Harnessing potential of Plant Growth Promoting Microbes (PGPMs) for zinc and iron fortification in Potato (*Solanum tuberosum* L.) and Lettuce (*Lactuca sativa* L.) for sustainable agriculture

A Summary Submitted to the
Babasaheb Bhimrao Ambedkar University, Lucknow
in fulfilment of Requirement for the Award of Degree of

Doctor of Philosophy

in Environmental Science

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

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(Enrolment No. -1155/18)

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2024

Summary

Malnutrition and hidden hunger pose significant challenges worldwide, affecting both developed and developing nations with widespread and devastating consequences. The recent implications of the global pandemic have underscored the urgent necessity for adapting our food systems to address deficiencies in the global food supply. Additionally, climate change projections pose an alarming scenario of increased inequality and heightened poverty, particularly in developing countries. In light of these challenges, there is a pressing need to enhance the nutritional content of Plant-Based Foods (PBFs). Biofortification emerges as a promising solution being not only sustainable but also cost-effective in the battle against malnutrition.

Potatoes and lettuce represent a substantial potential for biofortification to effectively address micronutrient malnutrition. Widely adopted as a dietary practices in numerous regions, they are highly regarded for their cultivation ease, modest land requirements relative to major crops, and efficient nutrient yield per unit area, time, and investment. Adaptable to diverse climates, including temperate, tropical, and subtropical zones, the production and consumption is high, particularly in developing countries. Beyond their role as a fresh vegetable, potatoes and lettuce exhibit notable industrial demand. With its intrinsic nutritional richness, potatoes and lettuce emerge as a cost-effective source of essential nutrients, surpassing various fruits and vegetables. Laden with carbohydrates, proteins, minerals, vitamins, and dietary fibers, they contribute significantly to a nutritionally balanced diet. While nutritional compositions may vary across varieties, the presence of vitamin B6, vitamin C, antioxidants such as polyphenols, carotenoids, anthocyanins, and flavonoids in potatoes and lettuce make them better option for biofortification as compared to cereal crops.

In the pursuit of sustainable agricultural practices and addressing global concerns about nutrient deficiencies in crops, this Ph.D. thesis delves into the comprehensive evaluation of plant growth-promoting microbes (PGPMs) as potential agents for enhancing the solubilization of essential nutrients, specifically zinc and iron. The study encompasses an extensive investigation involving eleven PGPM strains, with a focused aim to identify the most effective microbial candidates. The ultimate goal is to unravel the intricate connections between PGPMs, nutrient solubilization, and their impact on the growth and productivity of two vital crops, potato and lettuce. By exploring the symbiotic relationship between plants and these beneficial microbes, the research endeavours to contribute crucial insights into sustainable agricultural strategies that not only enhance crop yield but also address the critical challenge of nutrient biofortification. This summary encapsulates the key findings that emerge from a meticulous exploration of the interactions between PGPMs, carriers, and their influence on plant health, nutrient uptake, and soil quality.

The study's core hypotheses centered around anticipating a substantial increase in zinc and iron content within plants treated with PGPM, coupled with enhancements in yield, growth, and overall vegetable quality. The objective was to present a cost-effective and sustainable avenue for enriching the nutritional profile of vegetables, thereby contributing significantly to nutritional security in developing countries. The study encompassed a range of specific objectives, ranging from the isolation and characterization of beneficial soil microbes with zinc and iron solubilization capabilities to the development of a potential microbial formulation for fostering plant growth and nutrient solubilization. Additionally, our research delved into the creation of a carrier-based bioformulation with selected microbes, designed to enhance growth and nutrient levels in both potato and lettuce. The final phase involved a meticulous evaluation of plant productivity, crop yield, and iron and zinc bioaccumulation. This evaluation was carried out under controlled pot conditions and in real-world field settings, using the bioformulations meticulously prepared during the course of our study.

The study aimed to evaluate the efficacy of plant growth-promoting microbes (PGPMs) in enhancing zinc and iron solubilization in soil and their availability for potato and lettuce plants. Eleven PGPM strains were tested in pot trials, and the best-performing isolates were selected for further evaluation. The study included the assessment of single strains, consortia, and carrier-based bioformulations with talc and bentonite. The selected strains, including *B. altitudinis*, *T. flavus*, *B. siamensis*, *B. cereus*, and *A. faecalis* exhibited beneficial plant growth-promoting traits such as siderophore production and zinc solubilization. The study demonstrated that microbial inoculation positively influenced plant growth, productivity, and nutrient uptake, especially under zinc and iron supplementation. The use of consortia and carrier-based bioformulations further enhanced these effects. The findings indicated the potential of the selected PGPMs for biofortification, especially in regions where zinc and iron fertilizers are underutilized.

