Chapter-1

Introduction

Globally, food security is an emerging concern, and it creates a constant pressure on staple food crop to meet the global food demand. However, vital elements such as several micronutrients, minerals, vitamins, and amino acids are not available in adequate quantity in primary food crops, resulting in malnutrition among consumers [1]. The primary cause of malnutrition worldwide is the lack of many micronutrients, which causes a substantial influence on human nutrition. Micronutrient deficiencies are a major global health issue, especially in developing nations. Millions of people are adversely affected by these inadequacies, which have a detrimental effect on health and impede social and economic advancement. The inability to regenerate bodily tissues, the consumption of insufficient amounts of any nutrient, and insufficient energy intake are all considered signs of undernourishment [2].

Human need more nutrients than plants for their development and growth, and obtain the necessary mineral elements both directly and indirectly through the diverse diets that are made from different crops [3]. The edible sections of the crop intended for human consumption eventually absorb the mineral components from the soil and/or fertilisers added to the soil. However, it is predicted that almost two billion people are experiencing the insufficiency of micronutrients such as Fe (iron) and Zn (zinc), due to poor mineral element availability in soil and/or inadequate deposition of minerals in comestible components [4]. Micronutrient malnutrition affects people who rely on cereals-based food, because cereals like rice, wheat, and maize often have poor mineral content and low bioavailability. It is estimated that, approximately 820 million populations are suffering from hunger, 2 billion are malnourished, and another 2 billion are suffering from obesity worldwide [5]. The ever-expanding population also poses a threat to nutritional security in the forthcoming future [6]. For this, practically important and effective steps should be implemented, which will be beneficial for the humans, livestock as well as for environment [3]. These deficiencies also have devastating negative effects on health, economic productivity, and mental development. Globally, deficiencies in Zn, Fe, I, Se, Ca, and vit A are the most general insufficiencies of micronutrient and people are deficient in least one of these micronutrients [2].

More than 50% of people worldwide experience micronutrients deficiency, particularly those of zinc (Zn) and iron (Fe), as a result of their dietary dependence on staple crops, mainly rice, wheat, and maize. Among the top 40 risk factors for the burden of disease worldwide, Fe and Zn deficiencies are classified as the 16^{th} and 40^{th} primary causes, respectively. About 4% of child demises and worldwide disability adjusted life years are thought to be caused by zinc deficiency, while iron insufficiency is the major cause of global disability adjusted life years brought on by micronutrient deficiencies. Iron and zinc shortages are common in underdeveloped nations, notably in Sub-Saharan Africa, where diets are dominated by monotonous diet, which is high in mineral absorption inhibitors [7]. Despite the fact that humans only require trace amounts of micronutrients in their diets, still these nutrients are nonetheless crucial for the development and well-being of individuals. Amid top fifteen leading causes of disease globally, Fe scarcity considered as one of the major contributors, approximately 7.3% of all worldwide illness burden is caused by Fe deficiency [8].

Furthermore, soil micronutrient insufficiency has been recorded in several areas worldwide, which restricts crop and subsequently human nutrient uptake. Inadequate consumption of these micronutrients has unambiguously significant biological consequences since they are vital to human biological progressions. Additionally, production of low-quality, insufficient feed results in a substantial imbalance between supply and demand occurs for cattle as well. Consequently, nutritional guarantee is a matter of major relevance from the viewpoint of human and cattle health [9]. Researchers have documented deficiencies in micronutrients including iron, zinc, copper, boron, sulphur and manganese as well as macronutrients such phosphorus, potassium, and nitrogen [10].

The two essential nutrients for humans, Fe and Zn, are the most often deficient micronutrients worldwide. Zinc and iron deficiency causes a significant health issue that is prevalent in sub-Saharan African and South Asian nations [2]. Rapid rise in micronutrient malnutrition (hidden hunger) is predominantly due to the shortage in the micronutrients' bioavailability in staple food crops [11]. As of right now, 40% of expectant mothers and 42% of children (< five years) are anaemic, while 17% of the people worldwide are at risk for not getting enough Zn [12]. This in turn is due to the poor bioavailability of these vital micronutrients in agricultural soil. Zinc insufficiency is as high as 47 % and iron deficiency is up to 13 % in Indian soils [13]. [14] demonstrated 9.6% Zn deficiency in soils of Uttarakhand. Fe scarcity in the Uttarakhand state ranged between 0-5% with an average of 1.4%. Since the majority of Uttarakhand's soils have pH values below 7.0, there is relatively little Fe shortage in these soils. But as crops are grown continuously without being replenished with Fe, the amount of accessible Fe in these soils is decreasing. Although the availability of Fe is typically high in acidic soils, the plant cannot access it because of antagonistic relationships with Cu, Zn, Mn, and phosphate ions [14].

