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An insight in decoding the multifarious and splendid role of microorganisms in crop biofortification

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ABSTRACT

Agronomic practices of present era mainly focused on huge production of cereals and other food crops in more sustainable mode for challenging incessantly raising global population. However, rigorous cropping patterns and other environmental factors resulted into reduce micronutrients concentration in crop plants. Large human population relies on such crops for staple food engenders risk of micronutrient deficiency, also narrated as 'hidden hunger'. Then the strategy of biofortification is a leading solution to counteracting the problems of micronutrient deficiency in various crops. Agronomic, breeding and intervention through biotechnology are main approaches of biofortification for delivering important micronutrients (Zn, Fe, Se and Mn) with increase concentration in various crops, but these approaches are expensive, laborious and slower. However, application of microorganisms represents inexpensive and alternative approach of biofortification with ultimate solution for assisting plants by enhancing their growth with increase micronutrients concentration. Microorganisms have the capacity to solubilize zinc through wide arrays of mechanisms including acidification of nearby soil by organic acid synthesis, productions of siderophore, oxido-reductive system on cell membrane and chelated ligands. Inoculation of microorganisms especially plant growth promoting bacteria, is a better strategy making increase availability of micronutrients in various edible parts of plants. This review is focus to confer an insight on exploiting of microorganisms for increased micronutrient content in plants, using research studies conducted from last few decades to now, and presenting view on using such microorganisms as effective bioinoculant to drive biofortification of food crops in more eco-friendly impendent to improve sustainable agriculture.

Keywords

Crop
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Introduction

To address the problem of micronutrients deficiency in food crops, biofortification strategy is appreciable solution which consigns proper level of essential nutrients in

several edible parts of plants. Adverse environmental factors obstruct the uptake of micronutrients from soil to plants, and therefore inadequate amount of micronutrients affects the plant growth and development. Deficiency of micronutrients (zinc, iron,

selenium, copper, manganese and vitamins) in both humans and plants is narrated as 'hidden hunger' (de Valenca *et al.*, 2017) and set the threat of malnutrition among global human population. The goal of current agriculture system is to produce nutritious safe food crops with elevated level of micronutrients in the edible part of plants. Major human population is heavily dependent on crop based foods for basic diet, and having diet with inadequate level of essential micronutrients engenders serious health related issues in human beings. Therefore, adopting biofortification strategy provide better solution for producing crops with enhanced level of required micronutrients. Most literatures mainly suggested biofortification of food crops by three different ways: I) agronomic biofortification, II) breeding approach and III) genetic modifications. However, these approaches of agronomic and genetic biofortification to augment micronutrient concentration in crops for alleviating micronutrient malnutrition is considered to be lucrative and inappropriate in developing countries where rural population is most common (Mayer *et al.*, 2008). Moreover, rigorous application of chemical fertilizers presents several nuisance scenarios by disrupting soil microbial ecology and demolishing soil fertility, and ultimately drastic effects on both crop production and human health have been observed. Therefore, obvious demand arises for exploring another cost effective alternative strategies instead of genetic and agronomic approaches for fulfilling the aim of biofortification. Using of microorganisms, especially plant growth promoting microorganisms is an alternative approach for biofortification and better substitute of chemical fertilizers (Singh *et al.*, 2017). Moreover, utilization of plant growth promoting rhizobacteria as bioinoculants presents a low-cost and sustainable option for augmenting the micronutrient concentrations in plants (Shaikh and Sharaf, 2017). Soil

