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REVIEW ARTICLE

A review on the biofortification of crops using zinc, iron and selenium for sustainable quality production

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Abstract

Micronutrient deficiency, often termed "hidden hunger," poses a significant threat to both crop development and human health. This condition leads to stunted growth and diminished nutritional quality in crops, ultimately impacting food security and public health. Addressing this challenge requires innovative solutions, with agronomic biofortification emerging as a cost-effective and rapid strategy. However, agronomic biofortification faces several hurdles, including variable soil conditions, interactions between nutrients and environmental risks associated with excessive application, along with lack of awareness among farmers hampers its adoption. Also, few studies have looked at the combined effects of micro-nutrients on field crops; most research on agronomic bio-fortification of crops has focused on administering one or, in rare instances, two micronutrients. Thus, further research is essential to optimize these methods and ensure their efficacy and safety. Numerous studies have highlighted advancements in biofortification techniques since 2015; however, a comprehensive comparative analysis of micronutrient bioavailability remains scarce. Understanding these dynamics is crucial for improving crop nutrition and addressing the pressing issue of hidden hunger. By synthesizing existing research, we can identify gaps and propose enhanced agronomic practices that will contribute to healthier crops and improved nutritional outcomes for populations reliant on staple foods. Therefore, research was conducted with the objective of using of zinc (Zn), iron (Fe), and selenium (Se) to increase field crop yield and fortify them for long-term, high-quality output. Thus to increase the nutritional content of edible plant portions, this method applies micronutrient fertilizers via foliar sprays, soil application, chelated formulations, nanoparticles, and the employment of helpful microbes. The review findings indicated in order to address nutritional security in the realm of agriculture, the usage of agronomic fortification techniques were applied to generate biofortified crops . It provides scientists with information on the substantial potential of biofortification to boost agricultural yields while providing crops with more nutrients.

Keywords : biofortification; biosynthesis; hidden hunger; integration; micronutrients

Introduction

Second, only after moisture stress the most significant factor limiting crop productivity in these soils are nutritional problems. Deficits in phosphate and nitrogen are the main issues, but recent studies have shown that micronutrient issues are also impeding crop productivity. Through our past review, it tends to be seen that the utilization of natural and inorganic manures in the mix can altogether upgrade the effectiveness of local and applied supplements, at last prompting more significant returns and better yield quality (1-10). In agricultural contexts, micronutrient deficiencies in the soil can severely limit crop yields and the nutritional quality of produce (11-13).

Zinc is the most deficient nutrient in Indian soils and has been examined in every crop and cropping system in the nation followed by iron. Indian soils now have 48.1 % and 11.2 % of extractable zinc and iron (DTPA) deficient, respectively (14). It has also been reported that climate change threats are leading to selenium deficiency (15).

Soil deficient in micronutrients not solely restricts crop productivity but also affects the dietary best of foods, leading to malnutrition in the human populace (16). Low soil levels of Zn, Fe and Se can stunt plant growth and yield nutritionally deficient crops, contributing to human malnutrition. As a result of this, over 2 billion people globally experience the end result of attaining deficiencies in iron (Fe), zinc (Zn) and other micronutrients viz. selenium (Se). Micronutrients are necessary for plants, they are needed in comparatively lesser amounts at 100 µg/g of dry matter as compared to the 1000 µg/g requirement of macronutrients (17). But the importance of their participation cannot be overstated. When it comes to micronutrients, the range of sufficiency is less than that of macronutrients since even a small excess or shortfall can cause a decrease in output. The nutritional security of humans and cattle is also determined by its influence on the quality of the product Improving the soil's micronutrient level is crucial for the long-term sustainability of soil fertility and productivity (18). Addressing the 'hidden hunger' of micronutrient deficiencies is essential for improving crop productivity and human health. To mitigate these limiting factors, biofortification has proved as a suitable tool to enhance the bioavailability of essential nutrients in edible plant parts (19). Biofortification involves enhancing the nutritional content of food crops through traditional plant breeding, improved agricultural methods and advanced biotechnology. This is achieved without compromising the characteristics preferred by both consumers and farmers (20).

Under the aspect of improved agricultural methods, the research work done evidence underscores the importance of addressing micronutrient deficiencies through sustainable agricultural practices. By optimizing biofortification through nutrient management techniques for micronutrients, farmers can achieve higher yields, better profitability, and improved soil health, thereby contributing to overall food security and nutritional quality. Thus, to adjourn the gap the review paper is taken in to consideration.

The integration of organic and inorganic nutrient sources has been shown to enhance both the yield and quality traits of crops. For instance, a study conducted on fodder oat (*Avena sativa* L.) varieties under integrated management techniques in Jammu demonstrated that combining organic fertilizers with inorganic ones significantly improved green and dry fodder yields as well as forage quality traits such as crude protein content (21). Furthermore, this issue is also addressed through integrated nutrient management, which combines organic and inorganic fertilizers to enhance soil fertility and crop yields. A study conducted on the intercropping of maize with rajmash (*Phaseolus vulgaris*) demonstrated the effectiveness of such an approach (22). The maize + rajmash (1:1) intercropping system produced the highest maize equivalent yield (MEY) of 7772 kg/ha, with substantial net returns and a benefit-cost (B:C) ratio of 1.81, according to the results of the experiment, which compared various cropping systems and nutrient management strategies. Under new recommendations, which included the application of 75 kg/ha urea, 68 kg/ha DAP, 25 kg/ha MOP, and 3 t/ha farmyard manure (FYM), further validation through on-farm trials (OFTs) revealed a 46.27 % increase in MEY, a 55.64 % increase in net returns, and a 23.83 % improvement in the B:C ratio. This integrated approach not only improved productivity but also enhanced soil fertility through balanced fertilizer application, organic manure usage and including micronutrients, for crop production.

Agronomic biofortification has remained primely active in providing Zn, Fe and Se based nutritive support where the use of Se-enriched manures can increase the Se levels in crops such as maize and wheat (23). In the current scenario, managing the overall plant nutrient availability (including Zn, Fe, Se and other micronutrients) in food based crops is trivial. For instance, crops plagued by zinc (Zn) deficiency endure a cascade of detrimental effects: stunted growth, suppressed tillering, and the emergence of smaller, chlorotic leaves that signal distress. The repercussions extend to delayed maturation, compromised fertility, and ultimately, diminished product quality (24). The agricultural sector's research and innovation efforts are intensely concentrated on Zn, recognizing its pivotal role in both amplifying crop yields and addressing widespread nutritional shortfalls in populations. Groundbreaking research in Turkey, has illuminated the profound impact of Zn fertilization, which not only bolsters yields but also enriches grain Zn content in both cereals and dicotyledonous crops, heralding a significant stride in combating Zn deficiency (25). Field research in India has revealed a remarkable tripling in rice yields and zinc (Zn) content in grains when Zn-enriched urea is utilized (26). Similarly, a study across ten African nations found that Zn-enriched fertilizers boosted Zn levels in maize, rice and wheat (27). The increases were notable: 23 % in maize, 7 % in rice, and 19 % in wheat with soil application; and 30 % in maize, 25 % in rice, and a striking 63 % in wheat with foliar application. This Zn enrichment also reduced phosphorus uptake and phytate accumulation in grains, potentially enhancing Zn's bioavailability for human consumption. A modelling study in the same African countries, all facing Zn deficiencies, assessed the impact of Zn-enriched manures (27). The discoveries recommended that such composts could increment dietary Zn by 5 % and decline handicap changed life years (DALYs) lost to Zn lack by 15 % in Malawi. However, more research is needed to validate agronomic biofortification's effectiveness, particularly concerning micronutrient bioavailability and the metabolic pathways that affect absorption and health benefits.

