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Review Article

IRON FORTIFICATION IN LEAFY VEGETABLES: PRESENT STATUS AND FUTURE POSSIBILITIES

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ABSTRACT

Iron is an essential micronutrient, which carries oxygen in the blood. Iron deficiency anemia is a worldwide health problem, especially for women and children. Vegetarian people require 1.8 times more iron compare to non-vegetarian people. Dark green leafy vegetables (GLVs) are the valuable sources of iron, even better on a per calorie basis than meat. Many local, wild, and underexploited GLVs are the good supplier of iron and other valuable micronutrients. They is short duration in nature and accumulate the higher amount of biomass within small life periods without much care and precaution. An increase in iron content of up to 3 mg/100 g was obtained in some biofortified leafy vegetable. This was about 40 times higher than the traditional leafy vegetable. The best ways for increasing iron status and its bioavailability in daily diet in commonly grown leafy vegetables are through agronomic practices such as increasing iron levels through supplementation of iron-containing fertilizer, biofertilizer or microbial inoculants, and breeding approaches such as identification, development, and promotion of iron-rich varieties. This will help in reducing the emerging iron deficiency anemia among the women and children throughout the world.

Keywords: Iron deficiency, Green leafy vegetables, Iron biofortification.

INTRODUCTION

Iron is one of the essential micronutrients and the central part of hemoglobin, which carries oxygen in the blood. It plays a vital role to catalyze oxidation/reduction reactions. Iron also serves as a prosthetic group in proteins to which it is associated either directly or through a heme- or an iron-sulfur cluster. Iron is found that all plant organs include root leaves flowers, fruit, seeds, and storage organs such as tubers. In different food items, iron is available in two forms, heme- and non-heme iron. Heme iron, which makes up 40% of the iron of non-vegetarian diets such as meat, poultry, and fish, is well absorbed. Non-heme iron constitutes 60% of the iron in animal tissues, and all the irons in plants (fruits, vegetables, grains, nuts, etc.) are not absorbed properly. The vegetarian people require 1.8 times more iron than non-vegetarian people as they have to depend non-heme iron diet to fulfill iron demand; therefore, selection of iron-rich diet and better bioavailability techniques are crucial to meet the demand. Recommended dietary allowance for iron is 8 mg/day for adult men as well as for post-menopausal women, whereas it is 18 mg/day for pre-menopausal women. Throughout the world, iron deficiency anemia is a serious health problem, especially affecting the women and children. Even the WHO admitted that iron deficiency is one of the most prevalent nutritional deficiencies in the world today particularly for vegetarian women of reproductive age [1]. However, to combat iron deficiency and to improve iron absorption, selection of diet rich in iron is very important as well as techniques for better iron absorption and bioavailability can have a great potential for improving the iron status of women and children.

SCOPE OF IRON FORTIFICATION IN LEAFY VEGETABLES

Green leafy vegetables (GLVs) have occupied a unique place in our daily diet because of their color, flavor, and health benefits. They are rich in several nutrient elements, particularly iron, zinc, calcium, magnesium, etc., and the cheapest source of antioxidants, vitamins, and dietary fibers. Most of the leafy vegetables are short duration crop and accumulates the higher amount of biomass within small life periods. They can be grown throughout the year even on homestead areas. GLVs can be used as natural fortificants of iron as they are rich in iron among the vegetables [2]. However, in some cases, the presence of certain antinutritional factors or inhibitors the bioavailability of iron gets reduced.

To overcome the iron deficiency, existing iron-rich GLVs need to be characterized and promoted for wider use based on their iron availability (Table 1). Of which, *Amaranthus* (25.50 mg/100 g), fenugreek leaves (16.50 mg/100 g), palak (16.20 mg/100 g), spinach (15.50 mg/100 g), and basella (10.50 mg/100 g) were the commonly grown leafy vegetables throughout the India.

Hence, emphasis should be given to explore underutilized leafy vegetables where a higher amount of iron can be harvested. To enrich iron in common leafy vegetables, it can be fortified in the cultivated varieties through agronomic practices or breeding approaches. To increase desired iron content in leafy vegetables, development of iron-rich varieties should be given top priority. The main challenge of iron fortification is to remove the associated antinutritional factors, namely, phytic acid, tannins, and phenolic compounds. Utilization of conventional breeding in association with molecular techniques can help to overcome the barriers related to iron fortification.

STRATEGIES OF IRON FORTIFICATION

Agronomical strategies for biofortification

Adoption of iron management strategies has a positive impact on the concentration of iron in edible tissues of different crops.

