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Alleviation of Iron Deficiency in Plants, Animals and Humans through Biofortification: A detailed Review

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Abstract:- Iron (Fe) is essential for all biota and plays main role in various processes of photosynthesis, DNA synthesis and respiration. Iron (Fe) deficiency is a common metabolic deficiency worldwide, affecting an estimated 3.7 million individuals worldwide. Presence of Fe in food plantations is a major worldwide issue because of limited iron in routine intake food. Mostly, Fe is deficient in Pakistani soil and causes significant decrease in yield. This deficiency can be overcome through various regulatory mechanisms in plants that will fulfill the requirements for animals and human beings indirectly. Biofortification is one of the most effective technique to tackle the deficiency of Fe. In biofortifying various types of bacteria, fungi and other useful microorganisms are applied to soil for increasing nutrients uptake especialy iron. These rhizosperic inocula release siderophores which are responsible for nutrients releasing from the soil surfaces. Biofortification have various types like agronomic biofortification, genetically fortification and through microbes. Among these use of microbes is an effective and newly evolved technology that can eliminates diseases like anemia in pregnant womens and school going children indirectly through plants. This review study explained that Biofortification looks to be a viable method for underfed people in remote rural locations, bringing naturally fortified food items to individuals who lack access to commercially marketed goods, which are more widely available in urban areas.

Keywords:- *Iron deficiency, Anemia, Biofortification, Siderophores, Microbes.*

I. INTRODUCTION

1. Iron (Fe)

The 26th element of the periodic table is iron, having a molecular weight of 55.6. Ferrous (Fe2+) and iron (Fe3+) are the often occurring aqueous oxidation states. These states permit iron to take up or provide electrons to oxidation/reduction reactions which are crucial to energy metabolism. This characteristic allows free iron to catalyse oxidative processes, creating reactive molecules that are potentially harmful. Body iron should thus be chemically bonded and have a minimum of opportunity for free ion-iron to catalyse damaging oxidative processes in order to assist proper physiological activity, transit and storage. [1].

The majority of iron in the body occurs in hemo associated proteins including haemoglobin and myoglobin oxygen. When oxygen attaches to the iron atom in hemoglobin, the protoporphyrin and histidin residues stabilise oxygen in a Fe2+ state of oxidation. The iron in hemoglobin, which circulates in blood erythrocytes, quickly binds and releases oxygen. Myoglobin is a single molecule of haem and globin that permits transportation of oxygen from erythrocytes to muscle cytoplasmic mitochondria.[1]. There it involved in electron allocation, oxygen use, and ATP synthesis, smaller amounts of iron in the heme form are used. A tiny amount of body iron is used by heme-containing hydrogen peroxidases like catalase, which catalyse the conversion of hydrogen peroxide to hydrogen and oxygen and so guard against excessive hydrogen peroxide accumulation [1].

In non-heme proteins with an iron–sulfur link, iron also plays an essential role. It is the most prevalent form of iron in mitochondria, and it participates in various energy-metabolizing enzymes Aconitase is affected by iron levels in the mitochondria and cytosol. The aconitase enzyme develops the complete iron–sulfur cubic structure associated with metabolic activity when iron is abundant. The protein lose its aconitase activity and changes into an iron absorption molecule when iron levels are low [1].

1.1. Iron regulation mechanism

The body of a newborn baby contains approximately 250mg. An adult male's total body iron level ranges from 3 to 4g. The woman, on the other hand, has only 2 to 3g of iron in her body. This disparity is due to women's lower iron, lesser haemoglobin concentrations. Around two-thirds of this is used as functional iron. The rest is found in the form of ferritin (20%) and hemisoderin (10%) in storage [2].