Iron (Fe) presents challenges in agronomic biofortification due to its insoluble forms, which plants struggle to absorb from soil (28). The significant impact of nutrient fortification on nutrition and health is evident, yet only one case has been documented where agronomic biofortification has definitively improved human micronutrient levels. This case comes from Finland, where since 1985, a nationwide program has fortified fertilizers with selenium, leading to a marked increase in Se levels in cereals, and consequently, in human and animal diets (29). This has greatly reduced Se deficiencies among the Finnish population. The safety and efficacy of using sodium selenate in fertilizers have been thoroughly researched and confirmed, with over 70 % of the increased Se intake coming from animal-based foods. Despite these advances, there remains a gap in research directly linking agronomic biofortification to dietary micronutrient intake and its subsequent health effects, barring the use of genetically biofortified crops (30). Agronomic biofortification may be a viable approach to combating micronutrient deficiencies, according to modelling studies (31). For example, applying just 5 g of Se per hectare to maize crops in Malawi could potentially raise daily Se intake by 0.04 mg for those on a maize-centric diet. This highlights the untapped potential of agronomic biofortification in improving dietary micronutrient levels and enhancing public health. As Se plays a vigorous role in plant development by contributing to cell wall formation, maintaining tissue structure, and facilitating the transport of nitrogen, phosphorus and potassium (32).

Selenium (Se), like Zn and Fe, plays a vital role in metabolic processes, including DNA synthesis, respiration and photosynthesis (33). A fifteen-fold rise in the Se content of cereal crops has resulted from Finland's widespread use of Se to NPK fertilizers, exceeding nutritional guidelines (34).

Yet, litter fertilization and foliar application of mineral Fe have emerged as promising methods to enhance Fe content in crops. Studies indicate that foliar nutrient application can enrich wheat and rice grains with iron, although some reports suggest no impact from foliar-applied inorganic and chelated iron fertilizers (35, 36). In Australia, applying Se to soil or as a foliar spray has significantly increased wheat grain Se concentrations, with a 133-fold increase from soil application and a 20-fold increase from foliar application (37). Research has established a direct correlation between the application of selenium (Se) and the increase in Se content in maize grains, as well as its enhanced bioavailability in wheat flour and bread (38, 39). Studies conducted recently suggest that applying Zn, I, or Se topically has more effective effects than applying it in the soil, especially when it comes to increasing grain concentrations. (40). For example, adding zinc to nitrogen (N) and foliar spraying wheat grain and flour increases zinc concentration and bioavailability, indicating a potential strategy for wheat zinc fortification (41). The viability of foliar application relies upon elements, for example, plan type, source, and molecule size. Soluble Zn solutions (like $ZnSO_4.7H_2O$ and $ZnCl_2$) and chelated Zn formulations like Zn-EDTA are two types of Zn foliar sprays that are frequently used (42, 43). Another potential option is Zn conjugated chitosan nanostructures (Zn-CNPs) (44). Selenium is usually applied as selenate, selenite, selenomethionine (SeMet), methio-seleno-cysteine (MeSeCys) and nano-Se are the principal Se-forms applied to cereal crops (45). In an open field, controlled greenhouse, or *in vitro* experiment, the growth conditions are maintained by preparing the growth media with soil, hydroponics, or artificial growth media. These are the methods for prime application (45). However, despite diverse studies conducted the best suitable highly responsive methods of providing Zn, Se and Fe for enhanced nutrient support are tabulated and shown in Table 1. For combating worldwide malnutrition of zinc (Zn), iron (Fe) and selenium (Se), there is need to study the effects of soil, foliar or nano chelated treatments on concentrations of Zn, Fe, Se, N in crops and further on their bioavailability in grains. Hence a review was taken on the biofortification of crops with an objective to use Zn, Fe and Se for enhancing yield and grain their absorption in grain, which could further increase agricultural output and incomes of smallholder farmers' and thus sustained agricultural industry more economically as a result of these developments.

Case studies on biofortification

Zinc fortification in rice crop

A field study evaluating the effects of several zinc (Zn) fertilization methods on aromatic hybrid rice was performed at the IARI research farm in New Delhi in the monsoon seasons of 2007 and 2008 (46). As shown in Fig. 1, the study included seven different Zn treatments, ranging from no fertilization to the application of Zn-enriched urea (ZEU) and soil applications of Zn compounds. Significantly outperforming previous treatments, the application of ZEU (2.0 %) with zinc sulphate produced a biological yield of 19.22 t/ha and a grain production of 7.53 t/ha. The trial demonstrated that the solubility of Zn sulphate-enriched urea, along with the additional sulphur it provides, was more active in improving grain yield contrast to ZnO-enriched urea. The continuous release of nitrogen and Zn from the ZEU throughout the crop's growth period was key to achieving these results. The application of 2.0 % ZEU with $ZnSO_4.7H_2O$ resulted in the greatest Zn concentrations in the grain and straw, according to the study. This suggests that the treatment allowed the rice plants to absorb zinc for a longer period and more efficiently. As a result, the biomass above ground and the root system both increased, improving the plants' total absorption of zinc. The research highlighted the positive interaction between nitrogen and Zn fertilization, suggesting that the acidifying effect of nitrogen in the soil enhances the availability of Zn, thereby contributing to the synergistic benefits observed in crop yield and nutrient content.

Higher Zn availability to plants in treated plots compared to control (no Zn) plots may potentially be the cause of the improvement in rice quality metrics following Zn fertilization caused photosynthates to go to reproductive portions which in turn resulted to increased Zn absorption and biomass production. This may be due to impact of zinc synthesis on photosynthesis, sugar conversions and seed development, zinc influences the metabolism of carbohydrates. Also protein serves as a zinc sink in grains and increase crude protein content after

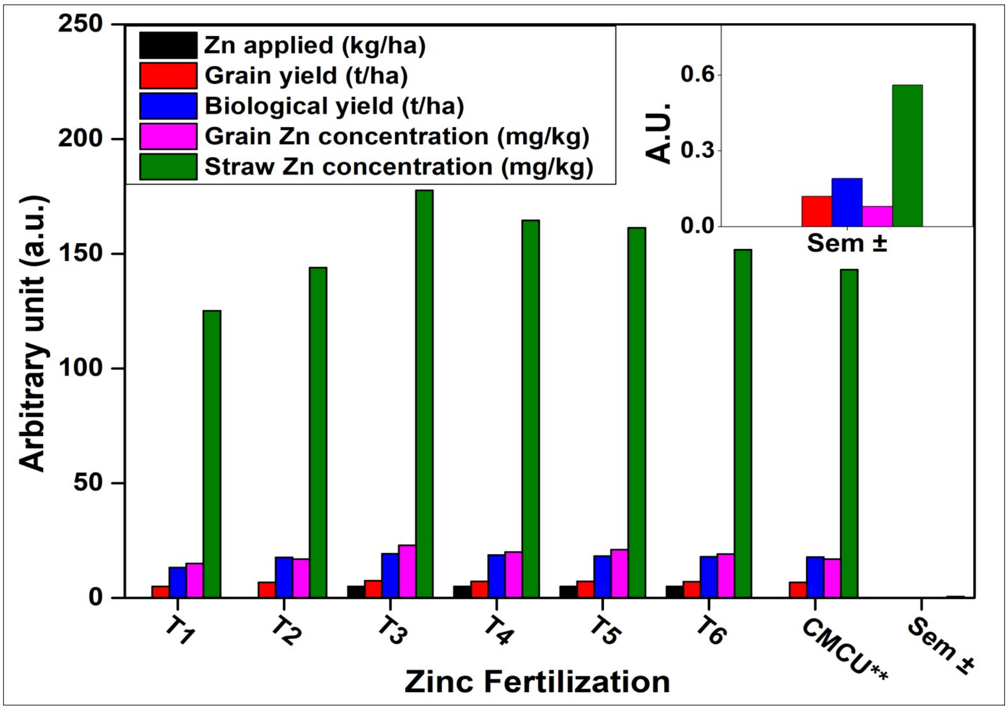

Fig. 1. Effect of Zn fertilization on yield and Zn concentrations of aromatic hybrid rice (pooled data of 2 years). Treatments Details: T_1- Absolute control (Zero N & Zn); T_2- Control (only N); T_3- 2.0 %ZEU* ($ZnSO_4.7H_2O$); T_4-2.0 %ZEU (ZnO); T_5- 5.0 kg Zn/ha($ZnSO_4.7H_2O$); T_6- 5.0 kg Zn/ha (ZnO); CMCU**; Sem± (46).