dwelling plant growth promoting microbiome maintains soil health, and facilitate the conversion of complex mixtures of micronutrients into simpler form which are ultimately uptake by plants (Singh and Singh, 2017; Shah *et al.*, 2018; Prasad *et al.*, 2016). Plant associated bacteria stimulate the growth of host plant through several ways such as increase mobility, uptake, and enrichment of nutrients in plants (Prasanna *et al.*, 2016; Singh and Prasad, 2014). Plant growth promoting bacteria/rhizobacteria (PGPB/R) enhance crop production through various mechanisms namely biological nitrogen fixation, solubilization of insoluble minerals (like P, Zn, Ca etc.), production of phytohormones and biocontrol (Glick *et al.*, 1999; Singh *et al.*, 2010, 2010a, 2010b, 2011; Singh and Goel, 2015). Microorganisms can also influence nutrient availability through exhibiting various characteristics including chelation, solubilization and oxidation or reduction (Khan, 2005; Singh *et al.*, 2013, 2018). Soil and rhizosphere associated microorganisms helps in improved nutrient acquisition of essential micronutrients from soil system to various portions of plants, and thus microbial assisted precise nutrient acquisition approach make microorganisms suitable candidates for crop biofortification with require nutrient elements essentially zinc (Zn), iron (Fe) and selenium (Se). Diverse form of microbiome including bacteria, cyanobacteria and fungi can be considered for efficient plants growth with deriving increased concentration of nutrients in crops at significant level, as achieved by genetic and agronomic based biofortification. However, plant growth promoting microbial entities are minutely explored for biofortification approach and they are needed to be put into the category of natural biofortification agent to develop biofortified crops. Present review is focused on role and application of microorganisms for decoding their admirable competence for biofortification of plants

especially food crops with selected micronutrients.

Major micronutrients and effects of their deficiency

High existence of micronutrient deficiency results in extensive disease burden in low and middle income countries (Black, 2014), and hence present devastating influence on human health. According to the United Nations System Standing Committee on Nutrition (UNSSCN), (2004) micronutrients malnutrition directly or indirectly associated with more than 50% of all child mortality and also associated with most foremost risk factors for maternal mortality. Iron (Fe), zinc (Zn) and selenium (Se) are measured as chief micronutrients and for maintaining life processes they are essentially required through diet. Deficiency of one or more micronutrients presents health related issues express in variety of diseases. Iron (Fe), a fourth most abundant and essential microelement on the earth crust (Tripathi *et al.*, 2015), which takes parts in various cellular processes (Puig *et al.*, 2005). Fe deficiency cause chlorosis in plants (Brown, 1961) and considered as major limitation for crop yield and which ultimately affects human health via food-chain, particularly to those people whose diets mostly rely on plant resources (Abadia *et al.*, 2011). Situation of iron deficiency is the common factor for causing nutritional anemia (Hurtado *et al.*, 1999) which are also associated with impaired neurocognitive development and as well impaired immune factions in children (Murray-Kolb, 2013). The micronutrient zinc (Zn) is vital for all organisms (Broadley *et al.*, 2007) and plays key structural roles in several proteins (Hershinkel, 2005). Zinc deficiency may engenders a number of human health related issues such as retarded growth, deferred wound healing, diarrhea, skeletal abnormalities, increased risk of abortion

(Salgueiro *et al.*, 2000), impairments of physical growth and greater risk of various infections (Hotz and Brown, 2004). Another example of profound dietary nutrient is selenium (Se), which involved in wide range of metabolic pathways including antioxidant defense and thyroid hormone metabolism (Rayman, 2012). Selenium (Se) deficiency may associated with a number of health disorders such as heart diseases, hypothyroidism, reduced male fertility, weakened immune system and increased risk of cancer, infections, oxidative stress-related conditions and epilepsy (Rayman, 2012; Hatfield *et al.*, 2014).

Strategies for biofortification

Importance of micronutrients is well understood and it is always advisable to consume foods that are nutrient rich for maintaining the health. Food is a basic source for micronutrients intake, and it is prerequisite to produce the food crops with elevated level of micronutrients concentration. Therefore, biofortification is a mean through which nutrient elements level could be increased directly in food crops through adapting agriculturally or core science based approaches. Biofortification strategies such as agronomic, plant breeding, genetic engineering and application of microbial inoculants are the key methods for improving nutritional content of plants. 'Agronomic biofortification' is the method for application of nutrient-rich fertilizers to soil or on foliage to increase the micronutrients level in edible part of the crops and consequently enhance the consumption of necessary micronutrients by consumers (Carvalho and Vasconcelos, 2013). Important approach for biofortification is "plant breeding" practiced by farmers for hundred of years where conventional plant breeding is designated to the crossing of plants to yield descendants with characters of both parents (Garcia-Casal *et al.*, 2016) and thus