Currently, there is widespread recognition that zinc is among the vital trace elements required for diverse physiological functions, and it holds significant importance in the development, growth, and reproductive processes of individuals. Zinc performs the function of membrane preservative and is necessary for immune system functioning [15]. Several biological catalysts require Zinc as a cofactor, it is also assumed that enzymes also participate in the regulation and storage of insulin, protein synthesis, and repair of tissue. Additionally, zinc contributes in the production of thyroid hormones and the development of bones, and prostaglandin. In crop plants, zinc is necessary for a number of enzymes responsible for the breakdown of auxin as well as carbohydrates, synthesis of proteins, and membrane integrity. The trace element Zn is especially important in these processes. Additionally, zinc also contributes to pollen formation, fertilization, and chlorophyll production [16].

Whereas, Fe is a crucial element that participates in ETC and cytochrome as well as helping in many enzymes functioning. Additionally, the onset of numerous catalysts used for a variety of biological processes depends on iron, as well as for the production of proteins like haemoglobin and myoglobin that are responsible for oxygen transport inside the human body [13]. Its deficiency leads to detrimental effects on children and women; therefore, the lack of crucial nutrients in staple diets has drawn the attention of the scientific community. Numerous studies have emphasized the significance of adding these trace elements to food chains in order to reduce the higher risk of micronutrient (Fe/Zn) deficiencies in population [17].

This highlighted the necessity of raising incredibly nourishing food to achieve nutritional security. Researchers and regulatory authorities (Food and Agricultural Organization [FAO] and World Health Organization [WHO]) have been contrived to propose a variety of solutions to deal with Zn and Fe deficiency due to the magnitude of the issue and its adverse impacts on both well-being and the economy [15].

There are many approaches, including food fortification, dietary diversity, and medicinal supplements for overcoming the Fe/Zn insufficiency in food [18]. Chemical fertilisers are used to improve crop health and soil fertility, yet this practise lowers soil fertility, which has detrimental impacts on both human and ecological health, lowering soil fertility, not cost effective, and deteriorating soil nutrients as well as micro and macroflora. Researchers are actively working to replace synthetic fertilizers with biofertilizers or microbial-based fertilizers by taking into account all of these issues [19]. In fact, use of microbial approach enables the accumulation of specific nutrients in the consumable portion of specific crop, therefore, biofortification has been suggested as a possible method to combat deficiency of micronutrients [6]. It is widely acknowledged, that the biofortification of crops is the least expensive method for reducing ‘hidden hunger’ in the world. It is a highly hopeful method for increasing the proportion of micronutrients in the edible parts of crops without compromising yield [1].

The application of endophytes as biofertilizers may be a more effective and practical strategic way to uptake and transporting the micronutrients to the plant from the soil. Due to the fact that they live inside their hosts, endophytic bacteria can colonise plant roots without causing damage to the plants. They promote plant development, function as a biological control agent to naturally keep pests away from the host, and helps host in mitigating a variety of biotic and abiotic challenges [20]. Additionally, they can augment the uptake of nutrients and minerals from the soil, including phosphorus, sulphur magnesium, zinc, iron, and nitrogen, and transferring such nutrients will increase the host plant's growth and survival [21].

Researchers have long focused on improving the bioavailability of Zinc and iron in cereal crops like rice (*Oryza sativa*) [22], and wheat (*Triticum aestivum*) [23]. Despite its ranking as the sixth major cereal after popular cereals like rice, wheat, sorghum, corn, and pearl millet, *Eleusine coracana* (finger millet) does not receive the same level of attention or recognition, even though it also contains essential nutrients and can contribute to the growth and health of consumers. Finger millet, despite being one of the primary cereals with exceptionally high nutritional content, has been recognized and considered as a "super cereal" by the "United States National Academies." This acknowledgment highlights its significant potential to provide essential nutrients and contribute to the overall health and well-being of consumers [24]. Further, 'International Year of Millets' has been recognised by the United Nations for the year 2023. Regarding nutrition, finger millet has a greater micronutrient density than the two most common cereal grains in the world, i.e., rice, and wheat, and is significantly richer in minerals. In addition, the people of Uttarakhand also consume Ragi scientifically known as '*Eleusine coracana*' (Finger millet) and commonly called 'Mandua'. Though the ragi abundant in calcium but it is also not much enriched with zinc and iron contents, therefore, biofortifying the crop with beneficial endophytes will solve the problem of these micronutrient deficiency.