Table 1. Micronutrient role and mode of application

Micronutrient	Role in plant	Deficiency /Excess effects	Sources (Dosage)	Method of application	Reference
Zinc (Zn)	Plant metabolism, the role of enzymes, and ion transport As a component of more than 300 enzymes from all six enzyme classes, zinc plays a significant role. All six classes of enzymes- lyases, transferases, hydrolases, isomerases, oxidoreductases, and ligases- share this element alone. As a basic or catalytic enzyme, zinc affects the folding, structural integrity, and activity of many proteins Zn in plants include gene regulation and activation, synthesis of proteins, involvement in phosphate and carbohydrate metabolisms, a key factor for ribosomal structural integrity, functional and structural involvement in bio-membranes. The multifunctionality of Zn in plant defense, involved superoxide dismutases (SODs) and zinc finger proteins. SODs altering reactive oxygen species catalyze the conversion of superoxide radicals to hydrogen peroxide, which is involved in abiotic and biotic stress . Further, Zinc finger binding domains are present in the well-known plant resistance proteins NBS-LRRs (nucleotide binding sites-leucine rich) that are involved in the effector-triggered immune response. Zn supply increases the expression of various antioxidant systems detoxifying the harmful level of free radicals upon infection thus promote plant's defence mechanism and reducing the severity of diseases.	Slowed development, chlorosis, lesser leaves, spikelet sterility Both Zn excess and Zn deficiency cause Zn to become prooxidant Under Zn-deficient conditions, reactive oxygen species (ROS) are considered to be the primary factor responsible for plant growth inhibition In tropical regions, where soil zinc availability is often limited or imbalanced, the PGPB, through different mechanisms such as Zn solubilization; siderophore production; and phytohormone synthesis, supports Zn uptake and assimilation, thereby facilitating the adverse effects of zinc deficiency in plants Excess Zn can provoke oxidative damage by enhancing the levels of reactive radicals Zinc toxicity provokes deficiency of other essential nutrients owing to similar ionic radii and interfering with their phytouptake and movement inside plants	$ZnSO_4.7H_2O$, ZnO and $ZnCl_2$ and chelated Zn formulations Zn oxide nanoparticles (ZnO-NPs) Dosage 25-50 kg per ha as soil tender 452 mg kg^{-1} as foliar spray	Soil/Foliar	(77–84)
Iron (Fe)	Iron is an essential cofactor in cellular redox reactions due to its ability to transition between ferrous and ferric oxidation states with moderate oxidation potential and its broad range of ligand-binding capabilities It is the chemically functional component of mononuclear iron complexes, dinuclear iron complexes, (2Fe-2S) and (4Fe-4S) clusters, (Fe-Ni-S) clusters, iron protophorphyrin IX, and many other complexes in protein biochemistry. Cells prioritize delivery of Fe to mitochondria to maintain the proper functioning of iron-requiring biochemical processes such as Fe-S cluster biosynthesis, apoptosis, and respiration. Numerous respiratory complex subunits utilize iron (Fe-S clusters, heme, and/or non-heme iron) as their cofactors. It readily accepts and donates electrons, and functions as a part of redox centers where it serves as a cofactor for various enzymes and proteins. The iron homeostasis network is sophisticatedly interwoven with the uptake and transport of other mineral nutrients . interactions between iron and phosphate and found that deficiencies in one nutrient resulted in transitory induction of the expression of genes encoding proteins involved in the uptake of the other. An antagonistic crosstalk between iron and phosphate signalling was also reported with respect to the accumulation of catecholic coumarins. whereas, silicon improved root-to-shoot iron translocation, resulting in an increased iron content in the youngest leaves.	Iron chlorosis is a widespread agricultural problem occurring in about 30–50 % of cultivated soils Many crops are sensitive to the iron chlorosis, such as citrus and fruit trees In response to iron limitation, phytosiderophores (PSs) are exported by transporter of mugineic acid (TOM1) and Fe-PS complexes are subsequently imported by YS1, a member of the oligopeptide transporter family Iron deficient plants may overaccumulate heavy metals such as cadmium Iron deficiency in the root proteome may produced morphological changes that include swelling of root tips and formation of lateral roots, root hairs, and transfer cells that increase the root's surface area. Biochemical changes result in an increased ability to acquire Fe, and include the induction of a plasma-membrane Fe (III)-reductase and an Fe (II) transporter, an enhanced proton extrusion capacity, and the release of low molecular weight compounds such as carboxylates, flavins and phenolic compounds. Excess, iron can react with hydrogen peroxide and trigger the formation of harmful hydroxyl radicals.	1 to 1.5 lb Fe per acre (IFSA) Fe-sequestrene, 0.25 % as foliar spray of Iron - EDTA iron-humic complexes Iron humic nano-fertilizers Iron fertilizers based on HSs extracted from lignites, such as leonardite, are used in the Mediterranean area (as liquid concentrates) in drip irrigation	Soil/Foliar	(85,86,95,96, 87-94)

Micronutrient	Role in plant	Deficiency /Excess effects	Sources (Dosage)	Method of application	Reference
Selenium (Se)	Reduces the harm that harsh weather, heavy metals, drought, and salinity brought on by climate change may do. Se concentration found to be higher in younger leaves as compared to older ones during seedling growth. Se is mostly accumulated in their vacuoles and can be effluxed through sulphate transporters present in the tonoplast Selenoproteins act as powerful antioxidants in plant metabolism through the glutathione peroxidase (GSH) pathway, and provide an increased activity for enzymatic (SOD, CAT, and APX) and non-enzymatic (ascorbic acid, flavonoids, and tocopherols) compounds that act in reactive oxygen species (ROS) scavenging system and cell detoxification. Selenium helps to inhibit the damage caused by climate changes such as drought, salinity, heavy metals, and extreme temperature. Also, Se regulates antenna complex of photosynthesis, protecting chlorophylls by raising photosynthetic pigments Se application at low concentration enhance photosynthesis in plants due to increase protection of antenna complex and induced the conversion of chlorophyll *a* into chlorophyll *b* and also increased carotenoids and pheophytin co ncentration As a phytofortifier, Se may improve the nutritional quality of food crops and fodder Se can access the sulphur (S) assimilation pathway and incorporated into the Se-amino acids Se-cysteine (SeCys) and Se-methionine (SeMet)	Se stress decreased level of glutathione, Increased lipid peroxidation Se at excessive concentrations leads to toxicity in plants, resulting in chlorosis and necrosis as well as restricted growth and reduced protein biosynthesis.	For wheat crops, 21 g Se ha^{-1}, and for rice crops, 100 g Se ha^{-1} of sodium selenite 100-200 g of sodium selenite containing Se ha^{-1}.For maize crop Na_2SeO_4 Foliar Se application at the concentration 50 g ha^{-1} applied as sodium selenate increases the antioxidant, photosynthetic metabolism, and yield of several crops Selenium nanoparticles (Se NP) have a larger advantage. Se NP is more bioactive and has enhanced solubility than bulk Se.	Soil/Foliar	(97–103)

Zn application. Zinc plays a crucial part in protein synthesis, and applying zinc fertilizers to the soil boosted the amount of zinc availability to plants (47).