this technique can be employed to produce descendents with admirable level of necessary nutrient elements. Rice varieties possessing high proportions of Fe and Zn were crossed with high-yielding rice varieties to produce descendants with both characteristics such as high yield and enhanced level of micronutrients (Khush, 2003). Biofortification made through “genetic modification” takes less time to generate crops those expresses in a stable way the trait of interest (e.g. nutritional content) and allows the transfer of particular genes or gene of interest (Garcia-Casal *et al.*, 2016). To exploit microbial inoculants for biofortification expresses cost effective approach and gives sustainable solution for enhanced micronutrient concentration in plants in environment friendly manner. Beneficial soil microorganisms make possible nutrients availability to plants through numerous mechanisms such as atmospheric nitrogen fixation, solubilizing the nutrients fixed into the soil, and by producing phytohormones (Yao *et al.*, 2008;). Such microorganisms make assure for further enhancement of micronutrients in plants, as they play a key role in organic material mineralization and as well as transforming inorganic nutrients (Rana *et al.*, 2012a).

Mechanistic insight intended for executing microbial based biofortification

Soil and rhizospheric region is main hub for innumerable microorganisms express various relation with plants and superiorly influence the plant growth and development. Microbial influence on micronutrients mobility is a significant module for biofortifying the food crops with several trace elements (Zn, Fe and Se). Microbial exudates carried out several practices such as chemical transformation, chelation and protonation for mobilization of trace elements. The influence of bacterial and fungal activity on trace elements mobility and its use for bioremediation has been reviewed

by Gadd (2004), where the contemporary knowledge on potential mechanisms of trace element mobilization by rhizobacteria has been summarized. Production of organic acid for Zn mobilization and synthesis of siderophore for Fe chelation through soil microorganisms are well established facts for transferring these nutrients in plants. Extensive persistence of zinc deficiency in crops due to inadequate supply of Zn from soil in to plants increased jeopardy of malnutrition at globe level. Zinc deficiency is expected to increase from 42% to 63% by 2025 due to continuous depletion of soil fertility (Singh, 2009) and presents serious concern about zinc deficiency in crops and as well humans. Soil is rich with plenty of zinc, but its deficiency prevailed in crops due to presence of unavailable fraction of Zn (Sunithakumarai *et al.*, 2016). Microorganisms employ the strategies for solubilization of Zn and other nutrients through production of wide range of organic acids. Organic acids are mainly CHO containing compounds characterized by the occurrence of one or more carboxyl groups with a maximum molecular weight of 300 daltons (Jones, 1998). Organic acids secreted by microflora increase soil Zn availability by sequestering cations and by reducing rhizospheric pH. However, Zn solubility increases with decrease in pH and its activity refuses upon precipitation as carbonate, hydroxide, phosphate and silicate at slightly acid to alkaline pH (Baruah and Barthakur, 1999). The Zn solubilizing *Bacillus* strains has been found to solubilize unavailable form of zinc through production of amino acid, chelating ligands, vitamins and phytohormones, oxido-reductive systems, proton extrusion and secretion of organic acids (Saravanan *et al.*, 2003). Plants incorporate iron from bacterial siderophores through different mechanisms such as chelate and release of iron, the direct uptake of siderophore-Fe complexes and by ligand exchange reaction (Schmidt, 1999). Hence,