Additionally, the impact of PGPMs on photosynthetic pigments, antioxidant content, and biochemical parameters such as nitrate, soluble sugar, and protein was analyzed. The results showed that microbial inoculation increased chlorophyll content, total phenolics, and various biochemical components, indicating a positive influence on plant physiological processes. Furthermore, the study assessed the impact of PGPMs on soil enzymes revealing increased activities of alkaline phosphatase, dehydrogenase, and urease in PGPM-treated plots. This suggested improved soil health and microbial activity, emphasizing the broader positive effects of PGPMs on agroecosystems.

The key findings from the discussion of the Ph.D. thesis on evaluating the efficacy of plant growth-promoting microbes (PGPMs) in enhancing zinc and iron solubilization, plant growth, and nutrient uptake in potato and lettuce can be summarized as follows:

Eleven PGPM strains were tested for their impact on the growth, productivity, and zinc/iron enhancement in potato and lettuce. Out of these five strains (*B. altitudinis*, *T. flavus*, *B. siamensis*, *B. cereus*, *A. faecalis*) were selected based on their positive effects on plant growth and nutrient solubilization. *B. altitudinis* and *T. flavus* exhibited excellent activity in solubilizing zinc, while *B. siamensis*, *B. cereus*, and *A. faecalis* were efficient siderophore producers for iron solubilization. PGPM consortium, when applied with talc and bentonite, enhanced the availability of zinc and iron in the soil for plant uptake.

Inoculation with PGPMs, especially *B. altitudinis* and *T. flavus*, significantly improved plant growth, productivity, and yield in both potato and lettuce. Plants treated with chemical salts (zinc or iron) without PGPMs showed lower overall growth compared to those inoculated with PGPMs. PGPM inoculation, particularly with zinc supplementation, increased chlorophyll content suggesting improved photosynthetic efficiency. Total phenolics increased in inoculated plants, indicating enhanced antioxidant activity. Soluble sugar levels were also significantly higher in inoculated plants contributing to improved metabolic regulation.

Bentonite and talc were effective carriers for the PGPM consortium, enhancing physiological growth, nutrient mobilization, and biomass production in potato and lettuce. Bentonite outperformed talc in several aspects possibly due to its absorbent and conducive microenvironment properties.

PGPMs, especially *T. flavus* and *B. altitudinis* demonstrated potential for zinc and *B. siamensis*, *B. cereus*, and *A. faecalis* for iron biofortification in potato and lettuce. The microbial consortium, when applied with carriers further enhanced nutrient uptake, improving plant growth and yield. Soil enzyme activities (alkaline phosphatase, dehydrogenase, urease) were positively influenced by PGPM consortium application, indicating enhanced microbial activity and organic matter decomposition. PGPM consortium application improved soil health, organic carbon content, and enzyme activities in the field, contributing to sustainable agriculture practices. The use of bentonite and talc as carriers for PGPM consortium proved effective, providing a potential solution for widespread application in improving crop performance.

The study concludes that the application of selected PGPMs, either individually or in consortia, significantly enhanced plant growth, productivity, and nutrient uptake, particularly in the context of zinc and iron supplementation. The use of carrier-based bioformulations with talc and bentonite further improves the efficacy of PGPMs, providing a potential strategy for practical application in agriculture. The findings also highlight the positive impact of PGPMs on soil enzymes and overall soil health, indicating a holistic approach to sustainable agriculture.

Moreover, the study emphasizes the potential of the identified PGPM strains, including *B. altitudinis*, *T. flavus*, *B. siamensis*, *B. cereus*, and *A. faecalis* for biofortification purposes. These strains exhibited zinc and iron solubilization capabilities making them valuable candidates for regions facing nutrient deficiencies in crops. The study contributes valuable insights into the mechanisms of nutrient solubilization and uptake by plants through PGPMs paving the way for future research and practical applications in agriculture.

The research provides a comprehensive understanding of the multifaceted benefits of PGPMs in promoting plant growth, enhancing nutrient availability, and improving soil health. The findings have practical implications for sustainable agriculture practices, emphasizing the potential of microbial inoculants for biofortification and enhanced crop production.

The study suggests the potential of using PGPMs for zinc and iron biofortification in regions where fertilizers are underused or not cost-effective. Further research is needed to understand the complex interactions between different plant species, soil properties, and mineral element concentrations. Overall, bioformulations with PGPMs and carriers like bentonite hold promise for enhancing nutrient uptake, soil health and overall crop productivity.

In conclusion, the study highlights the significant impact of PGPMs on plant growth, nutrient solubilization, and soil health. The findings provide valuable insights into the potential application of PGPMs and carrier-based bioformulations for sustainable and efficient agricultural practices, with implications for addressing nutrient deficiencies in crops.

Future research should explore diversifying the application of bioformulations across a range of crops to achieve comprehensive nutrient enhancement Emphasizing thorough bioavailability analysis, especially concerning the consumption of biofortified potato and lettuce, is crucial for ensuring effective nutrient absorption. Long-term monitoring of soil characteristics and in-depth molecular studies to identify crop-specific metal transporters will further optimize biofortification strategies and enhance our understanding of sustained ecological impacts.