Zinc biofortification and yield of baby corn

In 2018, during the Kharif season (September to November), a significant field experiment was conducted at the Tamil Nadu Agricultural University (TNAU) in Coimbatore (48). The experiment took place in Tamil Nadu's Western Agro Climatic Zone. Ammophilous mud soil with a pH of 8.74, moderate basicity (0.53 %), low convenient nitrogen (202 kg/ha), medium applicable dawn (20 kg/ha), and high accessible potassium were used in the experiment. All along the cutting season, a total of 324.4 mm of rain raze over 31 days. The experiment employed the baby corn mixture G-5414, which was planted at a 45 cm by 25 cm spacing. The plan employed a Randomized Complete Block Design, accompanying nine situations and three replications. Fig. 2 depicts the situations, that are different from foliar spraying $ZnSO_4$ and administering soil application indifferent consolidations to asking for no metallic mineral (control). The appropriate NPK portion of the drug or other consumable of 150:60:40 kg/ha was diffused everywhere in the form of muriate of dressing to aid the production of crops, urea, and sole super phosphate. Nitrogen and potassium were used in two equal splits, while the complete dosage of Planet seen at dawn was used as a basic quantity. Metallic mineral sulphate was applied as per the situations, two together as a basic and foliar use. The experiment written miscellaneous limits to a degree plant altitude, dry matter production, green forage yield and chlorophyll content of baby grain leaves. The number of days between seeding and when 50 % of the plants had projected tassels was also observed. Zinc fertilization resulted in a considerable increase in both green cob and green fodder production. The maximum green cob yield of 18637 kg/ha was achieved by applying 37.5 kg/ha of $ZnSO_4$ to the soil, followed by a 0.5 % foliar spray 20 and 40 days after sowing (DAS). This was comparable to the treatment that involved spraying 1 % foliar spray at 20 and 40 DAS and applying 37.5 kg/ha of $ZnSO_4$ to the soil. The control treatment without zinc application had the lowest green cob yield of 13578 kg/ha. The increase in yield might be attributed to the influence of zinc fertilizer on auxin and starch synthesis, administered both in soil and as a foliar spray. This led to favorable crop development, including increased plant height, dry matter production, and chlorophyll content, all of which improved yield qualities and yield, which can be compared to the previous report that was provided (49, 50). Green fodder output increased in tandem with green cob yield. This increase in green portion harvest was deducible to the revised switch of photosynthates, resulting in larger result of green forage accompanying appropriate amounts of metallic mineral as soil and foliar use (51, 52).

However in some studies of bioavailability it has been seen that the presence of anti-nutritional factors Phytic acid generate insoluble complexes and reduce the bioavailability of zinc and iron particularly found in legumes and cereals, this can be mitigated through genetic and agronomic biofortification where higher concentration of micronutrients in the edible portion vis a vis lowering the quantity of phytic acid prevent them from being absorbed and thus reducing the chelating and binding action of cationic minerals (zinc and iron) with phytins and thus increase their bioavailability in both ruminants and nonruminants (53).

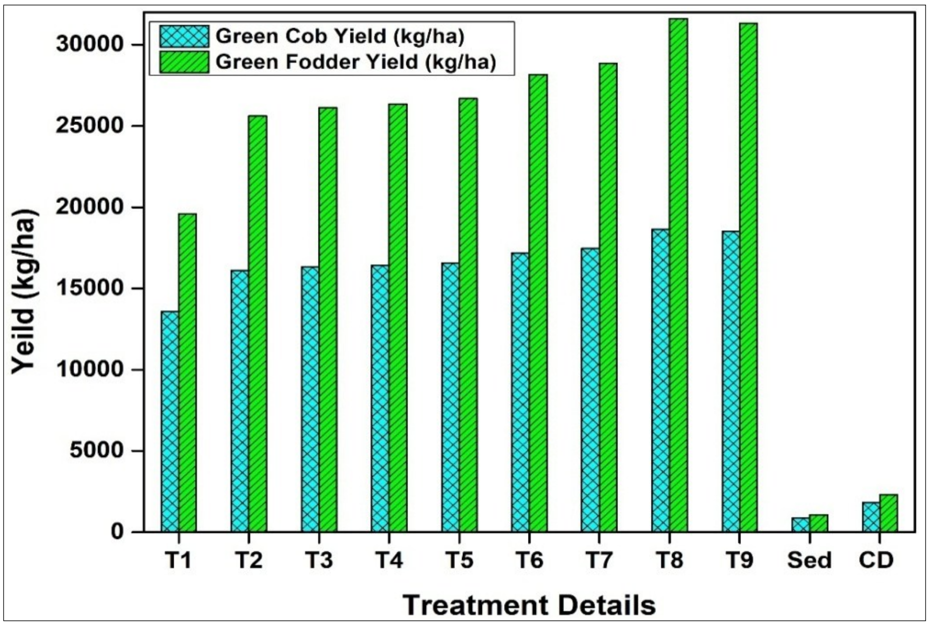

Fig. 2. Zinc fertilization's impact on baby corn's green cob and green fodder yields. Treatments details were followed from a previous study(48).

Zinc based fortification in edible grain

A previous study undertook a comparative analysis of various zinc-based nutrient treatments, focusing on their economic returns (54). Their findings, depicted in Fig. 3, revealed that the treatment termed T_5 was the most economically viable. This treatment involved the soil application of 7.5 kg of Zn/ha using $ZnSO_4$ (containing 21 % Zn). T_5 yielded a gross return of ₹117003 per hectare, a net return of ₹86605 per hectare, and a benefit-to-cost (B:C) ratio of 3.85. These impressive results were primarily driven by a significant increase in grain yield and gross income, which effectively offset the cultivation costs. The elevated B:C ratio in T_5 can be attributed to two key factors. Firstly, there were savings in input costs, likely due to the efficient use of zinc-based nutrients. Secondly, the treatment led to higher yields, which resulted in additional returns. This combination of cost savings and increased returns contributed to the higher B:C ratio (55). In another way, the situation popular as T_6, that involved the soil request of 1 kg of Zn/ha through chelated metallic mineral (Zn EDTA), earned the best help cost at ₹29517 per hectare. The high cost of the fertilizer itself is primarily to blame for this rise in costs. (56).