association of siderophore producing microorganisms with plants become important for facilitating iron into plants, especially in iron deficient conditions. Carrillo-Castañeda *et al.*, (2002) reported positive effects of siderophore producing *Pseudomonas*, *Rhizobium* and *Azospirillum* on alfalfa plantlet growth with special reference to increased germination as well as the root and stem dry weight after the inoculation grown in iron limited cultures. Mung bean plants inoculated with the siderophore-producing *Pseudomonas* strain GRP3 under iron scarce conditions showed less chlorotic symptoms and enhanced chlorophyll content when compared to uninoculated plants (Sharma *et al.*, 2003). Both organic and inorganic forms of Se are exist in agroecosystem, where inorganic form of Se presents four oxidation states denoted as Se^{2-} (selenide), Se^0 (elemental Se), Se^{4+} (selenite) and Se^{6+} (selenate) (Fernandez-Martinez and Charlot, 2009). In general, bacteria have the ability to reduce oxidized and methylated Se oxyanions to some different Se-compounds. The reduction processes have been illustrated as the capacity of these microorganisms to convert the Se^{6+} and Se^{4+} , to Se^0 and finally generate Se methylated compounds (Losi and Frankerberger, 1997) and organic Se compounds (SeMet and SeMeSeCys) (Duran *et al.*, 2015) and provide usable form of Se in agriculture system.

Biofortification strategies through microorganisms

Zinc

Application of Zn fertilizers to various crops was promoted to tackle down the crisis of zinc scarcity, but their transformation to various unavailable forms depending upon soil types and chemical reactions (Gontia-Mishra *et al.*, 2017) set forth a further issue of zinc deficit state. The unavailable Zn compound can be

converted back to available form through bioaugmentation of microbial inoculants bearing the ability to solubilize insoluble Zn compounds (Saravanan *et al.*, 2007). In the last few years, the ability to solubilize insoluble Zn compounds [ZnO , $ZnCO_3$, $Zn_3(PO_4)_2$] by plant growth promoting rhizobacteria (PGPR) has been reported (Krithika and Balachandar, 2016; Saravanan *et al.*, 2007). Bacteria isolated from rice rhizosphere solubilized zinc through lowering the pH of culture medium and used as a perspective inflector of Zn biofortification of rice (Gontia-Mishra *et al.*, 2017). Previous studies indicated that organic acids like gluconic acid, 2-ketogluconic acid, 5 ketogluconic acid and pentanoic acids are produced by microorganisms during Zn solubilization (Saravanan *et al.*, 2007). Cyanobacterial inoculants on different maize hybrids also showed significant effect on zinc accumulation in flag leaf (Prasanna *et al.*, 2015).

Tariq *et al.*, (2007) have described the potential effect of Zn mobilizing PGPR which significantly alleviated the Zn deficiency symptoms and also increased the total biomass (23%), grain yield (65%), and harvest index in addition to increased Zn concentration in the grain of rice. Consortium of two bacillus species (*Bacillus* sp. SH-10 and *B. cereus* SH-17) was marked as potent umpire to produce Zn fortified rice grains and showed highest zinc translocation index value (1.6 to 1.7) when compared with translocation index value of only Zn and consortium of Zn and bacterial strains (Shakeel *et al.*, 2015).

Endophytic fungus *P. indica* under supplemented concentration of 10 mg/l Zn significantly increased leaf Zn concentrations (7.6 fold) of lettuce plant with enhanced chlorophyll under greenhouse conditions and proved its representation for biofortification (Padash *et al.*, 2016).

Iron

Microorganisms also played the role of biofortifying tool for enhancing Fe content in palatable crops as suggested by various literatures. Siderophore producing microorganisms can be suitable candidates for iron biofortification as they provide additional iron availability to plants. Various plant growth promoting bacteria especially *Enterobacteria* sp, *Pseudomonas putida* and other bacterial strains potentially doubled iron content in rice grain with enhanced efficiency of Fe translocation from root to shoot and grains (Sharma *et al.*, 2013), and thus presented event of strong application of plant growth promoting bacteria for Fe biofortification.

Inoculation with *Trichoderma asperellum* significantly increased the Fe concentration in wheat plants grown on Fe deficient calcareous medium and revealed a positive effect of this fungal organism on Fe nutrition in wheat (de Santiago *et al.*, 2011). Increased concentration of Fe in different parts of chickpea plants (grains, shoots and roots) were reported after inoculation with PGPRs along with Fe, and provided rhizobacterial based cost effective strategy for improving the Fe content in chickpea (Khalid *et al.*, 2015).