The consumption of finger millet leads to several health benefits as mentioned in Figure 1.1 [25].

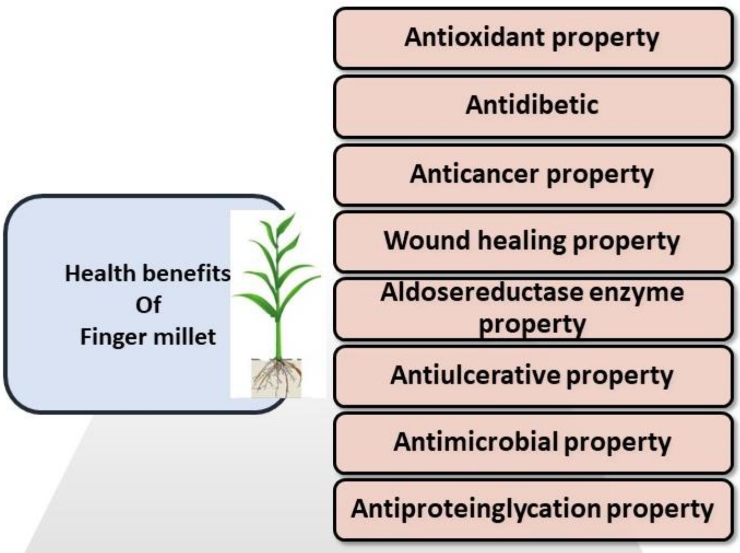

Figure1.1: Various health benefits of finger millet

Besides these health advantages, *Eleusine coracana* too has a low cost of production, as compared to cereals, and also accounts for diverse assurance in addition to food such as fiber, fodder, and environment, making ‘ragi’ the indispensable caretakers for agricultural guarantee.

In the process of biofortification, endophytic microorganisms use their machinery in delivering the micronutrient. This process is eco-friendly as well as cost-effective. Endophytic microbes enhance the micronutrient uptake via many processes like phytohormones production, production of siderophore, and mineral solubilization (zinc, iron, etc.) [19]. Endophytes have also been reported to solubilize phosphate, fixation of nitrogen, regulation of ethylene concentration, ACC-deaminase production, etc. [26].

On the other side, as a result of rising construction and industrial development, many soils used for growing crops are spoiled with harmful substances, endangering human health along with the entire food chain. Therefore, it is critical to enhance the vital nutrients' density and bioavailability while lowering harmful substances in grains' edible portions. For this, practically important and effective steps should be implemented, which will be beneficial for the environment as well as for humans [1].

Research Hypothesis

An approach for nutrient enrichment proposes several hypotheses to investigate the potential of bacterial endophytes in improving the bioavailability of iron and zinc in Finger Millet (*Eleucine coracana*). The central premise of the study is that specific bacterial endophytes, when introduced into the rhizosphere, establish symbiotic relationships with the plant, positively influencing nutrient uptake mechanisms. It is hypothesized that these endophytes play a pivotal role in solubilizing and mobilizing iron and zinc in the soil, thereby enhancing their availability for uptake by Finger Millet. The study also postulates that the selected endophytes contribute to improved nutrient translocation within the plant, facilitating the efficient transport of iron and zinc to edible plant parts, particularly grains. Furthermore, the research anticipates that Finger Millet grains cultivated in the presence of these bacterial endophytes will exhibit enhanced bioavailability of iron and zinc for human consumption, addressing nutritional deficiencies prevalent in communities relying on Finger Millet as a staple food. The study also explores the potential environmental sustainability and positive impacts on agricultural productivity associated with this approach, emphasizing the reduction of dependency on chemical fertilizers. Through rigorous experimentation and analysis, the thesis aims to validate these hypotheses, providing valuable insights into the application of bacterial endophytes as a sustainable strategy for nutrient enrichment in Finger Millet cultivation.