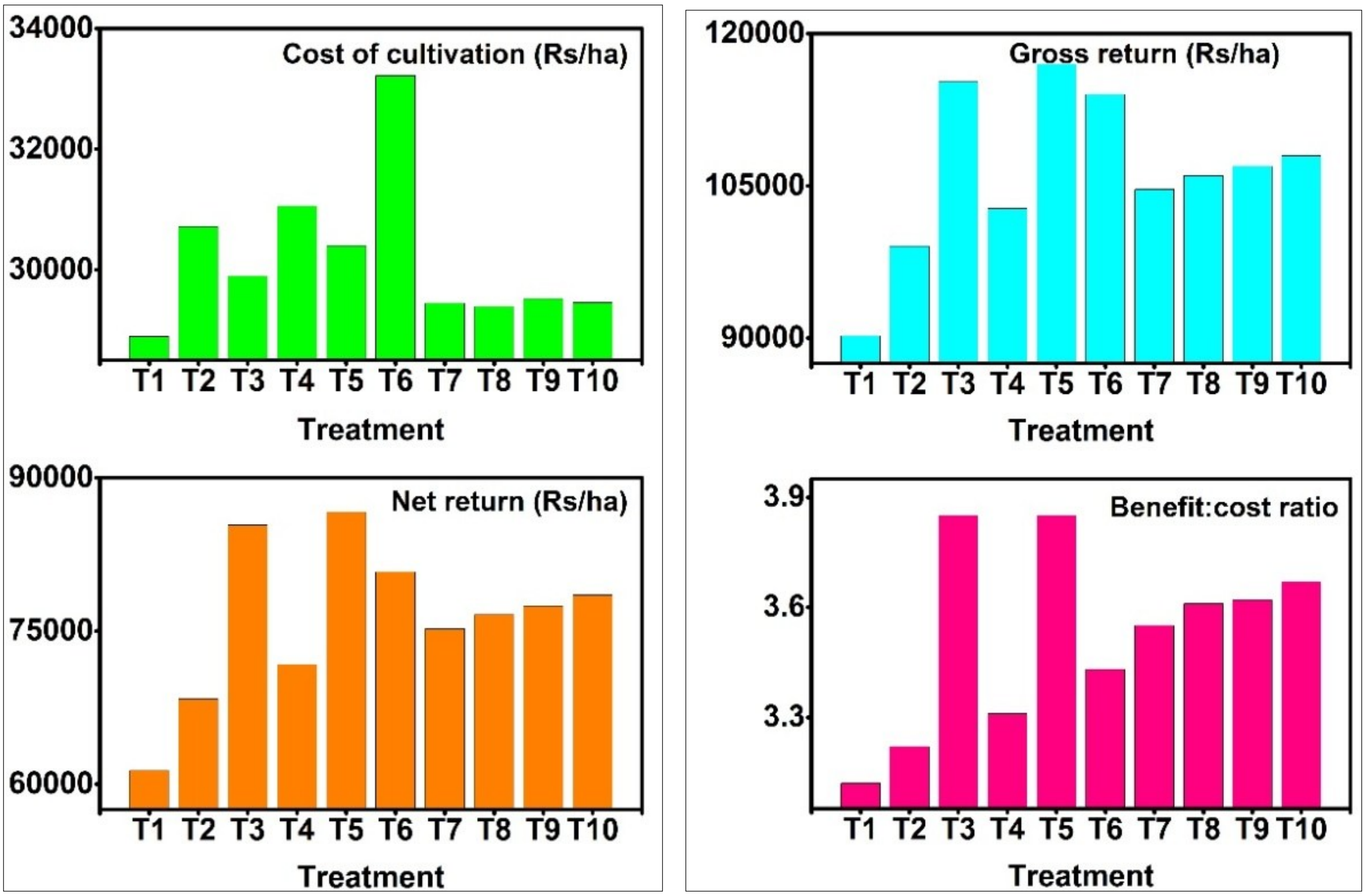

Fig. 3. Effect of metallic mineral fortress on worth of edible grain (54).

Iron deficiency leads on chlorophyll and N_2 metabolism in *Areca catechu* L.

The extensive research investigation involved employing of seedlings of the *A. catechu* cultivar (Reyan No.1), a variety created by the Coconut Research Institute (CRI) of the Chinese Academy of Tropical Agricultural Sciences (57). The Coconut Research Institute's *A. catechu* nursery is where these four-month-old seedlings were produced. Each seedling, which had three completely grown leaves, was placed in a plastic container with a growth medium made of silica sand, perlite and vermiculite. The seedlings were grown in a controlled environment within a growth chamber, which maintained a constant photoperiod of 16 hr of light and 8 hr of darkness, and an average temperature of 27 °C. A specially formulated nutrient solution for *A. catechu* was used, and three different iron concentrations were applied: 0.5 µM, 50 µM and 150 µM for iron deficiency (ID), normal level (CK) and as excessive iron (EI) respectively with pH value ~5.8. For each treatment, twenty seedlings were prepared. During the experiment, the nutrient solution was replenished every three days, and excess salt was removed every ten days. Leaf samples were collected 28 days after the start of the treatment. The results indicated that chlorosis, or yellowing of the leaves, wasn't evidenced in *A. catechu* seedlings under N and Zn deficiency till 80 days into the experiment. In contrast, deficiencies in boron, magnesium, and potassium resulted in slight chlorosis after 80 days. Chlorosis due to iron deficiency became apparent after just 15 days and intensified after 20 days. This chlorosis typically begins in the youngest leaf and gradually spreads to the older leaves. Seedlings exhibiting yellowing showed slow growth and did not develop further. A mineral analysis revealed that, except for iron, most elements did not show a significant difference between normal and yellowing leaves. Further experimentation with different iron levels confirmed that iron deficiency induced physiological chlorosis, while excessive iron led to high iron toxicity. Supplement examination uncovered that the degrees of nitrogen, iron and manganese were decreased in the aerial pieces of the plant under a lack of iron, while the degrees of nitrogen, potassium, magnesium and boron were diminished in the underground parts. Extreme iron brought about decreased levels of nitrogen and iron in the aerial parts and diminished levels of potassium, magnesium and copper in the underground parts.

Ammonium nitrate and nitric oxide help to alleviate iron deficiencies in pears

Researchers from Qingdao Agricultural University independently produced (*P. communis* L. x *P. bretschneideri* Rehd.) 'Qingzhen D1' - a pear variety (58). Shoots on a ingraining medium improved accompanying 0.2 mg/L naphthalene tart acid (NAA), 1.5 mg/L indolebutyric acid (IBA), organic compound composed of carbon 3 % (w/v), and 0.7 % (w/v) agar were used to develop the "Qingzhen D1" pear artificial. Later 20 days, seedlings presenting identical development and growth were picked for supplementary treatments, that endure for 60 days. The following directions were attended for the 20-epoch NO-removal exploratory situations: Fe + c PTIO (1 µmol/L Fe(III)-EDTA + 100 µmol/L 2-(4-carboxyphenyl) -Fe (1 µmol/L Fe(III)-EDTA), c PTIO (NO rat; tetramethylimidazoline-1-oxyl-3-oxide), -Fe + AN (1 µ mol/L Fe (III)-EDTA + 6.85 mmol/L NH_4NO_3) and -Fe + AN + c PTIO (1 µ mol/L Fe(III)-EDTA + 6.85 mmol/ L NH_4NO_3 + 100 µmol/L c PTIO).

Fig. 4 shows the environments under which the sophistications were of age: relative moisture (RH) ~65-85 %, light force grazing from 6K to 8K (lx), and hotness range of 22-27 °C with a 14-moment photoperiod. The educations were preserved in NN 69 medium accompanying pH 5.8 enriched accompanying 0.3 % (w/v) agar. From that time forward, the samples were fast stopped in liquid nitrogen and retained for further test at -80 °C.

Nitric oxide (NO) levels rose in the presence of ammonium nitrate when there was an iron shortage. Following that, an investigation utilizing exogenous NO therapy was carried out to assess the effect of NO. The observations demonstrated that ferric chelate reductase activity was enhanced by both NH_4NO_3 and NO. Furthermore, NO promoted the expression of several IRT genes, which helped iron move across membranes. It has been shown that ammonium nitrate and NO improve the capacity for absorbing nitrogen and activate the enzymes involved in nitrogen assimilation. These substances also increased the activity of glutamine synthetase. In the end, ammonium nitrate and NO application led to enhanced chlorophyll synthesis, which in turn improved plant photosynthetic capability and boosted biomass accumulation.

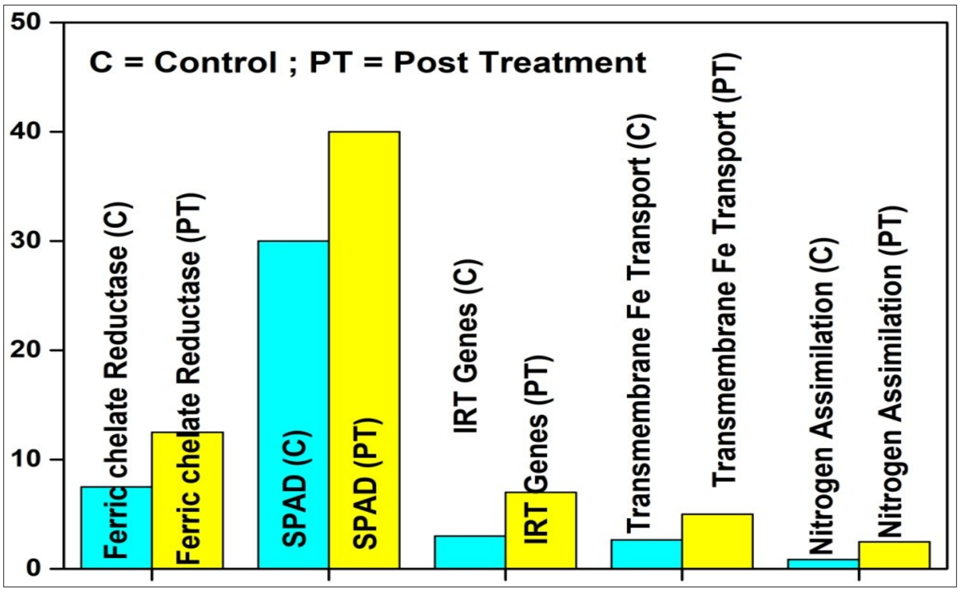

Fig. 4. Effect of ammonium nitrate and nitric oxide on Fe deficiency in pear.

