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Enhancing Zinc Content in Potato Using Microbe Assisted Biofortification Technique

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KEYWORDS

Zinc, Biofortification, *Potato*, ICP-MS, Microbial inoculants, Nutritional enhancement

ABSTRACT:

Introduction:

Aim

Zinc, the second most prevalent trace metal to iron in human body, is required in a variety of fundamental metabolic and physiological activities. We demonstrate that microbe-assisted biofortification is an effective strategy for increasing the zinc content of Solanum tuberosum L. (potato), a crop used to reduce dietary zinc deficiency globally. The scientific enquiry was aimed whether bio-inoculation with the zinc solubilizing bacterial consortium and zinc spray could effectively enhance tuber Zn concentration of potato along with increased vigor of plant.

Methodology

Potato plants received treatment with specific plant growth-promoting microorganisms, Bacillus altitudinis and Trichoderma flavus var. flavus, along with zinc. We measured the zinc content in the harvested tubers using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to standard trace mineral determination protocols.

Results

Microbe-assisted zinc biofortification significantly improved plant growth, biomass, yield, and tuber zinc concentration compared to untreated controls. Zinc levels increased from approximately 0.35% in non-biofortified potatoes to about 3.52%, representing a tenfold enhancement relative to USDA 2019 data.

Interpretation

The results demonstrate that microbe-assisted zinc biofortification is an effective, sustainable, and affordable strategy to improve the micronutrient content of potatoes. This approach has strong potential to combat hidden hunger, enhance nutritional security, and provide a scalable solution for populations reliant on potatoes as a dietary staple.

1. Introduction

Zinc is an essential micronutrient involved in a wide array of physiological processes, including immune function, enzymatic activity, wound healing, cellular growth and development, protein and nucleic acid synthesis, and regulation of gene expression (Jaiswal et al., 2022; Kumar, 2022)[1]. It is naturally abundant in a variety of plant- and animal-based foods. Additionally, zinc is often added to processed foods such as breakfast cereals and snack bars through fortification to prevent dietary insufficiency (Lo Dico et al., 2015)[2]. In low-income and developing regions where zinc deficiency is prevalent, biofortification has emerged as a promising strategy to enhance the zinc content in the edible parts of staple crops, thereby addressing micronutrient malnutrition at the population level (White et al., 2017) [3].

Zinc deficiency in these regions is largely attributed to monotonous diets dominated by cereals, which are inherently low in zinc content and bioavailability. Furthermore, modern cereal cultivars often lack sufficient zinc concentrations to meet human nutritional requirements (Thakur et al., 2022)[4]. This issue is compounded by the widespread presence of zinc-deficient soils-estimated to affect nearly 50% of wheat-growing areas globally-further limiting zinc uptake by crops.

Biofortification offers a sustainable solution through two primary approaches: genetic biofortification, which involves conventional breeding or genetic engineering to enhance zinc accumulation in crops, and agronomic biofortification, which relies on the application of zinc-containing fertilizers to improve zinc uptake and translocation within plants (Evrendilek GA et al., 2019)[5]. Both methods aim to improve the nutritional quality of food crops and contribute to long-term public health outcomes (Jaiswal et al., 2022). Biofortification techniques utilize biological processes throughout plant growth to increase the micronutrient content of the crops, whereas ordinary fortification refers to the addition of nutrients to food after harvest (Laurie et al., 2015)[6]. Among low- or middle-income countries, biofortification allows crops to naturally absorb, synthesize, and store vital elements within their edible tissues, providing an economical and sustainable way to address micronutrient shortages (Singh et al., 2022)[7]. In contrast to conventional fortification, biofortification may increase the nutrients' physiological efficacy and bioavailability because of their organic incorporation into the plant matrix (Thakur et al., 2022)[4].

The present study aims to determine the effectiveness of microbe-assisted biofortification in increasing the zinc content of Solanum tuberosum. This technique facilitates direct nutrient uptake, promoting their efficient transfer and accumulation in tubers (Jaiswal et al., 2022; Zhang et al., 2022)[8]. The approach seeks to combine modern biotechnological tools and advanced breeding techniques to develop crop varieties with improved nutritional density (Singh et al., 2022)[7]. This practice holds notable promise due to its minimal external inputs, long-term sustainability, and scalability across diverse agricultural systems (Kumar, 2022)[9]. Methods include agronomic biofortification (mineral fertilizer application), genetic biofortification (transgenic or marker-assisted selection), and conventional plant breeding to select nutrient-dense varieties (White et al., 2017;Vergara Carmona et al., 2019)[3,5].

