INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–944/2014/16–3–653–662
http://www.fspublishers.org

Review Article

Perspectives of Rhizosphere Microflora for Improving Zn Bioavailability and Acquisition by Higher Plants

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Abstract

Zinc (Zn) is an established micronutrient required for normal growth and functioning of plants. Its supply in adequate amount is considered indispensable for growth and development of plants. It is primarily taken up from soil through plant roots and translocated to other parts where it is needed to perform its specific role(s). In most cases, the soil (rooting media) have sufficient amount of total Zn, but plants still suffer from its deficiency constraints. Many factors affect and regulate the bioavailability of soil indigenous Zn as well as exogenously applied Zn enhancing bioavailability of the Zn to plants in soil matrix. Among these factors, soil microbial communities play a vital role in enhancing bioavailability of Zn to plants in soil matrix. Similarly, application of bioinoculants has been reported in the literature whose application to soil can promote Zn availability to plants. In this review, all possible strategies which can promote Zn availability to plants have been discussed comprehensively and critically.

Keywords: Zinc; Bioinoculants; Biofertilizer; Soil factors

Introduction

Zinc (Zn), a transition metal, is essentially required by plants for their growth and development (McCall *et al.*, 2000). It plays a vital role in photosynthesis, membrane integrity, protein synthesis, pollen formation and immunity system (Alloway, 2008; Hajiboland and Amirazad, 2010; Gurmani *et al*., 2012). It is also an important component of nucleic acids and Zn-binding proteins. It is documented that about 3,000 proteins of higher plants contain Zn prosthetic groups (Tapiero and Tew, 2003). Moreover, Zn is required as a co-factor for the activity of more than 300 enzymes (McCall *et al.*, 2000) and enhances the level of antioxidants within plant tissues (Luo *et al*., 2010). Furthermore, Zn plays a critical role in hormonal regulation in plants (Marschner, 1997). Zn finger proteins are involved in the regulation of signal transduction events. These proteins affect transcription by binding to DNA/RNA or other proteins, leading to cell death (Englbrecht *et al*., 2004; Ciftci-Yilmaz and Mittler, 2008). In addition, Zn is critical for the synthesis of phytohormones such as auxin, absicic acid, gibberellins and cytokinins. Its deficiency reduces level of these phytohormones in plant tissues resulting in an impairment of cell growth. Thus, its deficiency in plant tissues adversely affects various vital processes occurring within plant body.

Although Zn is required by the plant in micro-concentration, its bioavailable fraction in soil is very low due to various soil factors (Alloway, 2009). Some soils, despite having fair quantity of Zn cannot support plant growth because of poor bioavailable Zn. It is further documented that around 30% of the world's soils are Zn deficient (Kochian, 2000). The bioavailable content of Zn in soil can be increased using both chemical and biological approaches. Mineral fertilizers are considered a good source of Zn but it gets fixed quickly on soil matrix, resulting in poor availability to plants (Zia *et al.*, 1999). It has been estimated that about 90% of the total soil Zn exist in residual fraction, having no relevance to bioavailable fraction (Mandal *et al.*, 1988). It is crucial to increase bioavailability of Zn to plants by solubilizing fixed Zn and/or by reducing fixation of the applied Zn fertilizers. This can be achieved either by using organic amendments or potential Zn solubilizing bioinoculants. Organic amendments improve bioavailability of Zn by increasing microbial biomass, which not only enhance the rate of decomposition of organic matter (source of Zn), but also enhance the bioavailability of indigenous Zn by lowering the soil pH and by releasing chelating agents. Similarly, exogenous application of some potential Zn solubilizing microflora have shown huge capability to improve bioavailable Zn content in soil and its uptake by plant roots (Tariq *et al.*, 2007). This manuscript critically reviews

To cite this paper: Imran, M., M. Arshad, A. Khalid, S. Kanwal and D.E. Crowley.2014. Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. *Int. J. Agric. Biol.,* 16: 653–662

causes of poor Zn bioavailability in soil and the role of rhizosphere microflora as a potential tool in enhancing its bioavailability to higher plants.

Causes of Poor Zn Bioavailability to Plants

Zn deficiency is a ubiquitous problem of plants (Hotz and Brown, 2004; Welch and Graham, 2004). After application to soil, Zn is precipitated or adsorbed to the soil constituents. A number of factors including soil texture, pH, soil water content, organic matter and calcareousness of the soil are known to influence bioavailability of Zn in soil (Alloway, 2008). Heavy textured soils had shown more retention of Zn on their adsorptive sites than light textured soils. A negative correlation between DTPA extractable Zn and clay content of the soil was found (Sidhu and Sharma, 2010).

