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Role of Microbes in Biofortification of Crops

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With a growing global population (~9 billion by 2050), changing demographics and climate, pressure on natural resources, appearance of novel and more virulent pests and pathogens and changes in regulations with respect to the use of agrichemicals, food security is predicted to become increasingly vulnerable [1]. Thus, the pressure to produce sufficient nutritionally rich food in a sustainable manner has increased substantially. 'Sustainable Development Goals Project' (UN 2015) has identified 17 goals to transform our world emphasizing the goal to 'end hunger, achieve food security and improve nutrition and promote sustainable agriculture'. Nowadays, the undernourished population is about ~800 million people with the vast majority living in developing countries. It is assessed that malnutrition is responsible for the death of ~3.0 million children annually. Micronutrient deficiencies otherwise called hidden hunger is a global issue affecting ~2 billion people, where the intake of various vitamins and minerals (e.g. iron, iodine, selenium, zinc, folic acid and vitamin A) is too low for optimal health [2]. Nutritional deficiencies has an adverse effect on the individual's health and their economic prosperity. The annual economic cost of micro- and macronutrient deficiencies has been estimated at ~2 trillion US dollars. So, the major socio-economic challenge of this century is to improve the nutritional health. This enhancement of nutritional content in food is termed 'biofortification' which according to the World Health Organization (WHO) is defined as 'the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology' [3].

Perhaps, Golden Rice [4] is the most famous biofortification project using genetic modification. The benefits although clearly identified has faced much public opposition where it is perceived by many as the 'Trojan horse' for genetically modified crops. Golden Rice is still not commercially available [5] most probably due to genetic modification, which is anathema to many, especially in Europe. Above and beyond genetic transformation approaches to biofortification, other biofortification strategies, including initiatives such as those followed in programs by HarvestPlus [6] for the biofortification of rice, involving a combination of conventional breeding strategies for the exploitation of existing genetic variation in crop germplasm coupled with agronomic strategies, e.g. foliar and soil application of fertilizers [7].

More recently, research related to the role and application of soil microbes in the biofortification of crops has been fuelled by understanding the complex interactions between plant roots and microbial communities in the rhi-

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zosphere. These days, crop biofortification by bacterial- and mycorrhizal-mediated mobilization of the micronutrients iron (Fe), zinc (Zn) and selenium (Se) from the rhizosphere is gaining attention [8].

Application of plant growth-promoting rhizobacteria for the biofortification of crops

The rhizosphere is an important interface between plant roots and the soil, contributing to sustainable agriculture when the interaction between plants and beneficial bacteria is considered. About 35 years ago, Kloepper first described the role of plant growth-promoting rhizobacteria (PGPR) in plant growth and defence [9]. PGPR play a major role in the direct or indirect promotion of plant growth when associated with rhizosphere/plant roots. Biofertilization and phytostimulation are the direct plant growth promoter mechanisms, that simultaneously minimize the use of chemical fertilizers and promote plant growth, and bacteria with both biocontrol and biofertilization/phytostimulation properties to enhance nutrient supply and disease control in plants. The current scenario exemplifies work in the area of plant-microbe interactions has focused on the biofortification of staple crops using these PGPR. The WHO has acknowledged micronutrients which are essential to for proper functioning of the human body, i.e. selenium (Se), iron (Fe) and zinc (Zn), and making for significant portion of the current research on PGPR-mediated biofortification [10,11].

Selenium (Se) is a significant metalloid trace element in the human diet. Se is known to prevent cancer due to anti-oxidation properties. Seleno-proteins, *i.e*. proteins that contain a seleno-cysteine amino acid residue, facilitate adequate DNA synthesis, thyroid hormone metabolism and protection against diseases such as HIV, reproduction. Higher levels of Se can lead to seleno-toxicity, resulting in symptoms ranging from nervous system disorders to nail and hair loss and selenium levels that are too low can lead to inadequate levels of protection against radioactive damage to DNA, which can result in micronuclei formation and potentially cancer. Plant roots absorb Se as selenate, selenite or organo-selenium compounds such as selenocysteine and selenomethionine, but are unable to absorb metal selenides or elemental Se. Plant growth-promoting rhizobacteria (PGPR) have the potential to increase plant Se levels and subsequently benefit the Se status of humans and livestock in areas of the world which may be Se-deficient. Over the past two decades, a number of studies have examined the potential of PGPR to act as Se-biofortification agents. Studies revealed that Indian mustard (*Brassica juncea* L.) can be supported by PGPR to enhance selenium accumulation and volatilization In *B. juncea*, dimethyl selenide is the predominant volatilized form of Se which is 500 - 700 times less toxic than inorganic forms of Se making it a suitable vector for biofortification of the human food chain with Se. In the ash-derived volcanic andisol soil in Southern Chile, endophytic bacteria isolated from Se-supplemented wheat that shows potential for plant growth promotion, biofortification and biocontrol in wheat cultivation [12].