Potatoes (Solanum tuberosum L.) hold significant importance in global agriculture due to their high nutritional content, yield potential, and adaptability across diverse agro-climatic zones (Campos & Ortiz, 2020; Friedman, 2006)[10,11]. Globally, over 20 million acres are cultivated annually, producing approximately 366 billion pounds, with peak production in temperate Northern Hemisphere regions during early frost conditions (Campos & Ortiz, 2020)[10,12]. Nutritionally, potatoes are a good source of vitamin C, vitamin B6, vitamin K, folate, and iron, with dietary fiber and micronutrient density notably enhanced when consumed with the skin (Mishra et al., 2020)[13]. Coloured potato varieties, rich in phytochemicals such as flavonoids, beta-carotene, anthocyanins, and phenolic compounds, offer additional antioxidant benefits, although thermal processing may reduce the bioavailability of certain compounds (Yang et al., 2016)[14]. Given their widespread consumption and role as the most consumed non-cereal staple globally, potatoes are considered a promising candidate for biofortification to address micronutrient deficiencies (Kumar, 2022)[9].

Potato germplasm exhibits significant variation in size, pigmentation, nutritional content, and environmental stress resistance (Campos & Ortiz, 2020). This diversity provides potential for selecting or engineering cultivars suitable for biofortification (Singh et al., 2022). Compared to cereal grains, potatoes exhibit significantly higher iron bioavailability. Jongstra et al. (2020)[14] demonstrated that over 70% of iron released from potatoes remained accessible at the intestinal absorption level. Although zinc absorption from plant-based diets is typically low, potato tubers display high zinc bioavailability due to favorable organic ligand content and fewer absorption inhibitors (White et al., 2017;Vergara Carmona et al., 2019)[3,5]. Therefore, potatoes could be a substantial dietary source of both iron and zinc (Singh et al., 2022). One promising strategy for enhancing zinc content in potatoes is priming tubers in zinc-enriched solutions (Kumar, 2022)[7]. In controlled

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experiments, varying zinc concentrations and exposure times significantly influenced zinc accumulation and bioavailability in tubers (White et al., 2017; Vergara Carmona et al., 2019). Globally, zinc deficiency affects approximately 17% of the population, with high prevalence in developing countries like India (Hefferon, 2019; Wessells & Brown, 2012, as cited in Jaiswal et al., 2022)[1]. Interventions such as dietary diversification, food fortification, and biofortification have shown success in addressing hidden hunger (FAO, 2017, as cited in Jaiswal et al., 2022)[1].

Biofortification remains a promising approach for mitigating widespread micronutrient deficiencies (Laurie et al., 2015). The development of zinc-enriched potatoes through agronomic fortification has emerged as a viable strategy to address zinc deficiency . Among various biofortification techniques, potatoes are especially suitable for genetic improvement due to their natural micronutrient variability (Singh et al., 2022). Identification of high-nutrient genotypes and specific genes can accelerate breeding or transgenic development of biofortified potato cultivars (Zhang et al., 2022). This study evaluates the effects of various biofortification strategies on yield and nutritional attributes of potato tubers, aiming to enhance dietary intake of essential micronutrients in vulnerable populations by naturally increasing micronutrient density during cultivation, biofortification offers a sustainable, cost-effective, and long-term strategy to combat hidden hunger and improve global nutritional security.

2. Objective

Determine the effectiveness of microbes assisted biofortification strategy in increasing zinc accumulation within *Solanum tuberosum* (potato).

3. Methods

The current investigation was carried out in the departmental laboratory of Era University, Lucknow, employing a stratified, multi-factorial experimental design with three replications. The objective was to assess alterations in the nutritional profile of *Solanum tuberosum* following zinc biofortification treatment using standardized analytical parameters.

Estimation of Zinc Content in Biofortified Potatoes Using ICP-MS

The zinc content in biofortified *Solanum tuberosum* (potato) was estimated using **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**, following standard analytical protocols suitable for trace mineral determination in plant-based samples.

1. Sample Collection and Preparation

Fresh biofortified and non-biofortified potato samples were washed thoroughly with deionized water to eliminate any soil or contaminants. The samples were sliced, oven-dried at **60°C** to constant weight, and ground into a fine powder using a non-metallic grinder. Powdered samples were stored in clean, airtight polyethylene containers until analysis.