Relation to soil condition for iron uptake

Under acid soils, the bioavailability of iron gets enhanced as the organic acids of soil mobilize the iron transportation and solubility. Under alkaline and calcareous soil conditions, bioavailable iron concentrations get reduced. To fulfill the iron demand, plants need to mobilize iron in the soil by rendering it more soluble before they are able to take it up into their roots. Two effective Fe-acquisition systems known as Strategy I and Strategy II have evolved in higher plants, based on reduction and chelation of Fe^{3+}, respectively [4,5]. The group of Strategy I plants includes all dicotyledonous and all non-grass monocotyledonous plants. They acidify the soil, reduce Fe^{3+}, and take up divalent Fe^{2+} via specific divalent metal transporters [6,7].

Soil application of Iron fertilizers

Application of iron-based fertilizers and/or improving the solubilization and mobilization of iron in the soil will be helpful to enhance the iron

Table 1: Iron content of some commonly grown leafy vegetable crops

Crop name	Iron content (mg/100 g fresh weight)
Amaranthus (*Amaranthus* sp.)	25.50
Basella (*Basella alba*)	10.50
Celery (*Apium graveolens*)	6.30
Chinese cabbage (*Brassica chinensis*)	0.60
Chow chow leaves (*Sechium edule*)	0.60
Colocasia leaves (*Colocasia esculenta*)	0.90
Cowpea leaves (*Vigna unguiculata*)	20.10
Drumstick leaves (*Moringa oleifera*)	7.00
Fenugreek leaves (*Trigonella foenum-graecum*)	16.50
Kale (*Brassica oleracea* var. *acephala*)	1.60
Lettuce (*Lactuca sativa*)	2.40
Palak (*Beta vulgaris* var. *bengalensis*)	16.20
Pumpkin leaves (*Cucurbita moschata*)	2.10
Spinach (*Spinacia oleracea*)	15.50
Water spinach (*Ipomoea aqatica*)	3.10

Source: [3]

status of plant and bioavailability of iron. Soil application of iron fertilizer improves the available iron content of soil in iron deficient regions. The most commonly used iron-containing fertilizers for soil application is ferrous sulfate heptahydrate ($FeSO_4\ 7H_2O$) which is applied at 2-2.50 kg/ha before sowing of seed at the time of land preparation. However, application of common iron-containing fertilizers in soil is usually not very effective and throws a challenge as it rapidly becomes unavailable to plant roots through adsorption, precipitation, and oxidation reactions. To overcome the problem, chelated iron-containing fertilizers such as Fe-ethylenediaminetetraacetate (EDTA) are recommended (5.0 kg/ha).

Foliar application of iron fertilizers

To improve the iron availability of plants, foliar application is most commonly practiced. Soluble iron-rich fertilizers such as ferrous sulfate heptahydrate ($FeSO_4\ 7H_2O$– 0.5 g/lit water) or Fe-EDTA (Chelated iron-1 g/lit water) can be sprayed on the foliage for translocation to edible tissues for enhancing the iron content of the leaves. However, foliar applications of iron fertilizers are preferred in iron deficient soils for easy uptake and availability, as iron is not readily translocated within plants.

Application of elemental sulfur

Iron availability of plant can be further increased by applying elemental sulfur. This has the added benefit of crop sulfur fertilization. Some chemical compounds such as phytic acid can precipitate iron and act as antinutrient and decrease the bioavailability.

Besides GLVs, staple food crops such as wheat, maize, rice, sorghum, and beans can also be fortified with iron, and other minerals such as zinc, selenium, and iodine through agronomical approaches when grown in trace-element deficient soils, as has been practiced for low-zinc soils in Turkey and India and low-selenium soils in Finland.

Breeding strategies for biofortification

Adoption of conventional and transgenic breeding strategies needs to be exploited to produce edible products enriched in bioavailable iron. A systematic approach based on the identification of iron-rich genotypes among the cultivated varieties of commonly grown GLVs, collection of diverse germplasm of cultivated GLV rich in iron from the different geographical area, isolation of underexploited leafy vegetables rich in iron, exploitation of wild relatives and sister species for iron enrichment. Breeding approaches by crossing the high iron-containing varieties with the locality adaptive varieties can enhance the iron content of the leaves in existing crops. Utilization of mutation breeding for improving iron content can be examined for easy access in GLVs. Transfer of iron-rich gene from other crops or wild relatives can be made through transgenic breeding. Utilization of molecular markers that are closely linked with the traits of interest can be followed directly with molecular polymerase chain reaction and sequencing technologies. However, the growing challenges have to be overcome through better understanding of the physiological mechanisms of iron in plant system [8]. In transgenic breeding iron, coded genes are inserted into the seeds or plants of well-established varieties. Breeding initiative on iron enrichment of GLVs is under way.