Iron absorption management is certainly essential due to the lack of a controlled route of iron excretion. After food has been consumed and digested, dietary iron is absorbed mostly in the duodenum and proximal jejunum. Haem iron is absorbed more efficiently than non-haem iron due to endocytosis of the whole iron–protoporphyrin combination at the enterocyte brush border. Iron enters a common intracellular pool after digestion, from where it is reserved as ferritin, depending on the iron status of the individual. Ferroportin transports ferrous iron, which is rapidly oxidised to Fe3+ by transferrin and transported to cells having transferrin receptors.

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1.1.1 Regulation in plants

Iron (Fe) is a nutrient that is required by nearly all living organisms. As a component of numerous important enzymes, notably the cytochromes of the electron transport chain, it is necessary for a broad range of biological processes. Fe is necessary for the construction and operation of chloroplasts in plants, as well as for chlorophyll production. [3]. Fe is the fourth most prevalent element in the lithosphere and is found in large concentrations in soils, although itis less available in high pH soil. Fe is found mostly in Fe (111) form in aerobic soils, mainly as a component of oxyhydroxide with low solubility.

Young leaves with internal veins chlorosis and reduced root growth are visible signs of insufficient Fe feeding in higher plants. Because of the low redox potentials in wet soils, Fe solubility can rise by several orders of magnitude [4]. Excess uptake of Fe amounts in such circumstances, making it potentially hazardous. It increases the generation of reactive oxygen-based radicals that can cause lipid peroxidation, which can harm critical biological elements such as membranes i.e bronzing [5]. Iron readiness is thought to have an impact on species natural distribution [6, 7] and may hinder the growing of commercially important crops [8]. Plants must adapt their absorption mechanism to the environment in order to keep an appropriate Fe status. Because there is no evidence for a secretory pathway for Fe, the regulation of Fe absorption is the primary mechanism for maintaining Fe homeostasis. To avoid excessive absorption, wetland organisms have adapted mechanisms for oxidising ferrous Fe in the root system (i.e. formation of aerenchyma and release of oxygen).

1.1.2 Regulation in Animals and Humans

In the transfer of oxygen, iron acts as haemoglobin. It is an important part of enzymes engaged in biological mechanism, such as cytochromes and others, in cellular respiration [9]. Fe is a component of the haeme of haemoglobin (Hb), myoglobin, and cytochromes, as well as succinate dehydrogenase [10]. Iron is a cofactor for a variety of enzymes involved in neurotransmitter synthesis and is essential for normal myelination of the spinal cord [11]. Iron is involved in neurotransmitter synthesis and packing, as well as their uptake and breakdown into other iron-containing proteins, all of which can affect brain function directly or indirectly [12, 13] Fe is required for the synthesis of haemoglobin and is a pro-oxidant required by bacteria for growth [14]. Phosphate diets promote iron intake, but phosphate rich diets in high quantity reduce iron absorption by generating insoluble iron phosphates. The level of plasma iron is regulated by adrenocortical hormones (glucocorticoids). Regardless of the cause of stress, when the hypothalamus, adenohypophysis, and adrenal gland are stimulated, plasma iron decreases [15]. The ferrous form of iron is more soluble and digested than the ferric form. By generating iron phytate and iron oxalate, phytic and oxalic acids reduce iron absorption. Anemia is a sign of a deficiency condition (hypochromic, microcytic). Fe deficiency has been connected to developing brain as well as the pathophysiology of restless leg syndrome. [16, 17]. In addition, Fe deficiency is linked to changes in a variety of metabolic processes that can affect brain function [18]. The pig is born with limited iron and will develop anaemia if it is not given additional iron. Piglets develop anaemia as a result of their less iron stocks at birth [15]. Other species, such as dogs, cats, cattle, and rats, can develop iron deficiency anaemia at birth, but it is more severe in pig.