Impact of sulphur on biofortification and speciation of selenium in wheat grain grown in selenium-deficient soils

In this comprehensive research study, the top 15 cm of surface soil was collected from agricultural land in Condo Bolin, New South Wales, Australia, as documented in a previous research (59). The soil samples underwent a meticulous preparation process, which included the removal of extraneous debris, air-drying, sieving to a uniform size of 4 mm and thorough homogenization.

A subset of this prepared soil was then subjected to a detailed physicochemical analysis following the protocols established in an earlier research (60). This analysis involved the use of a CNS analyser (LECO, TruMac CNS) to measure the levels of organic carbon, total nitrogen and total sulphur. A gravimetric approach was employed to quantify the concentration of easily soluble sulphur (as sulphate), employing a 1:5 solid-solution ratio using both water and a 10 mm $CaCl_2$ solution (61). It is interesting to note that the calcium in the salt extract was not anticipated to have any effect on the solubility of sulphate in this particular soil sample (62).

To investigate the adsorption behavior of selenium in its hexavalent state (SeVI), the team followed the procedure outlined in a previous research with minor modifications (63). To sum up, a 0.01 M MES buffer (pH 6) and a 0.01 M NaCl suspension were combined to create a SeVI-enriched solution, which was applied to 1 g of soil and left for 24 hr. In the form of $MgSO_4$, two quantities of sulphate (SO_4) were added to each sample: 0 (control) and 100 µM. Introduced as sodium selenate, the quantities of SeVI varied from 0 µm (control) to 200 µm. The reaction containers were stirred for 24 hr at a constant temperature of 23 °C using a rotary shaker set at 150 rpm. Following agitation, the samples were centrifuged at 5000 rpm for 20 min. For each sample, a 10 mL aliquot was taken, filtered to a size of 0.22 µm and the measured amount of dissolved selenium was treated with an Inductively Coupled Plasma Mass Spectrometer. Later, the sorption isotherms were modeled using Langmuir's Equation and a non-linear fitting process in SAS (SAS, version 9.4). The results of the study provided a detailed breakdown of the percentage distribution of different selenium (Se) species in wheat grains after enzymatic digestion. The species that could be quantified included selenocysteine (SeCys), selenomethionine (SeMeCys), and selenate (SeVI), with two unidentified peaks observed at 12 to 14 min retention time under all treatments. Statistical analysis revealed significant differences (***$p \leq 0.001$) in the percentages of Se species between treatments with selenium and sulphur (S) amendments compared to control treatments.

Se-treated wheat increase total nitrogen and carbohydrate contents and lessen oxidative stress damage and preserve cellular homeostasis, which is crucial for maintaining cell membrane permeability, thus increased activity of the ascorbate peroxidase enzyme (APX enzyme) can be advantageous for phytomass production and accumulated Se in wheat grain (64).

Wheat biofortification through integration of zinc and iron

Wheat remains a staple crop for millions of people across most regions of the Indian sub-continent including arid landscapes of the Shivalik foothills. Typically, in Shivalik foothills, the growth and productivity of wheat in this region are often hindered by the lack of essential micronutrients such as zinc (Zn) and iron (Fe) in the soil. These micronutrients play a crucial role in plant growth and development and their deficiency can have significant impacts on crop yields and quality. In an effort to address this issue, researchers conducted a comprehensive study to investigate the influence of Zn and Fe nutrition on the growth, yield, and quality of wheat in the Shivalik foothills (65). The researchers designed a rigorous field experiment, involving four treatments: a control plot, plots receiving Zn alone, plots receiving Fe alone, and plots receiving a combination of Zn + Fe. The experiment was conducted over two consecutive years, with multiple replications to ensure the reliability and reproducibility of the results. The researchers measured a wide range of growth parameters, including plant height, number of tillers per plant, dry matter accumulation at different growth stages, and leaf area index. They also determined the grain and straw yield, as well as the Zn and Fe concentrations in the grains and straw. Additionally, they assessed the impact of Zn and Fe nutrition on important grain quality parameters such as protein content, sedimentation value, and gluten content. Compared to the control, Zn application alone or with Fe increased Zn concentration in wheat grains by 20-25 % and in straw by 15-20 %, while Fe application alone or with Zn increased Fe concentration in wheat grains by 18 -22 % and in straw by 15-20 %. These improvements in grain and straw nutrient concentrations were accompanied by substantial increases in grain and straw yield, with improvements of 12-15 % and 10-12 % respectively over the control. The researchers also found that Zn and Fe nutrition significantly enhanced plant growth parameters, including plant height, tillers per plant, dry matter accumulation and leaf area index. Moreover, the utilization of Zn and Fe nourishment further developed significant grain quality boundaries, for example, protein content, sedimentation worth and gluten content. The researchers observed that Zn and Fe nutrition increased protein content by 5-7 %, sedimentation value by 10-12 % and gluten content by 8-10 % compared to the control. These improvements in grain quality are significant, as they can have important implications for the nutritional value and processing characteristics of the wheat.

Sweet corn biofortification through integration of zinc and iron

During the 2017 Kharif season, a study experiment on sandy loam soils was conducted at S.V. Agricultural College's wetland farm in Tirupati. The research "Output and character of Sweet grain (*Zea mays* L.) as affected by Country Citadel accompanying Metallic mineral and Iron" aims to scrutinize the influence of metallic mineral and iron reinforcement on the yield and condition of sweet grain (66). As shown in Fig. 5, the trial followed a randomized block design with ten treatments, each replicated thrice. The treatments included a control (T_1), recommended dose of fertilizer (RDF) alone (180-60-50 kg N, P_2O_5 and K_2O ha^{-1}), and various combinations of soil application with spray based application of $ZnSO_4$ & $FeSO_4$. The results showed that the treatment combining 0.5 % foliar application of $ZnSO_4$ and 0.2 % $FeSO_4$ at booting and silking stages, along with RDF (T10), resulted in the highest zinc content (83.1 mg kg^{-1}) in the seed. This was significantly superior to the rest of the micronutrient management tried. The high zinc content may be the result of an increased zinc concentration in the seed as a result of the high mobility of foliar applied zinc ions within the plants. Foliar use of $ZnSO_4$ prompts an expansion in the grouping of zinc in both seed and vegetative pieces of the plants, which was essentially because

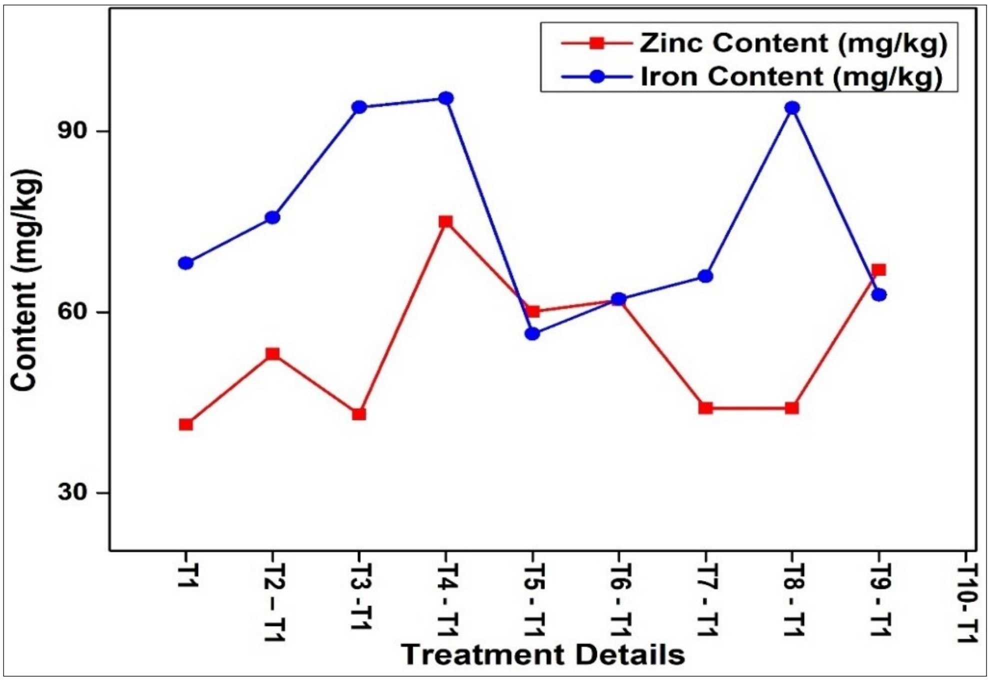

Fig. 5. Fortification with zinc and iron as influenced by quality of sweet corn. Treatments details were followed from a previous study (66).

