Chapter-7

Summary

Food security is a growing concern worldwide, and there is a constant pressure on staple food crops to meet global food demand. However, vital elements such as micronutrients, minerals, amino acids, and vitamins are not present in adequate quantity in primary food crops. Micronutrient deficiencies are a major global health issue, especially in low- and middle-income nations. Millions of people are affected by these inadequacies, which have a negative effect on health and impede social and economic advancement. Around two billion people are experiencing insufficiency of micronutrients such as Fe and Zn due to poor mineral element availability in soil and/or inadequate deposition of minerals in eatable portion. Micronutrient malnutrition affects people who rely on cereals-based food, as cereals like rice, wheat, and maize often have poor mineral content and low bioavailability. Around 820 million people suffer from hunger, 2 billion are malnourished, and 2 billion are obese. The ever-expanding population poses a threat to food security in the future. Deficits in Zn, Fe, I, Ca, and vit A are the most general paucities of micronutrients worldwide. Fe scarcity is considered one of the top fifteen leading causes of health issue globally, accounting for approximately 7.3% of the global disease burden. Soil micronutrient insufficiency has been recorded in several parts of the world, restricting crop and human nutrient uptake. Inadequate consumption of these micronutrients has significant biological consequences, as they are vital to human biological progressions. Nutritional guarantee is a matter of major relevance from the viewpoint of human and cattle health. Zinc and iron deficiencies are the most common micronutrient deficits globally, with 40% of expectant mothers and 42% of children anaemic and 17% of the world's population at risk of not obtaining enough Zn. Decreased bioavailability of zinc and iron, also affects agricultural soils in India. About 13% iron deficiency and 47% zinc deficiency have been recorded in Indian soils.

Zinc and iron deficiency in Indian soil is a prevalent issue that negatively impacts crop growth, quality, and human health. The deficiency of these micronutrients is attributed to factors such as low zinc/iron content in the parent material, high soil pH, inadequate organic matter, and imbalanced nutrient management practices. Zinc deficiency leads to stunted plant growth, reduced leaf size, and chlorosis, while iron deficiency causes yellowing of leaves and affects photosynthesis and metabolic processes. These deficiencies result in decreased crop yields, poor crop quality, and increased vulnerability to diseases and pests. Furthermore, inadequate intake of zinc and iron through food leads to health problems like impaired growth, anemia, weakened immune system, and cognitive impairments. Addressing these deficiencies requires soil testing, balanced nutrient management, appropriate fertilizers, crop diversification, breeding programs, and education to ensure sustainable agriculture and improved human nutrition.

The enhancement of iron and zinc bioavailability in finger millet (*Eleusine coracana*) using bacterial endophytes is an innovative approach with promising implications for addressing nutrient deficiencies in this important crop. Finger millet is a staple food in many parts of India, and is known for its nutritional value. However, it is often characterized by low iron and zinc bioavailability, limiting its effectiveness in combating nutrient deficiencies. Endophytic bacteria have been explored as potential biofortification agents to enhance the nutrient content and bioavailability in finger millet. These beneficial bacteria reside within the plant tissues and can play a significant part in enhancing nutrient uptake and utilization. Studies have shown that certain bacterial endophytes can promote the solubilization of iron and zinc in soil, making these micronutrients more accessible to the plant. The mechanism by which bacterial endophytes enhance iron and zinc bioavailability involves the production of organic acids, siderophores, and enzymes. These compounds aid in the release of iron and zinc from their insoluble forms in the soil, increasing their availability for uptake by the plant roots. Moreover, bacterial endophytes can also stimulate the production of plant growth-promoting substances and enhance root expansion, leading to enhanced nutrient absorption.

With the aforementioned in mind, it was intended for the current investigation to leverage possible finger millet endophytic bacterial species as a source of the microbiological biofortification technique. This may help increase the zinc and iron content in finger millet plants and improve crop quality, which would ultimately improve the health of consumers.