An increase in pH results in more retention by soil colloids due to increase in cation exchange capacity (adsorptive capacity), chemosorption on $CaCO_3$ and iron oxides (Cox and Kamprath, 1972; Yoo and James, 2002; Alloway, 2004). Harter (1983) reported that a decrease in Zn bioavailability with the increase in pH due to adsorption. Yoo and James (2002) also found a decrease in water-soluble Zn and increase in cation exchange capacity (CEC) of soil with an increase in soil pH from 4.0 to 7.0. The authors suggested that the decrease in water-soluble Zn occurred due to adsorption on exchangeable as well as at non-exchangeable sites. Zhao and Selim (2010) compared the sorption and desorption capacity of acidic and neutral soils. They found that sorption was less in acidic soils compared to neutral soil. Only 9-11% of sorbed Zn was released in neutral soils over time while it was 42-51% in acidic soils. Ghosh *et al.* (2009) reported a negative relation between soil pH and DTPA extractable Zn.

The bioavailability of Zn in soil is also strongly influenced by the calcareousness of soil. Sorption on surface of $CaCO_3$ reduces soil Zn bioavailability as $CaCO_3$ adsorbed Zn is difficult to be desorbed (Kiekens, 1995). Not only sorption to carbonates and clays contribute to poor Zn bioavailability but co-precipitation with carbonates and formation of calcium zincate are also very much important in calcareous soils. Xin-chun *et al.* (2008) investigated the effect of $CaCO_3$ on Zn availability in soil, using wheat as a test crop. They found a decrease in availability of Zn with $CaCO_3$. Most of the added Zn was transformed into an unavailable form as only 1.3% of the applied Zn was taken up by the plants. Sidhu and Sharma (2010) observed a negative correlation with soil $CaCO_3$ content and DTPA extractable Zn content. Thus, calcareousness is the major problem for agriculture and is the main factor of poor Zn solubility in soil causing Zn deficiency in plants

Water content in soil is very important factor, which determines the availability of Zn to plants (Patnaik *et al.*, 2008). Usually, flooding of soil reduces Zn availability most probably due to dissolution of indigenous P (Neue and Lantin, 1994), formation of insoluble compounds with manganese, iron, carbonate and sulfide under strictly anaerobic conditions (Alloway, 2004). Flooding also affects Zn availability through pH changes (Johnson-Beebout *et al.*, 2009). Dutta *et al.* (1989) documented a reduction in DTPA extractable Zn under submerged conditions and reported that this reduction occurred due to formation of insoluble Zn compounds such as hydroxides, carbonates, sulfides. According to Mandal and Hazra (1997), a high concentration of Fe^{2+} under submerged conditions contributed to reduced Zn availability in soils. Yoo and Jame (2003) compared the Zn extractability and uptake by rice (*Oryza sativa*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) on flooded and unflooded soils. They observed that flooding resulted in a significant reduction in Zn concentration in the leaves of all plants. Johnson-Beebout *et al.* (2009) determined DTPA-extractable Zn from oxidized and reduced soils cultivated with rice. They done extraction of field-moist soil and air dry soil and found a 60% reduction in DTPA-extractable Zn in case of field moist soil.

Soil organic matter increases solubility of Zn and reduces fixation, which results in its more uptake by plant roots (Marschner, 1993; Obrador *et al.*, 2003; Cakmak, 2009). According to Hodgson (1963), the production of complexing agent from organic matter is responsible for enhanced Zn solubility and extractability. Yoo and James (2002) in a laboratory study determined the extractability of added Zn as a function of organic matter. They noted that organic matter amendment increased the solubility of Zn in soils. Katyal and Sharma (1991) found a significant positive correlation between organic carbon and DTPA extractable Zn. Similarly, Sidhu and Sharma (2010) observed an increase in DTPA extractable Zn with soil organic carbon. Behera *et al.* (2011) determined the correlation between soil organic carbon and extractable Zn of Hariharpur, Debatoli, Rajpora and Neeleswarm having organic carbon 0.12-1.07, 0.11-0.85, 0.52-2.79 and 0.39-3.46, respectively. They found that with an increase in organic carbon, extractable Zn was also increased. Keeping in view causes of poor Zn availability and its potential impact on plant growth (Table 1), there is a dire need to review strategies which could be effective in enhancing availability of Zn to higher plants.