of the fundamental physiological job of zinc in the plant cell of seed was enlisted with a similar treatment (T_{10}) (67). This could be because the leaves are better able to absorb iron that has been applied to the leaves, which then moves to the source and is mostly stored as phytoproteins, which are ferric phosphoproteins (68). As a result, the study sheds light on the effects of zinc and iron fortification on sweet corn yield and quality. The strategic application of these nutrients can significantly enhance the yield and quality of the crop, potentially leading to increased agricultural productivity. However, for a more comprehensive understanding, additional information on the long-term impact of these treatments and their applicability to other crop types and geographical locations would be beneficial.

Rice biofortification through integration of zinc and iron

An exploratory study was conducted at Annamalai Academy's urban research field during the Navarai and Kuruvai seasons to assess the impact of iron and metallic mineral pollination on the development and incident of edible grain crop (69). The clay textured soil, with 234 kg ha^{-1} available nitrogen content of 20.50 kg ha^{-1} phosphorus content and 305.7 kg ha^{-1} of potassium content (Fig. 6). The experiment consisted of thirteen treatment combinations *viz.,* $ZnSO_4$, $FeSO_4$, Zn-EDTA and Fe-EDTA, arranged in a randomized block design replicated thrice. Basal treatment was given through $ZnSO_4$ and $FeSO_4$ While foliar sprays of Zn and Fe -EDTA were applied during the active periods of rice growth. The rice variety co-47 was spaced at 15 × 10 cm. Results disclosed that the situation place recommended measure of fertilizers (Radio direction finder), soil request of metallic mineral sulphate and iron sulphate as basic fertilizers, along with foliar use of metallic mineral EDTA and iron EDTA at the alive wheel, panicle start and milking stages were used surrendered the maximal seed yield of 5.82 t/ha and hay yield of 7.85 t/ha. This might be due to integrated effect of zinc and iron, when provided in mixture with RDF done, promote the distribution of Fe and Zn within the rice plant. This classification happens through the xylem and re-fluctuation in the phloem, that increases the establishment of produce tissues and enhances photosynthetic venture. This in turn, boosts the growth of plant parts and leads to an increase in dry matter and ultimately promoting yield (70). Accordingly, the study specifies valuable understandings into the impact of metallic mineral and iron procreation on the development and growth of edible grain. The strategic application of these nutrients can significantly enhance the yield and quality of the crop, potentially leading to increased agricultural productivity. Similar findings were reported in an earlier research (71).

Biofortification of millets through integration of zinc and iron

Field research was performed at the Instructional Field, Junagadh Agricultural University, Junagadh during the summer season of 2019 (72). The model crop was millet (Var. GHB-732). The trial consisted of three foliar sprays of $FeSO_4$ and three foliar sprays of $ZnSO_4$ in a factorial randomised design, replicated 3 times. The foliar sprays of $FeSO_4$ and $ZnSO_4$ were applied at different stages after sowing (DAS). The soil at the experimental site was clayey in texture, slightly alkaline with a pH of 7.9, and had an electrical conductivity (EC) of 0.33 dS/m. The soil had medium levels of available nitrogen, phosphorus, potassium, zinc and iron. Using a variety of methods, soil samples were collected and analyzed for available nitrogen, phosphorus, potassium, iron and zinc. Separate estimates were made of the plant samples' nitrogen content. The Atomic Absorption Spectrophotometer was used to examine the iron and zinc content of the grain and stover samples (73). The foliar spray of $FeSO_4$ at a concentration of 1.0 % applied at 25 and 50 DAS resulted in the highest iron content and uptake in both grain and stover (Fig. 7a & 7b). Pearl millet's iron content and uptake increased when higher iron levels were applied via foliar spray. The significantly higher iron content found in both stover and grain accounts for the increased iron uptake (74). When iron was sprayed on the foliar surface more often, pearl millet showed a notable decrease in zinc concentration. The zinc content of the pearl millet was lowest when 1.0 % $FeSO_4$ foliar spray was given at 25 and 50 DAS. Zinc absorption by the grain and stover initially rose when the foliar spray of iron increased gradually, but non-significant effects were seen after a certain point. This could be

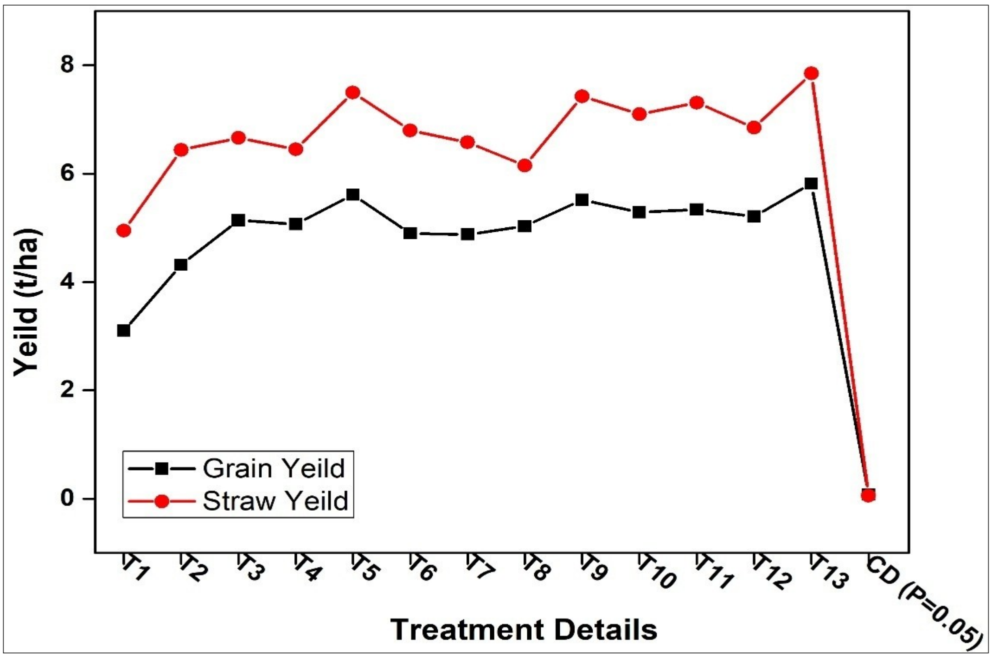

Fig. 6. Zinc and iron applied topically and in the soil affect rice yield characteristics. Treatments details were followed from a previous study (69).