The results of the current study helped in exploring and evaluating the efficiency of iron and zinc-solubilizing endophytic bacteria and their consortia that enhanced plant growth in addition to fortifying finger millet crops with iron and zinc micronutrients under pot conditions, and proves to be a significant step in resolving the issue of zinc/iron related malnutrition. The following summarizes the main findings of the study:

Isolation and analysis of iron and zinc solubilizing endophytic microbes:

- In order to isolate bacterial endophytes for this investigation, plant and seeds of three cultivars of finger millet (VL-348, VL-352, and PRM-1) were obtained from the VCSGU University of Horticulture and Forestry in Bharsar, Uttarakhand. All three varieties of the finger millet plant, including the roots, shoots, leaves, and seeds, yielded a total of 112 endophytic bacteria. These isolates were selected on the basis of various morphological types for further characterization and identification, and they were purified and given names in accordance with the crop variety names.

- The study assessed endophytic isolates' ability to solubilize iron and zinc by measuring the zone of clearance. Six isolates were selected from 112 endophytic bacterial isolates based on zone of clearance development on modified basal medium and Tris minimum medium agar plates. All six isolates solubilized the three zinc (i.e., zinc carbonate, zinc oxide, zinc phosphate) and iron (ferric phosphate) salts, showed varied SI.

Plant growth promoting attributes and other features of bacterial endophytes:

- The selected six endophytes were further assessed for the plant-growth promoting traits such as IAA, HCN, siderophore, ammonia, organic acid, extra cellular enzyme production and solubilization of phosphate. Bacterial endophytes were also examined for their stability at various abiotic stresses such as different temperature, pH and NaCl concentrations.
- Iron and zinc solubilizing isolates showed changes in colour with Salkowski's mixture, indicating phytohormone synthesis. Bacterial endophytes produced IAA differently, with EC3B-22 synthesizing the highest amount (117.2 µg/ml).
- Bacterial endophytes, except EC2B-21, synthesized hydrogen cyanide in various amounts. Qualitatively, EC1B-24 and EC3B-22 showed strong reactions, while EC3B-12, and EC3B-23 showed moderate reactions. Spectrophotometrically, isolate EC3B-22 synthesized HCN significantly, with the maximum absorbance value of 0.043.
- Selected bacterial endophytes were tested for ammonia synthesis, only the endophytic isolates EC3B-23 and EC2B-21 exhibited the ammonia synthesis.

- All of the bacterial endophytes produced siderophores and had an orange zone surrounding the colony except EC1B-24. Quantitative assessment displayed that the bacterial endophytes, exhibited siderophore in varying amount and maximum percent siderophore unit (psu) was showed by EC3B-22 (71.8 psu) followed by EC3B-1 (66.09 psu), EC3B-12 (61.4 psu), EC2B-21 (51.4 psu), EC3B-22 (23.74 psu), and EC1B-24 (13.4 psu).
- The study assessed bacterial endophytes' phosphate solubilization potential by examining halo zones surrounding colonies on NBRI-BPB medium. Bacterial isolate EC2B-21 showed the highest solubilization index (3.6 mm), followed by EC3B-22 with the least (2.4 mm). Endophytic bacteria EC3B-1 also solubilized phosphate with a solubilization index (3.3 mm).
- All the bacterial isolates showed formation of yellow zone round the colonies which shows that they were capable to synthesize organic acids except isolate EC3B-1.
- All of the endophytic isolates displayed a variety of enzymatic activity.
- The finger millet endophytic bacterial isolates' adaptability was evaluated at various pH levels, temperatures, and NaCl concentrations. They grew well in pH ranges of 5.0-9.0, but preferred 6.0-8.0 for optimal growth. Temperatures ranged from 15°C to 35°C, and EC3B-23 survived at 40°C and 50°C. NaCl concentrations between 0.5% and 3% were ideal for all isolates.
- Each bacterial isolate displayed unique colony morphology, size, pigment, margin, and shape attributes.

Identification of potential isolates

- Further on the basis on iron and zinc solubilization index, out of six screened, two promising isolates were selected EC3B-22, and EC3B-23 for further examination.

- Based on 16S rRNA gene analysis endophytes EC3B-22 and EC3B-23 displayed 99.85% and 99.51% sequence similarity to *Pseudomonas bijieensis* L22-9T and *Priestia megaterium* NBRC 15308T, respectively.
- Additionally, the influence of diverse sources of nitrogen and carbohydrates on the development and growth of bacterial endophytes were assessed, and it was observed that both endophytes utilized diverse nitrogen/carbohydrate sources.