Potential Strategies to Enhance Zn Bioavailability to Plants

Various strategies such as application of chemical fertilizers, chelated Zn, organic amendments and bioinoculants are used to increase Zn concentration in the rhizosphere. However, there are certain limitations in using chemical and chelated Zn sources. The fertilizer-use-efficiency of inorganic Zn sources is very low. On the other hand, despite the fact that synthetic chelated-Zn compounds are a source of readily available Zn (Mortvedt *et al.*, 1999), their application is limited because they are expensive and pose a

Table 1: Impact of Poor Zn bioavailability on various physiological processes of plants

Crop	Physiological Parameters	Zinc treatment	Impact of Zn deficiency	Reference
Soybean	Proline Content	+Zn	12.48 ± 0.990 (mg/g dry wt.)	(Weisany *et al.*, 2012)
		-Zn	13.77 ± 3.660 (mg/g dry wt.)	
Cabbage	Photosynthetic rate	+Zn	9.28 ±1.01 (µmol $CO_2/m^2/s$)	(Hajiboland and Amirazad, 2010)
		-Zn	7.32±0.7 (µmol $CO_2/m^2/s$)	
Broad bean	Soluble protein content	+Zn	157.27±0.30 mg/g dry wt.)	(Sharaf *et al.*, 2009)
		-Zn	126.21±0.20 (mg/g dry wt.)	
Broad bean	Chlorophyll (*a,b*) content	+Zn	15.23±0.12 (mg/g fresh wt.)	(Sharaf *et al.*, 2009)
		-Zn	10.89±0.04 (mg/g fresh wt.)	
Wheat	Protein content	+Zn	14.2 (mg/g dry wt.)	(Seilsepour, 2006)
		-Zn	12.7 (mg/g dry wt.)	
Maize	Photosynthetic rate	+Zn	91.88±4.52 [µmol CO_2 (g fr.wt.h)$^{-1}$]	(Salama *et al.*, 2002)
		-Zn	62.35±5.13[µmol CO_2 (g fr.wt.h)$^{-1}$]	
Chickpea	Photosynthetic rate	+Zn	198.5±9.3 [µmol CO_2 (g fr.wt.h)$^{-1}$]	(Salama *et al.*, 2002)
		-Zn	124.4±5.7 [µmol CO_2 (g fr.wt.h)$^{-1}$]	
Maize	Chlorophyll (*a,b*) content	+Zn	2.78±0.11 [mg chl (g fr. Wt.)$^{-1}$	(Salama *et al.*, 2002)
		-Zn	1.09±0.09 [mg chl (g fr. Wt.)$^{-1}$	
Rice	Chlorophyll content	+Zn	00.59± (g m^{-2})	(Sasaki *et al.*, 1998)
		-Zn	0.45±0.14(g m^{-2})	
Rice	Photosynthetic rate	+Zn	22.9±1.701 (µmol $CO_2/m^2/s$)	(Sasaki *et al.*, 1998)
		-Zn	20.4±2.301 (µmol $CO_2/m^2/s$)	
Cauliflower	Stomatal opening	+Zn	6.78±0.47 (µm)	(Sharma *et al.*, 1995)
		-Zn	3.43±0.28 (µm)	
Cauliflower	Transpiration rate	+Zn	7.42±0.53 (µg cm^{-2} s^{-1})	(Sharma *et al.*, 1995)
		-Zn	4.78±0.38 (µg cm^{-2} s^{-1})	

socio-economic constraint to farmers, particularly in developing countries. It is documented that soil microflora had good potential to improve availability of nutrients in soil through various mechanisms. Therefore, use of organic amendments, which enhance activities of indigenous microflora and exogenous application of potential bioinoculants are able to solubilize soil Zn, improve bioavailable fraction of Zn and reduce fixation of applied Zn might be very helpful.

Organic Amendments

Soil organic matter is considered a very crucial factor in nutrient mobility in soil. Various organic amendments such as compost, farmyard manure (FYM), poultry manure, olive husk etc., are applied to soil to improve soil health, fertility and crop yields. The organic material can improve the availability of Zn by releasing Zn with time and through changes in physico-chemical properties of soil. These properties may increase soluble/available fraction of Zn in soil for plant uptake. Moreover, application of organic amendments also improve biological properties of soil (Tejada *et al.*, 2006). For instance, microbial biomass and soil enzyme activities are substantially increased with application of organic amendments (Blagodatsky and Richter, 1998; Liang *et al.*, 2003). This increase in microbial population and activities is an important indicator of soil health and soil productivity.