1.2 Mechanism of iron Enhancement:

Siderophore secretion (non-protein amino acid) (found in the Gramineae family) and soil chelate isolation (reduction of trivalent iron ($Fe3+$) to bivalent iron ($Fe2+$) by proton permeability) are the two mechanisms by which plants in aerobic soils access iron compounds (found in other monocotyledon and dicotyledons plants) [19, 20]. The first technique involves the secretion of a complex chemical known as phytosiderophores, which when combined with iron in the surrounding roots generates solution iron compounds. Gramineae (grasses), which also include main agronomic crops like barley and wheat, may effectively take iron by discharging phytosiderophores into the rootzone through their roots [21]. Iron availability and absorption can be improved by chemical compounds generated by rhizosphere bacteria. These microbial siderophores can be used by plants to absorb iron, such as oats.

All of these compounds, which may be naturally in soils, leaves, fresh water, residues, and the ocean, are generated by rhizospheric bacterial inocula with low dietary needs [22]. In adding to this, iron chelate can be formed spontaneously through the breakdown of organic waste by microorganisms [19, 23]. The second approach includes proton leakage and material resurrection by a plant that transforms trivalent iron ($Fe3+$) to divalent iron ($Fe2+$) (with more solubility).

This strategy is used by other monocotyledon and dicotyledon plants. Higher plants undergo physiological and morphological modifications that allow them to convert extracellular $Fe3+$ to $Fe2+$ in response to intracellular iron levels. This fundamental change will check the effectiveness of ion transport by plant species. Plant roots absorb divalent ions ($Fe2+$) following complex fragmentation and metal ion emission. Some or all of the iron revival is due to the chelate reductase enzyme [19, 23].

The pH of the rhizosphere affects nutrient delivery and organism activity significantly. The pH of the rhizosphere can vary by more than two units of soil mass based on the nutritive status of the plants, soil buffering capacity, and plant age. Variations in cation and anion absorption, as well as nitrogen metabolism, can induce an acidic reaction in the root region, by increasing protons. Through the weakening band of iron and oxygen, this proton can change the amount of iron oxide solution. Iron stress also stimulates $Fe\ 3+$ reductase enzyme activity, in addition to promoting proton leakage [19,24,25,26]. In response to proton leakage, the activity of the ATPase pump in the cucumber root membrane rose twice in iron deficiency [27].

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Organic compounds such as resurrection sugars, amino acids, phenols, and organic acids are released as energized or deactivated chemicals from plant roots. Although hereditary factors contribute to leakage, environmental factors may have a greater impact on the volume and content of leakage than genetic factors [28]. Abiotic factors and presence of microorganisms all impact the volume and content of leakage materials.

In the presence of a nutrient deficiency (especially iron and phosphate), phenols and organic acids leakage may be enhanced. By acidifying the rhizosphere and reviving Fe3+, root leakage has a direct influence on the availability of micronutrients for plant roots. Riboflavin is produced by some plant species and helps iron absorption [29,30,31]. Because of the low redox potential, iron solution concentrations in flooded soils may be many times higher. Plants may be exposed to high amounts of iron in these settings, which can be harmful to them. Iron poisoning appears in brown plant tissues, black roots, and soft roots [32,33].

Plants undergo several morphological and physiological changes as a result of iron deficiency. Formation carrier cells those can serve as a technique for improved nourishment transfer in the apoplast-symplast band. These cells are with significant secondary wall growth that extend into lamina cells at a greater level than volume. These are liable for enhanced proton emission and revival movement in low iron roots.

