j.plaphy.2015.10.005>
110. Inaba S, Kurata R, Kobayashi M, Yamagishi Y, Mori I, Ogata Y., et al., Identification of putative target genes of bZIP19, a transcription factor essential for *Arabidopsis* adaptation to Zn deficiency in roots. *Plant J.* 2015; 84: 323–334. doi: <https://doi.org/10.1111/tpj.12996>
111. Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, et al. OsZIP4, a novel zinc-regulated zinc transporter in rice. *J Exp Bot.* 2005; 56: 3207–3214. doi: <https://doi.org/10.1093/jxb/eri317>
112. Suzuki M, Bashir K, Inoue H, Takahashi M, Nakanishi H, Nishizawa NK. Accumulation of starch in Zn-deficient rice. *Rice.* 2012; 5: 1–8.
113. Sasaki A, Yamaji N, Ma JF. Transporters involved in mineral nutrient uptake in rice. *Journal of experimental botany.* 2016; 67(12):3645–53.

114. Bashir K, Ishimaru Y, Nishizawa NK. Molecular mechanisms of zinc uptake and translocation in rice. *Plant Soil*. 2012; 36: 189–201. doi: <https://doi.org/10.1007/s11104-012-1240-5>

UNDER PEER REVIEW