Soils having more microbial biomass and microbial activities are supposed to be productive soils as they have good nutrient mobility and availability to plants. Organic amendments improve soil microbial biomass carbon (Cmic) (Fließbach and Mäder, 2000) and the Zn content in soil and plant tissues (Saviozzi *et al.*, 1999). For instance, Garcia-Gil *et al.* (2000) observed 29 and 39% increase in soil Cmic and soil Zn content with the application of manure compared to control. Likewise, substantial increase in Cmic and Zn content in soil was recorded with application of FYM and compost (Leita *et al.*, 1999). Application of olive husk was also found to increase Zn concentration in soil and tissues of *B. vulgaris* and *B. maritime* plants (Clemente *et al.*, 2007). Quality of organic amendments is variable, which has high importance (Tu *et al.*, 2006). More proliferation of microflora prevails in soils treated with organic substrates having easily decomposable carbon. While comparing different organic sources, Chowdhury *et al.* (2000) found highest Cmic in soils treated with manure compost than those soils treated with saw dust and rice husk. Das and Dkhar (2011) also observed a significant increase in Cmic after the addition of plant compost and integrated plant compost. Tu *et al.* (2006), found maximum Cmic by the addition of cotton grain trash compared to animal manure and rye/vetch green manure. Cmic represent the active fraction of soil organic matter and serves as a source of nutrients to soil microbial community, which in turn release plant available nutrients by the decomposition of organic materials, hence increasing nutrient availability in the rhizosphere.

Improvement in Cmic and microbial activity in soil with the addition of organic amendments ensures better soil quality and also has a beneficial impact on soil fertility. By mineralization process, these organic amendments also ensure the availability of those macro and micronutrients (including Zn), which are generally ignored by the farmers.

As microbial activity in soil is associated with organic matter decomposition, release of chelating agents and organic ligands improve Zn availability by forming soluble complexes with inorganic Zn.

No doubt, organic matter is a good source for improving soil health and level of Zn in plant tissues, but these results are dependent on type, nature and composition of organic amendment. Some organic amendments like poultry manure, sewage sludge, municipal solid waste compost etc., contain substantial amount of heavy metals, which may pose a risk of metal contamination of agricultural soils and underground water (McBride, 1995; Tlustos *et al.*, 2000). Since these metals are highly toxic to biological system, the application of organic amendments contaminated with metals may disturb biological transformations in soil by suppressing the activities of various enzymes like urease, phosphatase etc. (Garcıa-Gil *et al.*, 2000). Furthermore, from the soil these metals will enter into the plant tissues and from where, ultimately they will reach the human body causing serious disturbance in human systems. It has been documented that organic matter may also reduce available Zn concentration in soil (Mora *et al.*, 2005). According to Harter and Naidu (1995), organic matter may form insoluble complexes with metals (Zn) and is a major cause of Zn deficiency in organic soils (Stevenson, 1991). Likewise, organic amendments can increase the population of microbes, but not the specific group (Zn solubilizers). Therefore, exogenous application of some potential Zn solubilizing microbes enhances Zn content in the rhizosphere and ultimately in the plants. Thus, application of organic amendments along with potential bioinoculants is a dire need of the hour to overcome health problems associated with Zn deficiency in food.

Rhizosphere Microflora

Rhizosphere is the narrow zone of soil around roots that is directly influenced by root secretions and is considered a hot spot of microflora, having manifold increase in microbial population than bulk soil. All the microbial communities residing in this region constitute rhizosphere microflora. The rhizosphere microflora may benefit plants through multifarious mechanisms including fixation of atmospheric nitrogen, mobilization of nutrients, production of phytohormones, altering indigenous level of phytohormones, improving plant stress tolerance to salinity, toxicity, drought, metal and pesticide load and also acts as a biocontrol agent (Glick and Bashan, 1997; Lucy *et al.*, 2004; Khalid *et al.*, 2009). Although, each and every mechanism has its own significance, but mobilization of nutrients by microflora has considered the most crucial function they perform in order to improve nutrient content in plant tissues. There is a good deal of research on mobilization of phosphorus in the rhizosphere through these tiny creatures, but increase in Zn bioavailable fraction in the rhizosphere due to the activities of rhizosphere microbes has not been well explored yet. However, there are sufficient reports indicating substantial potential of these microbes in improving Zn bioavailable fraction in the rhizosphere of plants and Zn content in plant tissues (Kothari *et al.*, 1991; Whiting *et al.*, 2001; Chen *et al.*, 2003; Tariq *et al.*, 2007; Biari *et al.*, 2008; Subramanian *et al.*, 2009). Since these microbes play an important role in improving food quality, therefore, they would be given prime importance in future while devising strategies to mitigate Zn malnutrition in humans through food, especially in developing countries where diverse food is not available to common people and they can also not afford food supplements. Among microbes, both bacteria and fungi have shown tremendous ability to improve plant available Zn in the rhizosphere and also increase Zn in plant parts (Whiting *et al.*, 2001; Fasim *et al.*, 2002; Biari *et al.*, 2008; Subramanian *et al.*, 2009). The ways through which rhizosphere microflora may cause mobilization/solubilization of Zn include reduction in soil pH (Koide and Kabir, 2000; Subramanian *et al.*, 2009), chelation (Whiting *et al.*, 2001) or through improving root growth and root absorptive area (Burkert and Robson, 1994). These mechanisms vary from one microorganism to other. Some organisms may use single of them while others may have more than one mechanism(s) to improve Zn in soil and ultimately improve Zn acquisition/uptake in plant tissues. Importance and examples of these mechanisms have been discussed in detail here.