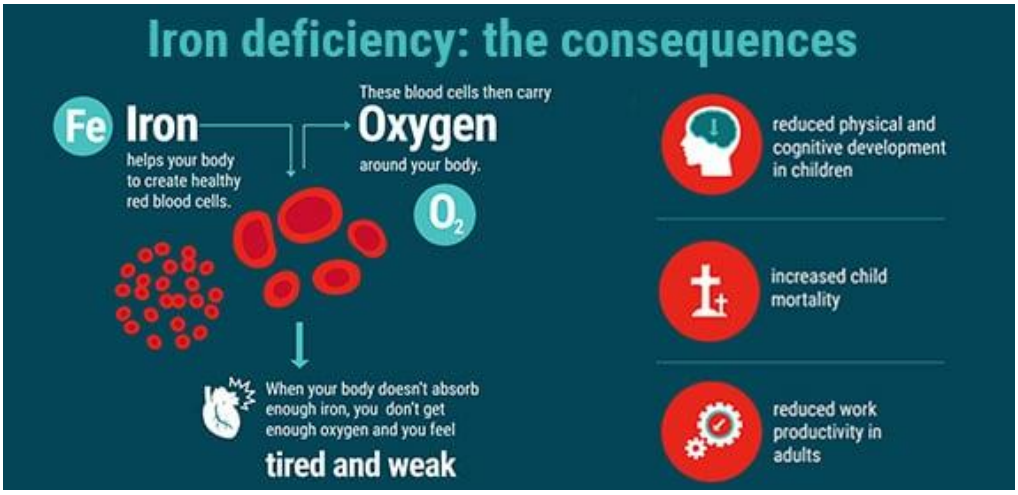
1.3 Iron Inhibitors:

Iron in soil is the fourth most abundant metal on the globe, but its availability to plants and microorganisms has been limited due to the poor solubility of iron-containing minerals in many parts of the world, particularly in arid regions with alkaline soils. Iron is commonly present in aerobic soils in the form of trivalent (Fe3+), which has poor solubility and, in most cases, is insufficient to fulfil the demands of plants. Because most soils have a high pH and are also calcareous, micronutrient solvability is low, resulting in reduced absorption of these elements and, as a result, an increase in plant needs for these elements [34,35,36]. Uneven use of phosphatic fertilizers on soils deficient in micronutrients such as iron (Fe), zinc (Zn), and manganese (Mn) results in an imposed shortfall of these elements; as a result, micronutrient concentrations in agricultural products and dry matter will decrease [37, 38].

When examining the effect of pH on iron (Fe) solubility, the proportion of water soluble iron at pH = 7 is around 10-18 mol/ L (moles per litre), while the levels required for proper plant development are approximately 10-8 mol L. As the pH rises, the solubility of trivalent iron decreases [39,40]. Elevated apoplast pH in leaves may impede the movement of plasma membrane reductase, resulting in insufficient iron absorption [23]. Plants in cardio soils have two methods for accessing iron compounds: first, siderophore release (non-protein amino acid) (located in the Gramineae family); and second, soil chelate removal or healing of trivalent iron (Fe3+) to bivalent iron (Fe2+) through proton leaking (located in different flora) [19,20].

II. IRON DEFICIENCY

Iron deficiency can range from iron-depleted levels without anaemia to more severe situations with severe haematological symptoms, which correlate to the most clinically serious case. However, with the exception of significant blood loss, the progression from iron deficiency to iron-deficiency anaemia is typically gradual. It is also recognised that iron deficiency can be caused by a combination of causes. Poverty, malnutrition, starvation, hookworm infections, and schistosomiasis, which causes persistent blood loss, are all apparent causes of anaemia among iron-deficient populations in poor nations. The major causes of iron deficiency anaemia in industrialised nations, on the other hand, include extreme vegan and vegetarian diets, malabsorption, and chronic blood loss caused by menstrual cycles. Anemia is more common in elderly individuals as they age, and it is linked to a variety of diseases such as inflammation, decreased erythropoietin levels, and cancer. Other diseases, such as obesity, congestive heart failure, and hereditary factors, may be at the root of iron deficiency anaemia.



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2.1 Strategies to overcome iron deficiency

There are some traditional approaches that are being used to alleviate deficiency of micronutrients in human like food fortification, food supplements and some dietary diversification [41]. However, poor residents of rural areas cannot afford any of these strategies, mostly for the people that belongs to developing countries, these strategies are so expensive. So keeping these facts in view a new strategy known as "Biofortification" was introduced that deals with crops having higher contents of micronutrient in their edible parts for uptake of human [42]. It is considered as most practical as well as a sustainable approach, it is cost-effective strategy that is involved in improving the daily iron intake for the people facing micronutrient malnourishment by the economic analyses [43]. Due to biofortification benefits and its availability, some useful strategies like genetic or agronomic could be used as measures to improve iron content in staple food that is taken on daily basis by the rural residents.