Iron (Fe) is undisputably one of the most important micronutrients in the human diet, the basis for the correct function of haemoglobin, and deficiency in iron can lead to anaemia. According to the World Health Organization (WHO), the only micronutrient deficiency that is prevalent in both the developing and developed worlds is Fe deficiency [13]. In developing countries, up to ~50% of pregnant women and ~40% of preschool children suffer from anaemia. Plants procure Fe through one of two strategies, conditional on whether the plant is monocotyledonous or dicotyledonous. In dicotyledonous plants, the reduction strategy, involving the secretion of H^+ and organic acids to acidify the rhizosphere which reduces Fe^{3+} to $Fe^{2+}$is predominant making it available for uptake into the plant. The chelation strategy, adopted by monocotyledonous plants, involves the secretion of phytosiderophores (e.g. mugineic acid) that can bind Fe^{3+} which is subsequently taken up by the root cells. Presumably, bacteria can also act to help plants acquire Fe as the enzymes involved in the mugineic acid pathway were deduced from sequence comparison with bacteria. These microbes can promote Fe acquisition in plants by inducing Fe-deficient responses, as the production of hormonal compounds similar to plant hormones, which microbes can produce autonomously. Adak., *et al.* [14] also observed increased acquisition of Fe through cyanobacteria inoculation in rice of ~13 to 46% compared to uninoculated controls. The potential for Fe biofortification in chickpea (*Cicer arietinum* L.), a major pulse crop has been highlighted by the recent intensive research on the biofortification of chickpea predominantly grown under semiarid conditions.

Zinc (Zn) is the only metal resident in all enzyme classes and characteristically the most abundant transition metal in living organisms after Fe. It plays a critical role in human health and strengthening of the immune system. Being involved in protein synthesis, metabolic homeostasis and modulation of gene expression it plays a critical role in male fertility with Zn deficiency resulting in inhibition of spermatogenesis and abnormal sperm production. The micronutrient concentration of the grain, especially in the case of Zn in wheat is largely reduced by processing. Reports reveal the potential of a PGPR consortium to increase Zn concentration in rice; the consortium, named 'BioPower', consists of two *Azospirillum lipoferum* strains, two *Pseudomonas sp*. strains and one *Agrobacterium sp*. strain [15]. Microbes have differential abilities to fix or solubilize nutrients within the rhizosphere for plant growth promotion.

Below ground symbiotic associations between with AMF that supplies the host plant with water and mineral nutrients is common in most plants, including all major food crops. Mycorrhizal colonized plants can efficiently acquire nutrients from a larger soil volume, beyond the nutrition depletion zone. Thus, promotes the plant growth increasing its productivity. In return, the host plant provides AMF with carbohydrates required to complete its life cycle. The AMF acquire essential nutrients such as P, Zn, Cu, Fe, N and K from the soil, and thus AMF contact with roots provides the plant with access to essential nutritional elements, leading to solubilization, mobilization and uptake of the essential nutrients needed by plants for their growth [16].

CONCLUSIONS AND FUTURE PERSPECTIVES

The global threat of food security, eradication of world hunger and improved nutrition can be addressed by crop bio-fortification. Aside from genetic modification, other successful strategies including conventional breeding strategies, use of natural germplasm diversity, foliar application of micronutrients and/or the exploitation of the natural microbial diversity in soils, i.e. plant growth-promoting microbes (PGPM) most especially bacteria and mycorrhizal fungi have been identified for crop bio-fortification. The proliferation of PGPM throughout agriculture is environmentally safe and cost-effective. Biofortification of crops using foliar and soil application of micronutrients is challenging particularly in relation to Fe has been the low uptake of the micronutrient post-application. Proper elucidation relating to regulatory mechanisms in the plant-microbe micro-/macronutrient cycling pathways and their reciprocal interactions is essential to resolve to the best for formulation of appropriate treatments to specific soil/climate/temperature/crop scenarios. To understand the role of microbes in bio-fortification identifying changes in gene expression during microbial interactions by using genomics is critical. Investigations to study the influence in the nutritional content of economically important crops due to microbes; identifying appropriate microbial strains; exploiting synergistic microbial activity, e.g. between AMF and other beneficial bacteria; and assessing constant field efficacy under different environmental regimes, to ensuring adequate uptake by plants and improving crop quality are of extreme importance. The problem of hidden hunger can be alleviated to a considerable extent by delivering essential nutrients using beneficial microbes naturally found in soils across the planet, such as AMF and PGPR and can provide a promising sustainable agricultural strategy for improving current crop micronutrient content and in developing future bio-fortified crops.

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