Reduction in pH

Availability of micronutrients in soil is very much sensitive to soil. A little change in soil pH may have a great impact on micronutrient mobility/solubility in soil. It has been reported that availability of Zn decreases 100 times with one unit increase in pH (Havlin *et al.*, 2005). Thus by decreasing the pH of alkaline soil, bioavailable fraction of Zn can be enhanced to an appreciable level. Rhizosphere microflora has been reported to lower the soil pH to a good extent (Wu *et al.*, 2006), which may occur due to secretion of some organic acids and protons extrusion (Fasim *et al.*, 2002). For instance, *Pseudomonas fluorescens* secreted gluconic acid and 2-ketogluconic acid in the culture during solubilization of Zn phosphate. In addition, concentration of protons was also found higher in the culture after incubation period (Di Simine *et al.*, 1998). Likewise, Fasim *et al.* (2002) observed that solubilization of Zn oxide and phosphate was accompanied by proton extrusion and production of 2-ketogluconic acid. Martino *et al.* (2003) documented that ericoid mycorhizal fungi secreted organic acid to solubilize Zn from insoluble ZnO and $Zn_3(PO_4)_2$. A change in pH was observed when *Pseudomonas* and *Bacillus* spp. were used to solubilize ZnS, ZnO and $ZnCO_3$ in broth culture (Saravanan *et al.*, 2004). Koide and Kabir (2000) proposed that mycorrhizal plants facilitate Zn availability by lowering the pH of soil by the release of some organic acids. Subramanian *et al.* (2009) also stated that microbial activity and acid phophatase activity in arbuscular mycorhizae (AM) inoculated soil would have reduced the rhizospheric pH and contributed the release of Zn from mineral fraction. However, extent of reduction in rhizosphere pH vary among microorganisms as Giri *et al.* (2005) observed a 1.1 unit reduction in pH of rhizosphere soil with mycorrhizal inoculation, while Wu *et al.* (2006) observed a decrease in pH up to 0.47 unit with bacterial inoculation. Thus, pH goes down due to the release of organic acids and H^+, which facilitates Zn solublization and uptake by plants.

Zn-chelation

Zinc ions have high interaction with the soil constituent due to which its persistency in the soil solution is very low (Alloway, 2009). Due to low persistency/high reactivity of Zn in soil solution, plant available fraction of Zn in the soil is poor. However, bioavailability of Zn could be increased by means of Zn chelating compounds (Obrador *et al.*, 2003). These compounds are either synthetic or synthesized and released by the plant roots and potential rhizosphere microflora into the rhizosphere to chelate the Zn and improve its bioavailability. The chelates of microflora are the metabolites, which form complexes with metal cations like Zn^{2+} (Tarkalson *et al.*, 1998), which reduces their reaction with the soil. These Zn chelates subsequently move towards the roots and release chelating ligand (Zn^{2+}) at the root surface, making them free to chelate another Zn ion from the soil solution. In some microorganisms, chelation has been observed as dominant phenomena to improve bioavailability and uptake by plant roots. For instance, Whiting *et al.* (2001) suggested that possible mechanism used by bacteria (*Microbacterium saperdae*, *Pseudomonas monteilii*, and *Enterobacter cancerogenes*) for increasing water soluble Zn (bioavailable) in soil was the production of Zn chelating metallophores. In another report, Tariq *et al.* (2007) found that *Azospirillum lipoferum* (JCM-1270, ER-20), *Pseudomonas* sp. (96-51) and *Agrobacterium* sp. (Ca-18) mobilized Zn and made it bioavailable for longer period of time when they were applied as a biofertilizer to rice by producing chelating agent like ethylene diaminetetraacetate (EDTA). According to the reports of Kucy (1987), inoculation of *Penicillium bilaji* increased Zn solubilization and uptake in plant to greater extent which might occur through chelating mechanism.