RESEARCH ACHIEVEMENTS IN IRON BIOFORTIFICATION

Reduction of phytic acid content in iron-rich diet

Phytic acid is an important antinutritional factor that reduces the bioavailability of iron in the vegetable-based diet. In plant, it accumulates as phosphorous and iron complex metabolites. Even though the desirable amount of phytic acid is essential for human health as it has health-promoting effects on the immune system and in preventing kidney stones, research evidence suggested that the prevalence of phytic acid in the plant-based diet is believed to contribute to the high rate of iron deficiency and anemia. Phytic acid content of plant tissues can be reduced by disrupting the biosynthetic chain and biochemical pathways. Several mutant lines have been identified in various plants species including soybean [9,10], maize [11], wheat [12], rice [13,14], and *Arabidopsis* [15,16] where phytic acid content have been reduced to a considerable extent. The drawback of the conventional breeding method on phytic acid reduction is associated with poor germination and reduced seedling growth. Research work is under progress to create better mutants which can suppress the activity of genes that are responsible for phytate synthesis.

Augmentation of ferritin content

Ferritins are major iron-containing proteins that store iron for short-term and long-term use. Ferritins exist in all organisms as a store of iron. Ferritins in general and ferritins in plant food items provide a high iron bioavailability [17-19]. Ferritin genes were used in biofortification approaches. For example, leguminous ferritin genes, especially from soybean and bean, were overexpressed in plants, and subsequently, an accumulation of ferritin protein was observed in the plants. Ferritins from legumes had been used since this plant family contains high ferritin levels in seeds, and the legume seeds serve in human and animal nutrition. Overexpression of ferritins in seeds and cereal grains resulted in increased iron content in these edible parts [20,21]. More research on ferritin overexpression is required for the GLVs to increase iron uptake and availability by the plants.

Increase of nicotianamine content of plants

Nicotianamine is a non-proteinogenic amino acid derived from S-adenosyl methionine by the action of the enzyme nicotianamine synthase. Nicotianamine is able to bind a number of different metals including ferrous and ferric Fe, depending on the pH environment. Nicotianamine ensures that Fe remains soluble inside the cells. Thus, Fe can be transported to the multiple compartments, and Fe toxicity effects are reduced. Nicotianamine contributes to all important sub-processes of plant metal homeostasis: Mobilization and uptake, intercellular and intracellular transport, sequestration, storage, and detoxification of metals. Several studies presented positive effects of nicotianamine on Fe uptake and accumulation in seeds [22]. Therefore, nicotianamine can be considered to be a potential biofortification factor for iron uptake in plants. It was also found that nicotianamine synthase overexpression could result in increased levels of iron in leaves but not consequently in seeds.

Limitations and challenges of iron fortification

The research work on iron fortification is going on few GLVs in some selected research institutes, and till date, the success rate is far below the expected level. There are certain limitations that hinder the large-scale use of iron fortified crops for the benefit of women and children.

- Very limited information is available to understand the physiological

mechanism of iron uptake in leafy vegetables
- The desired iron enrichment in most of the fortified leafy vegetables was much lower than the actual demand
- Iron fortification associated with antinutritional factors such as phytic acid, tannins, and phenolic compounds which are difficult to remove from the plants
- Some of the iron-fortified leafy vegetables are not very high yielder to be acceptable by the farmers
- Agronomic biofortification sometimes accumulates more amount of iron from soil which may be toxic for human consumption
- Iron fortification through plant breeding is still underexploited
- Desirable gene transfer sometimes linked to undesirable characters
- Implementation of transgenic biofortified plants has regulatory hurdles, which prevents commercial cultivation.

CONCLUSION

Iron enrichment in common GLVs will increase the iron status and its bioavailability in the daily diet which will help in reducing the emerging iron deficiency anemia worldwide. However, the biofortified GLVs will be more acceptable if the fortified leaves contain higher iron density than the others. Again on regular consumption of fortified leaves, the iron status of the consumer needs to be improved. Then, only the growers will be encouraged to grow and adopt the fortified varieties or fortification technologies for the benefit of those suffering from iron malnutrition. More awareness needs to be created to consume iron-rich GLVs, particularly for women and children.

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