Iron deficiency is widespread sensation among the plants as well as animal kingdoms. In some crops, especially in such crop that are grown on the calcareous soils. Deficiency of iron iscommon nutritional disorder, involvedin decreasing ofplant's vegetative growth and yield. It also cause loss in plant quality [44]. An emerging approach "agronomic biofortification"is involved in application of fertilizers that are enriched in micronutrients. It increase bio-available contents of micronutrients in edible parts of crops [45]. To overcome iron deficiencies in plants, micronutrients are applied or mixed with fertilizers, mostly in such fertilizers that are applied in granular form to the soils, or maybe as foliar sprays. Fertilizers that are applied in to the soil are mostly fixed within the interlayer spaces of clays and bind to soils sites that are charged negatively in low Ph soils, or fixed to the calcium carbonates in soils with high pH [46]. To overcome problem of iron fixation, iron chelates are used as a soil iron fertilizers [47]. Typically, plants need lower amounts of iron fertilizers so it is mostly applied in foliar forms, albeit at higher application cost. To reduce such costs, another strategy is commonly used to combine iron foliar with pesticides to apply crops [43].

Iron deficiency in animals is usually occurs due to chronic blood losses. Animals at young age faced primary deficiency of iron and have low capacity to store iron. So Addition of iron compounds like iron ammonium citrate to the farm soils where iron related diseases occurred, resulted in uniform and large increase in iron percentage in the grasses grown on farm soils. This suggests is a cheap and easy method to prevent disease associated to iron deficiency [48].

Other hand, some common approaches to treat iron deficit animals include prevention of further blood losses, correcting anemia if severe, iron supplements, and addressing underlying disease.

III. BIOFORTIFICATION

The process of developing micronutrient-rich staple crops employing the finest conventional breeding procedures and modern biotechnology with the goal of developing staple crops such as wheat, rice, maize, and millets is known as biofortification [41,49,50]. The technique aims to include the micronutrient-rich trait in cultivars with other desirable agronomic and consumer characteristics, such as high yield [51].

The definitive objective of the biofortification approach is to lessen death rate associated with micronutrient malnutrition, as well as to improve food safety, efficiency, and quality of life for meager inhabitants in emerging nations, by breeding staple crops that facilitate better-quality levels of bioavailability of nutrients present in low concentration at a low cost in a long-term sustainable manner [52]. Biofortified staple foods cannot provide the same amount of minerals and vitamins per day as additives or industrially fortified meals, but they wouldaid people for getting enough micronutrients every day of their lives [41].

The main goal is to introduce new features into the productive material so as with the intension to its market influence is guaranteed and growers will cultivate it for getting highproduce. It is specially critical in a market that isn't willing to pay more for more nutritional value [53].

Following attentions should be given for success of biofortified crops:

1. A biofortified should be highly productive giving more benefit to the grower
2. Crops should be suitable to growers and consumers in targeted areas where individuals suffer from malnutrition
3. The amount of basic food should be eaten daily by age and gender
4. Nutrients should not be wasted after harvest (nutrients can be severely depleted during storage and cooking)
5. The nutrients should be captivated by the body [55,56].

3.1 Forms/types of Biofortification

The importance of plant nutrients may be increased through various biofortification approaches, namely, agronomic biofortification, the development of plant species through normal and molecular breeding, and by genetic modification [41, 56].