Changes in Root Architecture

Zinc is immobile in soil and is taken up by plant mainly by diffusion (Havlin *et al.*, 2005). Due to poor native bioavailable Zn and low exogenous supply, depletion zones are formed around roots. Therefore, to improve Zn uptake it should be in close proximity of roots. This can be achieved either by application of more Zn or improving root growth and surface area so that roots can take nutrients beyond the depletion zone. A rhizosphere microflora especially mycorrhizal fungus is widely known for its impact on root architecture. Mycorhizal plants uptake Zn over more distances, crossing the depletion zone. According to Burkert and Robson (1994), arbuscular mycorrhizae can acquire Zn from a distance of 40 mm from the root surface. Jansa *et al.* (2003) noted that *Glomus intraradices* can take up Zn from a distance of 50 mm from the roots of maize. In the absence of Zn fertilization, Subramanian *et al.* (2009) observed that mycorrhizal fungus significantly increased root length, spread and volume of roots compared to the plants without fungal inoculation and this increased the Zn concentration in the grain up to 4%. Likewise, Tariq *et al.* (2007) observed a substantial increase in root weight, length and volume and Zn uptake in straw and grain with bacterial inoculation in rice.

Bioinoculants

Several studies have revealed that bioinoculants help in mitigation of Zn deficiency in plants through improving mobilization of Zn in soil. Many bacterial and fungal strains have been found capable of solubilizing fixed Zn and consequently increasing its uptake by plants (Table 2). The role of fungal and bacterial inoculants in improving availability of Zn to plants is comprehensively discussed below.

Fungal Inoculants

Among the fungal inoculants, AM fungus is considered highly effective in improving the availability and absorption of immobile nutrients by higher plants (Ikram *et al.*, 1992; Tarafdar and Marschner, 1994; Liu *et al.*, 2000). AM fungi are well known in improving the availability of phosphorus to plant roots. It has also been reported that mycorrhizal symbiosis is also very effective in improving availability of Zn to plants (Ortas *et al.*, 2002; Gao *et al.*, 2007; Ryan *et al.*, 2007; Subramanian *et al.*, 2009). A substantial increase in soil bioavailable content of Zn has been reported in fungal inoculated soils compared to uninoculated soils (Table 3). This bioavailable Zn is taken up by the plant root and accumulates in root, or translocated to other plant parts. Thus, concentration of Zn in plant tissues is directly dependent on its availability in soil. There are good reports about increase in Zn uptake by the application of bioinoculants (Smith and Read, 1997; Liu *et al.*, 2000; Ryan and Angus, 2003; Subramanian *et al.*, 2009), which might have occurred through increase in bioavailable Zn in soil. For instance, Swaminathan and Verma (1979) observed a great improvement in bioavailable Zn fraction in soil through fungal (*Glomus macrocarpus*) treatment which subsequently increased the Zn concentration in the leaves of wheat, maize and potato grown on Zn deficient soils. Likewise, Subramanian *et al.* (2009) found that inoculation of *Glomus intraradices* caused an overall increase of 43% in bioavailable Zn in soil after 75 days compared to

Table 2: Potential of bioinoculants in improving soil bioavailable Zn (mg kg^{-1} soil)

Bioinoculants	Crop	Extraction Method	Soil bioavailable Zn		Reference
			Exenic Control	Inoculation	
Glomus intraradices	*Z. mays*	DTPA-Zn	1.08	1.43	(Subramanian *et al.*, 2009)
Consortium of five strains (*Azospirillum lipoferum* JCM-1270, *Azospirillum lipoferum* ER-20, *Pseudomonas* sp. K-1, *Pseudomonas* sp.96-51 and Agrobacterium sp. Ca-18)	*O. sativa*	DTPA-Zn	0.3	1.7	(Tariq *et al.*, 2007)
Consortium of three strains (*Microbacterium saperdae, Pseudomonas monteilii and Enterobacter cancerogenes*)	*Thlaspi caerulescens*	WE-Zn	50.68	73.85	(Whiting *et al.*, 2001)
Glomus macrocarpus	*Z. mays* *T. aestivum* *S. tuberosum*	Zn-*L*	1.33 0.96 1.86	2.85 2.03 3.17	(Swaminathan and Verma, 1979)