Pot experiment for plant growth and micronutrient (Fe/Zn) uptake

- Results of a pot trial in a plant growth experiment displayed that both endophytic bacterial isolates enhanced shoot/root parameter (dry weight, and length of shoot-root) as compared to uninoculated plants.
- Endophytic bacterial strain EC3B-23 (*Priestia megaterium*) significantly increased root length and plant height (22.1 and 68.3 cm) in cultivar VL-352 as compared to the control and zinc carbonate treatment, while finger millet cultivars VL-348 and VL-352 achieved maximum shoot height (67.2 cm; 66.8 cm) and root length (22.1 cm; 20.1 cm) respectively, which was higher than the uninoculated treatments.
- With regards to the shoot and root dry weight maximum plant dry weight was showed by endophytic isolate EC3B-23 (21.4 g) in cultivar VL-348, while maximum root dry weight was showed by similar isolate in cultivar VL-352 (6.49 g), which was higher than the control.
- Endophytic inoculation significantly impacts grain zinc concentration compared to uninoculated control treatments. Maximum zinc content was observed in variety VL-348 by the inoculation of EC3B-23 (*Priestia megaterium*) i.e., 2.78 g, which was 15.35% more than control, followed by variety VL-352 (2.75 g, 16.03% higher than the uninoculated treatment) by the inoculation of EC3B-22 (*Pseudomonas bijieensis*)

and PRM-1 (2.73 g) by the EC3B-23 (*Priestia megaterium*), which was 18.18 % higher than the control treatment.

- Zinc content in finger millet plants were also significantly influenced by endophytic inoculation. In cultivar PRM-1, EC3B-23 (*Priestia megaterium*) treatment showed the highest zinc content in shoot and root, with 16.7% and 15.7% higher than the control. In variety VL-348, EC3B-23 (*Priestia megaterium*) showed the maximum zinc content (16.5% shoot and 17.06% root), while in variety VL-352, EC3B-22 (*Pseudomonas bijieensis*) inoculation increased shoot and root zinc content by 16.2% and 17.3%, respectively.
- With regards to the enhancement of iron content in finger grains, both the endophytic bacterial treatments improved the Fe content in all three finger millet varieties. Maximum uptake was observed by the inoculation of EC3B-23 (*Priestia megaterium*) in variety VL-348 (3.65 mg; 4.58 % higher than the control) followed by PRM-1 (3.63 mg; 6.14 % higher than the control) and VL-352 (3.62 mg; 6.47 % higher than the control). Treatment B1 (EC3B-22 (*Pseudomonas bijieensis*) also enhanced the iron content in finger millet grains as compared to the control treatment.
- According to our findings, endophyte inoculation also enhanced the iron content of roots and shoots in all three finger millet varieties. In case of PRM-1, bacterial isolate EC3B-23 showed maximum iron content in shoot and root i.e., 2.43 mg and 2.35 mg respectively as compared to control (2.22 mg and 2.19 mg).
- Similar to this, in variety VL-348 bacterial isolate EC3B-23 resulted in enhanced shoot and root iron content (2.42 mg and 2.39 mg); EC3B-22 was also helpful in increasing shoot and root iron content (2.39 mg and 2.31 mg). Similar pattern was observed in variety VL-352, endophyte EC3B-23 (shoot; 2.45 mg and root; 2.4 mg) contributed

in maximum iron content enhancement, which is higher among all three variety and all sets of treatments.

- Along with iron and zinc content, bacterial endophytes also enhanced the NPK concentration in FM grains as compared to the uninoculated treatments. Maximum NPK content was showed by isolate EC3B-23 in cultivar VL-348 followed by PRM-1, and VL-352 (1.36, 0.251, and 0.642 mg respectively).

The study's findings demonstrated the beneficial benefits of bacterial endophytes inoculants with $ZnCO_3$ and $FePO_4$ supplementation on plant development and micronutrient (Fe/Zn) enhancement in the edible part of finger millet. Microorganisms and plants can interact in ways that are advantageous for plant health also. Additionally, it can be crucial in low-cost agricultural applications for the development of food crops enriched in Fe and Zn. In the near future, more experimental research will be needed to construct zinc/iron solubilizing bacterial-based formulations and determine their efficacy in boosting multiple micronutrients in edible plant parts for a more long-lasting treatment of nutrient deficiencies.