With the aforementioned in mind, the current investigation was planned. to use potential finger millet endophytic bacterial isolates as a source of the microbiological biofortification strategy. 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 (Figure 1.2). The specific objectives include:

- ✓ To screen finger millet bacterial endophytes for Fe and Zn solubilization.
- ✓ Screening of bacterial endophytes for plant-growth promoting attributes and selection of best plant growth promoting endophytes (PGPE) having Fe and Zn solubilization attributes. The selected endophytes will be identified using polymorphic techniques.
- ✓ To enunciate the mechanism(s) of such endophyte-host-interaction relative to Fe and Zn.
- ✓ To figure out the role if any, of bacterial endophytes in enhancing bioavailability of Zn and Fe in finger millet grains.

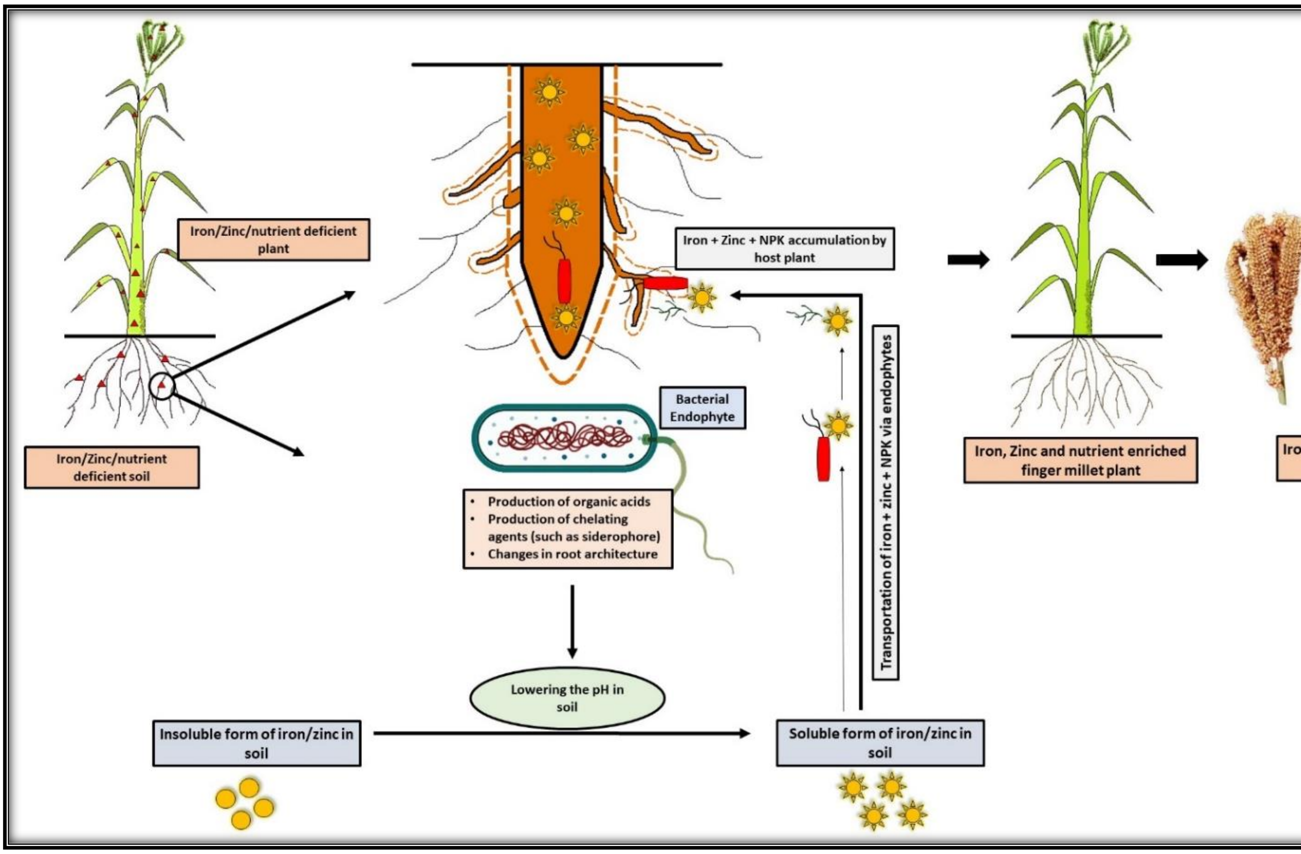

Figure 1.2: Graphical abstract illustrating that finger millet endophytic bacteria improve zinc and iron content, enhancing crop quality and consumer health.