Table 3: Effect of bioinoculants on plant Zn Acquisition under no Zn application

Bioinoculants	Crop	Plant Tissue (mg/kg dry weight)	Zn Acquisition		Reference
			Uninoculated Control	Inoculation	
Azospirillum brasilensel; Azotobacter chroococcum Bacillus polymyxa	*T. aestivum*	Straw	44.96 - -	52.45 49.5 47.88	(Eleiwa *et al.*, 2012)
Anabaena sp., PW1 *Providencia sp.* PW5		Grain	31.60	36.27 41.73	(Rana *et al.*, 2012)
Pseudomonas sp. NARs1+Rhizobium leguminosarum-PR1	*Lens culinaris*	Shoot	0.013	0.028	(Mishra *et al.*, 2012)
Glomus mosseae	*Prunus persica*	Root; Leaf	0.12; 0.04	0.18; 0.22	(Wu *et al.*, 2011)
Glomus intraradices	*Z. mays*	Grain	39.8	41.5	(Subramanian *et al.*, 2009)
Pseudomonas aeruginosa 7NSK2	*T. aestivum*	Root	151	185	(Sadaghiani *et al.*, 2008)
Azospirillum sp. Strain 21	*Z. mays*	Grain	2.73	5.66	(Biari *et al.*, 2008)
Consortium (*Azospirillum lipoferum* JCM-1270, *A. lipoferum* ER-20, *Pseudomonas* sp. K-1, *Pseudomonas* sp.96-51 and Agrobacterium sp. Ca-18)	*O. sativa*	Grain	9.2	23.6	(Tariq *et al.*, 2007)
Bacillus M-13	*Hordeum vulgare*	Whole Plant	66.2	73	(Canbolat *et al.*, 2006)
G. macrocarpum	*Cassia siamea*	Shoot	0.115	1.777	(Giri *et al.*, 2005)
G. mosseae	*Trifolium pratense*	Shoot	44.2	54.2	(Chen *et al.*, 2003)
Consortium of three strains (*Microbacterium saperdae, Pseudomonas monteilii and Enterobacter cancerogenes*)	*T. caerulescens*	Shoot	500	1000	(Whiting *et al.*, 2001)

uninoculated soils at various Zn levels. This increase in available Zn resulted in more acquisition of Zn and its partitioning to maize grain. Similar enhancement impact of mycorrhizal inoculation on grain Zn acquisition was observed by Jensen *et al.* (1982), Al-Karaki and Al-Raddad (1997) and Purakayastha and Chhonkar (2001) in barley, wheat and rice, respectively. Like grain, other plant parts like root, shoot and leaves also accumulated more Zn due to mycorrhizal infection (Table 3). Chen *et al.* (2003) observed 11% higher Zn in root of red clover plants inoculated with *G. mosseae* while Wu *et al.* (2011) observed 450, 225 and 200% more Zn accumulation in roots of peach plants inoculated with *G. mosseae*, *G. versiforme*, and *Paraglomus occultum*, respectively compared to uninocualted plants. Response of AM fungus inoculation has also been found very promising in terms of Zn accumulation in shoot and leaves. Giri *et al.* (2005) found about 15 times more Zn in the shoots of +AM compared to -AM plants. Chen *et al.* (2003) also recorded substantial increase in shoot Zn concentration through fungal inoculation. Similarly, mycorhizal infection also improved concentration of Zn in the leaves of AM plants compared to uninoculated plants (Swaminathan and Verma, 1979; Wu *et al.*, 2011).

However, response of AM fungi on Zn acquisition is comparatively low when Zn is applied at higher rate to soil. It is generally more effective under low Zn supply. For instance, Purakayastha and Chhonkar (2001) found low response of AM fungi inoculation when Zn was applied compared to no application. Even when Zn supply is abundant, impact of AM inoculation becomes negative as Chen *et al.* (2003) observed more Zn in shoots of red clover plants having mycorrhizal symbiosis with 0 and 50 mg kg^{-1} soil Zn application, but when Zn was applied at the rate of 100 and 300 mg kg^{-1}, Zn concentration was recorded even less in inoculated plants. This is fortunate, because in most of the developing countries, either no Zn is applied to soil or applied at very lower rate. So under, such circumstance, AM fungi inoculations is very useful in improving the Zn content in plant tissues.

Like AM fungi, free living fungi are also capable to convert insoluble Zn compounds into soluble compounds and improve Zn concentration in plant tissues. These include fungi belonging to genera *Aspergillus* and *Penicillium* (Burgstaller and Schinner, 1993; Sayer *et al.*, 1995). Kucey (1988) reported that inoculation with *Penicillium bilaji* resulted in a significant increase in Zn uptake by wheat plants. It increased from 161 (uninoculated control) to 232 µg pot^{-1} under green house conditions while from 9.61 to 10.7 mg $plot^{-1}$ in field study with fungal bioinoculant. These studies clearly demonstrated that fungal inoculants could be effectively used to increase Zn availability to plants.