2. Acid Digestion

A portion of **0.5 grams** of the dried powdered sample was accurately weighed and transferred into **Teflon digestion vessels**. To each vessel, **5 mL of concentrated nitric acid (HNO_3)** and **2 mL of hydrogen peroxide (H_2O_2)** were added. The samples were digested using a **microwave digestion system** under controlled pressure and temperature conditions (typically up to 180°C for 30 minutes), ensuring complete breakdown of organic material.

3. Sample Dilution

After cooling, the digested samples were filtered (if necessary) and diluted with **ultrapure deionized water** to a final volume of **50 mL** in trace-metal-free volumetric flasks. Blank and certified reference materials were prepared and digested similarly for quality control.

4. ICP-MS Analysis

Zinc quantification was performed using an **Inductively Coupled Plasma Mass Spectrometer (ICP-MS)**. The instrument was calibrated using multi-element zinc standards prepared in the same acid matrix. Zinc was detected based on its **isotopic mass (m/z = 66 or 68)**.

5. Data Interpretation

All analyses were conducted in triplicates, and results were expressed in **mg/kg dry weight**. Accuracy and precision were confirmed using recovery rates and standard reference material. This method offers high

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sensitivity and precision for trace zinc quantification in complex biological matrices like potatoes.

4. Results

The present study demonstrated that biofortification of *Solanum tuberosum* with zinc effectively resulted in a twofold increase in zinc concentration, alongside an improvement in overall crop yield. The application of zinc-based fertilizers significantly enhanced the accumulation of this essential micronutrient in potato tubers, indicating the efficacy of agronomic biofortification strategies. This approach serves as a **cost-effective, sustainable, and scalable solution** to address zinc deficiency in human diets, particularly in regions where micronutrient malnutrition is prevalent.

Unlike post-harvest fortification methods, agronomic biofortification enriches the nutritional profile of crops directly during cultivation, thereby reducing nutrient loss and enhancing bioavailability. This method supports long-term nutritional security by naturally increasing micronutrient density within edible plant parts, without altering the crop's genetic makeup. Moreover, it aligns with eco-friendly agricultural practices by promoting nutrient-rich food production at the source. The findings highlight zinc biofortification as a promising tool in integrated nutrient management systems and underline its role in improving both crop nutritional value and productivity in a sustainable manner.

Zinc content

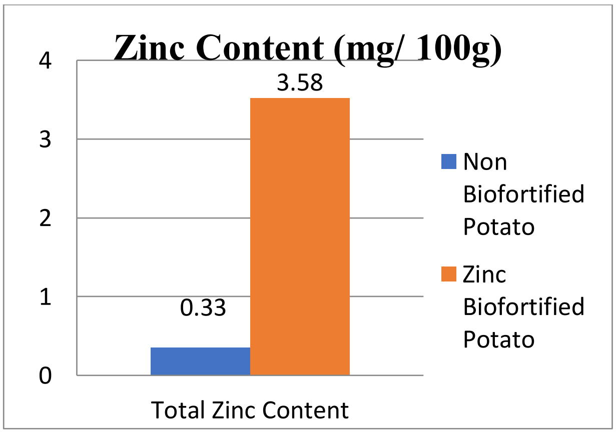

Fig1. Availability of zinc content

Based on the comparative analysis of the nutrient composition in ZBP (Zinc Biofortified Potato) and NBP (Non Biofortified Potato), the data highlights several significant differences across key parameters such as moisture, fat, fiber, ash, protein, carbohydrates, energy, and zinc content.

Comparative analysis of the nutrient composition of ZBP (Zinc Biofortified Potato) and NBP (Non Biofortified Potato), with a particular emphasis on zinc content. One of the most significant observations is the substantial increase in zinc concentration in ZBP. While NBP contains only **0.33% zinc**, ZBP shows a notable enhancement, recording **3.58% zinc**. This indicates a more than **tenfold increase** in zinc levels due to biofortification. Such a remarkable rise in zinc content underlines the effectiveness of fortification strategies aimed at addressing micronutrient deficiencies. Zinc is a critical trace element required for immune function, enzyme activity, growth, and development. Populations suffering from zinc deficiency, especially in developing regions, could benefit immensely from incorporating biofortified foods like ZBP into their diets.

The enhanced zinc presence in ZBP makes it a superior alternative to standard products, particularly for vulnerable groups such as children, pregnant women, and the elderly. In conclusion, the data clearly highlight that ZBP offers a significantly enriched source of zinc, which can play a vital role in combating nutritional deficiencies and promoting better public health outcomes through regular dietary intake.

