Chapter-6

Conclusion

Research has demonstrated the effectiveness of bacterial endophytes in enhancing iron and zinc bioavailability in finger millet. Inoculation with specific endophytic bacteria has resulted in increased iron and Zn uptake by the plants and improved nutrient accumulation in the eatable sections, such as grains. Bacterial inoculation also enhanced zinc/iron in plant parts. This biofortification approach not only increased the nutrient content in finger millet but also enhanced the bioavailability of iron and zinc, making them more easily absorbed by the human body upon consumption. Implementing bacterial endophytes biofortification in finger millet cultivation involves the cultivation and characterization of suitable endophytes from healthy plants/seeds. These endophytic bacteria were then applied to the roots/seeds of finger millet using inoculation techniques. A total 112 bacterial endophytes were isolated from finger millet plant parts and seeds. On the basis of the iron and zinc SI, six isolates were chosen, and further characterized and examined for plant growth promoting attributes such as IAA, hydrogen cyanide, ammonia, organic acid, siderophore production as well as for phosphate solubilization and extra cellular enzyme synthesis. The stability of these isolates was further evaluated over a range of environmental conditions, including pH, temperature, and NaCl. For the plant experiment, the two most promising bacterial isolates were chosen based on characteristics that promote plant growth. The endophytes were identified using the 16S rRNA gene sequence analysis. Pairwise sequence alignment of the endophytic isolates 'EC3B-22' and 'EC3B-23' (EZbiocloud database) exhibited 99.85% and 99.51%, respectively, sequence similarity to *Pseudomonas bijieensis* L22-9^T and *Priestia* *megaterium* NBRC 15308^T. According to the phylogenetic study, isolate EC3B-22 did not cluster with other *Pseudomonas* species, suggesting that the isolates may actually be a new species. Both bacterial endophytes utilized a variety of nitrogen and carbohydrate sources for their development and growth. These endophytic bacterial isolates increased root and shoot metrics (dry weight and shoot-root length) in a plant growth experiment, according to the findings of a pot trial in comparison to uninoculated plants. In variety VL-352, treatment B2, isolate EC3B-23 (*Priestia megaterium*) showed the maximum shoot and root length (68.3 cm; and 22.3 cm respectively), treatment B1, isolate EC3B-22 (*Pseudomonas bijieensis*) also showed improved results (shoot; 66.7 cm, root; 20.4 cm) in comparison to the control treatment. Maximum plant dry biomass was demonstrated by endophytic isolation EC3B-23 (21.4 g) in cultivar VL-348, and maximum root dry weight was demonstrated by a similar isolate in cultivar VL-352 (6.49 g), both of which were higher than the control. Zinc content in the grains of all three cultivars was enhanced by both treatments (B1 and B2) in considerable amount between 11.61 % to 18.18 % as compared to the uninoculated control. Overall, highest zinc content was found in variety VL-348 by the inoculation of isolate EC3B-23 (*Priestia megaterium*) i.e., 2.78 mg, 15.35 % higher than the control. Similar results were observed with regards to zinc content in shoot and root part of finger millet cultivars. Bacterial endophytes enhanced zinc concentration in plant part between 11.46 % to 17.39%. Interestingly, similar pattern was observed in the context of iron content in the grains. Though both the isolates increased the iron content but isolate EC3B-23 (*Priestia megaterium*) showed the maximum increase (3.65 mg) in variety VL-348 with 4.58 % higher enhancement than the control. This is followed by isolate EC3B-22 (*Pseudomonas bijieensis*) with 3.72 % increase in the same variety. The Fe content in the grains of all the varieties of finger millet was enhanced, ranging between 3.72 % to 6.47 %. In addition, endophyte inoculation increased the Fe content of shoots and roots in all three cultivars of finger millet ranging between 5.67 % to 12.7 % higher than the control. In variety VL-352, endophyte EC3B-23 contributed in maximum iron content enhancement (shoot; 2.45 mg and root; 2.4 mg), which is higher among all three variety and all sets of treatments. In comparison to the control, bacterial endophytes enhanced the levels of NPK in grains along with the iron and zinc levels. The results of the study showed that micronutrient (Fe/Zn) augmentation and plant growth in the edible section of finger millet were positively impacted by bacterial endophytes with $ZnCO_3$ and $FePO_4$ supplements. This is the first study to describe the association of '*Pseudomonas bijieensis'* and '*Priestia megaterium'* for biofortification of Zn, and Fe in the cereal and augmentation of NPK content in finger millet.

The augmentation of Fe and Zn bioavailability in FM using bacterial endophytes offers significant potential for addressing nutrient deficiencies and improving human health, particularly in regions where finger millet is a dietary staple. By harnessing the natural symbiotic relationship between plants and beneficial bacteria, this biofortification strategy has the potential to enhance the nutritional value of FM and contribute to combating iron and zinc deficiencies in vulnerable populations. Further research and development efforts are necessary to fully understand the precise mechanisms involved, optimize the application protocols, and assess the long-term sustainability and effectiveness of bacterial endophytes biofortification in finger millet cultivation under field conditions. However, this approach holds great promise for promoting nutrient-rich crops and enhancing India's food security and nutrition.