Bacterial Inoculants

Like fungi, bacterial bioinoculants are also helpful in increasing solubilization/availability of Zn in soil and its further uptake by plants to improve plant Zn content. Several bacterial species have been reported that are able to solubilize insoluble Zn compounds in liquid medium (Di Simine *et al.*, 1998; Fasim *et al.*, 2002; Saravanan *et al.*, 2007) and in soil (Whiting *et al.*, 2001; Tariq *et al.*, 2007). For instance, in an *in vitro* study, Saravanan *et al.* (2004) found that *Pseudomonas* and *Bacillus* can solubilize various Zn compounds like ZnS, ZnO and $ZnCO_3$ to a good extent in liquid medium. Likewise, in another study, Saravanan *et al.* (2007) demonstrated Zn solubilizing potential of *Glomus diazotrophicus* PA15. Inoculation with *G. diazotrophicus* PA15 resulted in 41, 15.7 and 60 times increase in soluble Zn content in case of ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$, respectively after 48 h of incubation compared to uninoculated control. Similarly, Fasim *et al.* (2002) found a high potential of *Pseudomonas aeruginosa* to solubilize ZnO in liquid medium. Bacteria have also shown high mobilization of soil Zn as Tariq *et al.* (2007) observed almost 5.6 time higher bioavailable Zn in inoculated soil compared to uninoculated soil. Whiting *et al.* (2001) have also documented about 0.45 fold increase in bioavailable Zn in rhizosphere soil through bacterial inoculation.

It has also been widely reported that bacterial inoculation improves plant Zn content (Whiting *et al.*, 2001; Sadaghiani *et al.*, 2008; Biari *et al.*, 2008). For instance, Whiting *et al.* (2001) observed a 2-fold more Zn concentration in the shoot of *T. caerulescens* compared to control while uptake was increased up to 4-fold. Eleiwa (2005) reported that under no Zn application, inoculation of *Azotobacter* and *Azospirillum* was helpful in controlling Zn deficiency in wheat as up to 18%i increase in Zn uptake in response to inoculation was measured compared to uninoculated control. Similarly, inoculation of corn with *Azotobacter* and *Azospirillum* caused a significant increase in grain Zn content (Biari *et al.*, 2008). They observed up to 107, 85, 95 and 107% increase in Zn content in seed with *Azospirillum* sp. strain 21, *Azospirillum brasilense* DSM2286, *Azotobacter* sp strain 5, *Azotobacter chrooccoccum DSM2286* as compared to uninoculated control. Mishra *et al.* (2012) found consortium of *Pseudomonas* spp. and *Rhizobiu*m leguminosarum-pr-1 improved the shoot Zn content to a greater extent. While conducting experiment on rice, Tariq *et al.* (2007) observed 133% increases in Zn concentration in grain of rice by inoculation. The bacterial application also alleviated the deficiency symptoms of Zn in plant. Canbolat *et al.* (2006) and Sadaghiani *et al.* (2008) also found a substantial increase in Zn acquisition in wheat and barley with *Bacillus* M-13 and *Pseudomonas aeruginosa* 7NSK2, respectively. Thus use of such inoculants could be useful to increase solubilization of Zn in soil and its consequent availability to plants.

Conclusions and Future Prospects

Zn is a very important micronutrient for plant health and yield**.** It is involved in various plant physiological processes. Therefore, it should be available to plants in sufficient quantity. Although, most of the soils having fair quantity of Zn, but unfortunately posses extremely low plant available fraction due to various soil factors. Therefore, Zn should be applied from exogenous sources to increase soil solution concentration. Chemical fertilizers, chelated Zn and organic fertilizers are applied to soil to improve soil bioavailable Zn content, but all these have some limitations. Potential of microbial biotechnology have also been tested to improve the indigenous Zn availability and reduced the fixation of applied Zn. Application of various fungal and bacterial bioinoculats to soil had shown very promising results in terms of improving Zn content in soil and plant tissues and improving yield. Despite the fact that work related to impact of these bioinoculatns on increasing Zn in soil and plant tissues is limited and potential of such bioinoculants has not much explored and only few bacterial and fungal strains have been tested, even then there are a reasonably good reports about the importance of these bioinoculants in improving bioavailable Zn fraction in soil and mitigating Zn malnutrition in plants and also to reduce use of chemical fertilizers. Therefore, in future, biofertilizers using potential Zn solubilizing microbes should be developed. In addition, identifying genes involved in the Zn solubilizing activity is imperative so that more efficient mutants could be developed. Transfer of these genes to plants will also be highly helpful to address the problem of poor Zn bioavailability in rhizosphere and in plant tissues.

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(Received 10 July 2013; Accepted 26 August 2013)