3.1.1 Agronomic biofortification

Agronomic strategies for improving mineral content in food crops include applying minimal fertilizer to plants that are easily accessible, improving soil availability, implementing crop rotation practices, or introducing micro-organisms that are beneficial to soil [47,57]. The most striking agronomic biofortification approach is the use of mineral fertilizers on plants in an easily accessible area [57,58]. The addition of nutritious fertilizers i.e., zinc, nickel, iodine, and selenium) improves their concentration in palatable plants and demonstrated the truth in several studies [58,59]. Intense supply of zinc in pea plants (*Pisum sativum* L.) improve concentration of zinc not found in seeds of pea

[58]. [59] summed up the improved availability and deposition of zinc up to 60 mg / kg of entire wheat grains by increasing zinc fertilizer in the soil. Through foliar application, zinc can be taken up by epidermis of leaf, regenerate and transfer to rice grains through phloem which develop mineral contents.

[60] also reported phytic acid decrease that improved bioavailability between rice varieties. Foliar application of Zn preserves yield of grain, protein quality and nutrients (iron and calcium) for refined rice. Iodine enrichment in China, selenium in Finland, and zinc in Thailand have yielded effective outcomes.

3.1.2 Conventional and molecular breeding and genetic engineering

Agronomic approaches of biofortification need rigorous land management in developing countries because of the expenses involved and the use of certain agricultural practices. Another way for strengthening through farming management is genetic technology and normal breeding and cell plant formation [61]. Different cultivars of the same plant that exhibit different nutritional traits are commonly produced to promote a new improved food yield. Traditional and molecular breeding; and genetic engineering techniques are two important routs to supplement plants with minerals such as iron and zinc [62,63,64]. Since the detection and collection of micronutrients in palatable parts of plants is controlled by polygenes with minimal effects, conventional biofortification-based breeding methods have achieved only side effects [65]. In addition, the success of this approach depends largely on the variation in gene in genetic makeup. In the absence of sufficient genetic diversity and major effects of genetic modification, genetic engineering will be another way to develop nutrients to the required level [66]. Breeding of crops is principally engrossed on increasing the staple food crops with adequate levels of iron, zinc, and provitamin A carotenoids to overcome the requirements of at-risk populations specially in the Global South [67]. These two approaches are widely accepted as genetically modified and rapidly resistant to the 1960's; seed banks are designed to collect and calculate these mutations. Center for Corn and Wheat Development in Mexico (CIMMYT) is an important illustration [68]. The most remarkably successful instances of biofortified plants are usually made with sweet oranges (OFSP) and high quality maize.

3.1.3 Biofortification through microbes

Plant biofortification can be achieved by infecting microorganisms such as fungus, bacteria, and mycorrhiza with plant growth-promoting rhizobacteria (PGPR) [69]. It, in conjunction with the breeding variety, has the potential to increase micronutrient concentration in wheat crops while maintaining yields and soil fertility [70]. Experimental studies show an increase in iron levels in wheat and red peppers when Trichoderma asperellum and mycorrhizal fungi, namely Methylobacterium oryzae, are treated, respectively [71,72].

IV. ADVANTAGES AND DISADVANTAGES OF BIOFORTIFICATION

4.1 Advantages of Biofortification

1. It is performed on staple foods that poor households already enjoy
2. Biofortification technique reaches rural and urban populations where supplementation or fortification may be difficult.
3. It is a sustainable approach.

4.2 Disadvantages of Biofortification

1. It takes time to implement
2. Public awarness is necessary for it

V. CONCLUSIONS AND FUTURE TRENDS

The scientific community's primary research goal is to eliminate iron's "hidden hunger." Biofortification looks to be a viable method for underfed people in remote rural locations, bringing naturally fortified food items to individuals who lack access to commercially marketed goods, which are more widely available in urban areas. It is critical to define the major components included in the iron-fortified foods approach. The primary built-up features are the iron assortment to be used as a fortifier and the revolution in the research for new items.Furthermore, even in biofortified crops with the highest iron enrichment defined thus far, the iron content has been marginally greater than the RDA and lower than that in pharmaceutical formulations. As a result, future difficulties will be associated with the creation of crops with greater iron levels and bioavailability for human consumption.

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