Zinc biofortification has been shown to significantly enhance the zinc content in potatoes, making it an effective strategy for improving the nutritional quality of this staple crop. According to data from the USDA (2019), the zinc concentration in non-biofortified potatoes is approximately 0.35%. However, following the application of zinc biofortification techniques, this concentration can increase remarkably to around 3.52%, representing a tenfold enhancement. This substantial rise highlights the potential of agronomic interventions to address micronutrient deficiencies in human diets, particularly in regions where zinc deficiency is widespread. Zinc plays a critical role in immune function, cell growth, and enzyme activity, and its deficiency is associated with stunted growth and impaired immune response. By increasing zinc levels in commonly consumed crops like potatoes, biofortification offers a sustainable and low-cost solution to combat hidden hunger. Moreover, this method improves

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nutritional outcomes without the need for dietary changes or industrial fortification processes.

Statistical analysis

To assess statistical significance, all collected data were initially evaluated based on qualitative characteristics and subsequently analyzed using paired-sample t-tests. The results revealed that, apart from zinc content, all other nutritional parameters exhibited p-values less than 0.05, indicating statistically significant differences in mean values between zinc biofortified (ZBP) and non-biofortified (NBP) potato samples. This suggests that biofortification led to meaningful changes in most nutritional components. The low p-values confirm that the observed variations are unlikely due to chance, thereby validating the effectiveness of the biofortification process in enhancing the overall nutritional profile of potatoes.

Nutritional Content	**MEAN**		**STANDARD DEVIATION**		**t-test Value**
	NBP	ZBP	NBP	ZBP	0.000334
Zinc	0.33	3.58	0.02	0.13	

Table1- Statistical data presentation

The table presents a comparative statistical analysis of zinc content in Zinc Biofortified Potato (ZBP) and Non Biofortified Potato (NBP). The **mean zinc content** in ZBP is **3.58%**, whereas in NBP, it is significantly lower at **0.5%**. This reflects a substantial enhancement in zinc concentration due to the biofortification process.

In addition to the mean values, the **standard deviation** provides insight into the variability of zinc levels in both samples. ZBP has a standard deviation of **0.13**, while NBP shows a much lower variability with a standard deviation of **0.02**. This suggests that while ZBP contains a higher zinc level, there is slightly greater variation in its zinc concentration across samples, which is common in fortified products due to variability in absorption and distribution. A key statistical measure shown is the **t-test value**, which is **0.000334**. This extremely low p-value indicates that the difference in zinc content between ZBP and NBP is **statistically significant** at a confidence level well above 99%. Such a result confirms that the increase in zinc concentration is not due to random variation but is a consistent and reliable effect of the biofortification process.

Overall, the data strongly support the effectiveness of zinc biofortification. The marked increase in zinc levels, combined with the statistically significant difference, suggests that ZBP could be a valuable intervention in combating zinc deficiency. This enhancement is especially important for populations at risk of micronutrient malnutrition, reinforcing the role of biofortified crops in public health nutrition strategies.

Conclusion

This study scientifically evaluates the nutritional composition of *Solanum tuberosum* (potato) biofortified with zinc, in comparison to its conventional, non-biofortified counterpart. The findings reveal a notable improvement in the nutritional profile of zinc-fortified potatoes, underscoring the effectiveness of biofortification as a strategic intervention to combat micronutrient deficiencies and hidden hunger. These enhancements reflect the positive impact of zinc supplementation on the crop's nutritional value.

Zinc is an essential trace element involved in numerous physiological functions, including immune response, thyroid regulation, wound healing, blood clotting, and sensory perception. It also plays a critical role in growth and development during pregnancy, infancy, and adolescence. Recommended daily intake levels suggest 8 mg for adult women and 11 mg for adult men, with slightly higher requirements during pregnancy (11 mg) and lactation (12 mg).

Potatoes, being one of the most widely consumed non-grain staple foods globally, offer a practical and accessible platform for micronutrient biofortification. Their affordability and dietary prevalence make them ideal candidates for enhancing public health through nutrition-sensitive agriculture. The present findings validate that biofortifying potatoes with zinc can sustainably improve their nutrient density and contribute to addressing widespread nutritional inadequacies. Hence, zinc biofortification emerges as a promising approach to reduce hidden hunger and support better

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health outcomes, particularly in resource-limited populations.

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