Selenium

Selenium (Se) is considered as an essential micronutrient because of its antioxidant capacity and positive effects on human health (Cartes *et al.*, 2005). The main source of Se for humans and animals is the soil-plant system, and a mineral imbalance can lead to Se-deficient food with consequences for human and animal nutrition (Govasmark and Salbu, 2011). In general, soils around the world have little Se quantities. Therefore, agronomic Se fortification by using inorganic Se source is a current technology in order to

maintain optimized level of Se status in human diet for reducing chronic disease risks (Bañuelos *et al.*, 2016). The promising strategy for selenium biofortification was set in wheat through co-inoculation of selenobacteria mixture (*Stenotrophomona* sp. B19, *Enterobacter* sp. B16, *Bacillus* sp R12. and *Pseudomonas* sp. R8) and arbuscular mycorrhizal fungi (*Glomus claroideum*), and produced Se enriched wheat grains (up to 23% increased selenium content) for supplementing foods for human consumption (Duran *et al.*, 2013). Duran *et al.*, (2014) reported the potential use of three endophytic bacterial strains such as *Bacillus*, *Klebsiella* and *Acinetobacter* for selenium biofortification of wheat plants. Another study showed co-inoculation of endophytic selenobacteria and AM fungus *Rhizophagus-intraradices* enhanced the Se content in lettuce plant and protected plants against drought stress conditions (Duran *et al.*, 2016). These studies set forth the beneficial effect of symbiotic interacted selenobacteria and AM fungi for micronutrient biofortification of food crops. *Bacillus cereus*-YAP6 and *Bacillus licheniformis*-YAP7 increased Se uptake by wheat plants, and high concentration of Se was reported in Stem (375%) followed by kernel (154%), (Yasin *et al.*, 2015) and determine the role of *Bacillus* for the selenium biofortification.

Biofortification with other micronutrients

Besides the Fe and Zn, other important micronutrients are Mn and Cu which are essential for plants and animals. Increasing the micronutrient density of staple crops or biofortification can play a vital role in improving human nutrition on a global scale (Rana *et al.*, 2012b). Mn deficiency is reported worldwide and differences in Mn efficiency among the crops are related to their ability to affect the solubility of Mn in the rhizosphere (Hassan Sayyari-Zahan *et al.*, 2009).

The availability of Mn in the rhizosphere is affected by several factors including redox condition and pH, moisture, temperature and concentrations of other nutrients and heavy metal in soil solution. Mn^{4+} is the main oxidized form of Mn that exists in oxidized soil and considered as low soluble mineral pyrolusite. Some rhizobacteria such as *Bacillus*, *Pseudomonas* and *Geobacter* reduce oxidized Mn^{4+} to Mn^{2+} , which is metabolically useful for plants (Dotaniya *et al.*, 2015). Mn reducing bacteria (*Pseudomonas sp.*) and fungi (*Gaeumannomyces graminis*) play an important role in enhancing Mn availability and crop uptake from the soil (St. Clair and Lynch, 2010). The microbial strains *Providencia sp.* (PW5) enhanced the Mn uptake and brought about a 36.7% increase in Mn content in wheat as compared to $N_{60}P_{60}K_{60}$ fertilizer control (Rana *et al.*, 2012b).

In conclusion, food based crops with enhanced micronutrients concentration are required globally for eradicating hidden hunger. Plant associated microorganisms reside on soil and root interface and commence enhance uptake of macro as well micro nutrients for plants vitality. Various microorganisms efficient for siderophore production and zinc solubilization were considered for boosting Fe and Zn in food crops, respectively. Plant growth promoting microorganisms were found to be capable to significantly augment zinc and iron content in rice and wheat, which are globally required in major food crops where the crops containing less concentration of micronutrients. Microbial inoculants established their potent role for biofortification and lessen the dependency on costlier approaches such as agronomic intervention and genetic modification for enhancing micronutrients concentration in edible part of crops. Multiple traits of plant growth promotion with various

nutrients acquisitions processes made microorganisms able to formulate biofortification strategy. Improved content of micronutrients in crops in response to bioinoculants confirm trueness of microorganisms as biofortifier agents.

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