explained by iron's hostile relationship with zinc. The plants have less zinc available because of the restricted mobility of accessible zinc caused by the greater iron content (75). The application of $FeSO_4$ at a concentration of 1.0 % at 25 and 50 DAS resulted in a notable increase in the nitrogen content and absorption in pearl millet. This is explained by the way that iron and nitrogen work in concert to promote improved root and shoot development (56). The foliar application of 0.5 % $ZnSO_4$ (two sprays) resulted in significantly increased zinc and nitrogen content and absorption in pearl millet's grain and stover, as shown in Fig. 7c and 7d. The treatment that displayed the most elevated levels of zinc and nitrogen content and retention in grain and stover was the foliar shower use of $ZnSO_4$ at a measurement of 0.5 % at 25 and 50 days in the wake of planting (DAS). Zinc sulphate treatment resulted in higher nitrogen and zinc content and absorption in the grain, which can be linked to several zinc's advantageous functions in plant physiology. Zinc helps increase the cation exchange capacity of roots, which aids in greater amount of nutrients absorption from the soil. Additionally, zinc plays a beneficial role in chlorophyll formation, regulates auxin concentrations, and stimulates various physiological and metabolic processes in plants. The improved absorption of nutrients from the soil is facilitated by these variables. Increased nutrient concentrations in the seeds are the result of zinc's beneficial effects on photosynthesis and metabolic activities, which include the synthesis and transport of photosynthates to various plant components, including the seeds. Similar findings were published in 2022 (76). As shown in Fig. 7c and 7d, foliar spraying 0.5 % $ZnSO_4$ at 25 and 50 days after sowing (DAS) produced the lowest iron (Fe) content in the pearl millet grain and stover (76). The transfer and absorption of iron from the roots to the aboveground portions of the plant may have been hampered by the higher quantity of zinc. The antagonistic link between iron and zinc was noted in an earlier research (66). Millets frequently exhibit iron and zinc antagonism, a condition in which the presence of one mineral might decrease the absorption or availability of the other. In the root and plant tissues, iron and zinc compete for identical transporters, which is the main cause of this antagonism. Reduced availability of both minerals can result from excessive concentrations of one mineral impeding the absorption and translocation of the other. As a result, the incompatible interaction between these two elements may be used to explain the drop in iron concentration in pearl millet caused by the application of zinc.

Biofortification of different maize cultivars with integrated foliar fertilizer applications of selenium, zinc and iron

Embraced the field tests to research the impacts of foliar medicines on the centralizations of zinc (Zn), iron (Fe) and selenium (Se) in the grains of three particular maize cultivars filled in three unique areas trying to battle worldwide ailing health connected with these components (41). Standard $ZnSO_4$, ZnO nanoparticles (ZnO-NPs), Zn complexed chitosan nanoparticles (Zn-CNPs) and a drink answer containing Zn, Fe, and Se were undeniably decided for effectiveness. All situation was executed topically at a 452 mg Zn L^{-1} + urea dosage. According to the study, adding urea in the form of ZnO-NPs, Zn-CNPs, or ZnSO4 and ten times less zinc (at a rate of 45.2 mg Zn L^{-1}) to grain Zn concentrations in comparison to deionized water either had no effect or very slightly increased them. However, traditional $ZnSO_4$ was the most effective in increasing grain Zn concentration when combined with urea sprayed on leaves. Intriguingly, the application of a beverage solution to the leaves effectively increased grain concentrations of Zn, Fe, Se and N as a whole-without compromising the piece result. For example, by applying a mixed drink arrangement topically, the typical grain focuses were simultaneously expanded for Zn, Fe, Se and N from 13.8 to 22.1 mg kg^{-1}, 17.2 to 22.1 mg kg^{-1}, 21.4 to 413.5 µg kg^{-1} and 13.8 to 14.7 g kg^{-1}, respectively. The aggregation of phytic acid (PA), in addition to the percentages of PA/Fe and PA/Zn ingrain, was dramatically weakened by foliar application of the answer. Fe and Zn grant permission to be more bioavailable for human energy, in accordance with this. The study raises that applying a beverage resolution containing metallic mineral, iron, selenium and nitrogen topically was the ultimate direct habit to biofortify crops. But the grains with hostile yield had the topmost aggregation of these elements. Thus, it is owned by breed maize types that can achieve both extreme seed productivity and good piece pertaining to food character in consideration of addressing challenges connected to human well-being and cuisine protection.

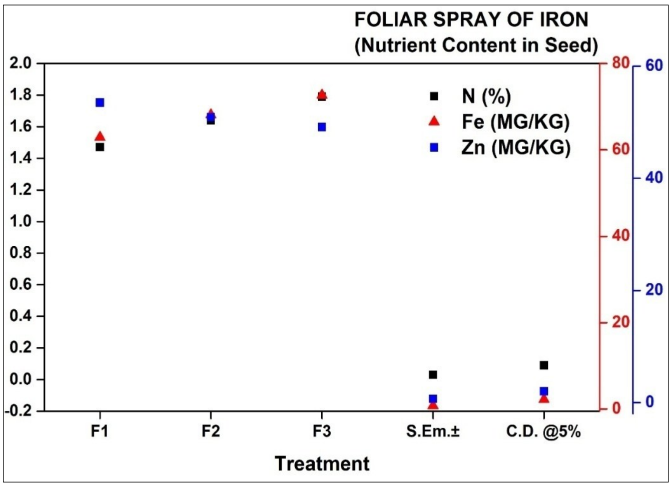

Fig. 7a. Effect of foliar spray of iron form analysing nutrient content in millet seed. Treatments Details: F_1: Control (No spray); F_2: $FeSO_4$ at 1.0 % at 25 DAS; F_3:$FeSO_4$ at 1.0 % at 25 & 50 DAS (72).

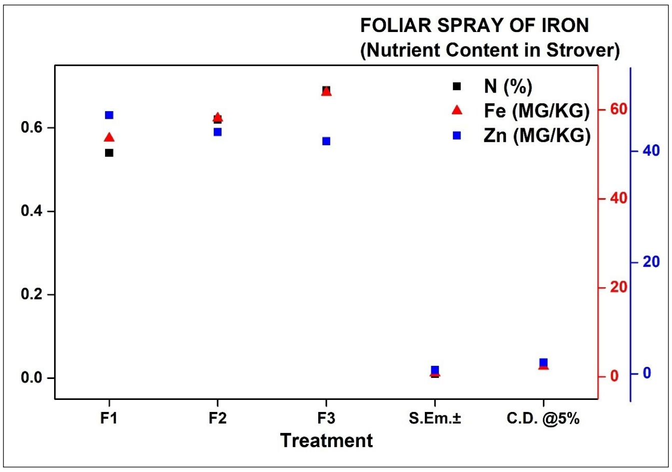

Fig. 7b. Effect of Foliar spray of iron form analysing nutrient content in millet strover; Treatments Details: F_1- Control (No spray); F_2- $FeSO_4$ at 1.0 % at 25 DAS; F_3- $FeSO_4$ at 1.0 % at 25 & 50 DAS (72). S.Em.± C.D. at 5 % (72).

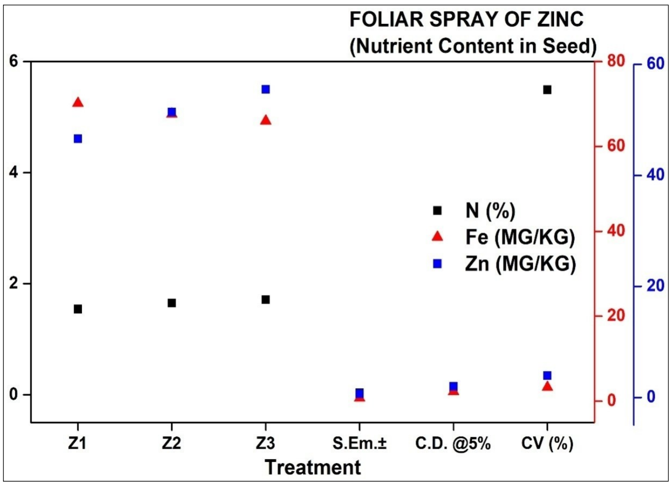

Fig. 7c. Effect of foliar spray of zinc form analysing nutrient content in millet seed. Treatments Details: Z_1- Control (No spray); Z_2- $ZnSO_4$ at 0.5 % at 25 DA, Z_3: $ZnSO_4$ at 0.5 % at 25 & 50 DAS (72).

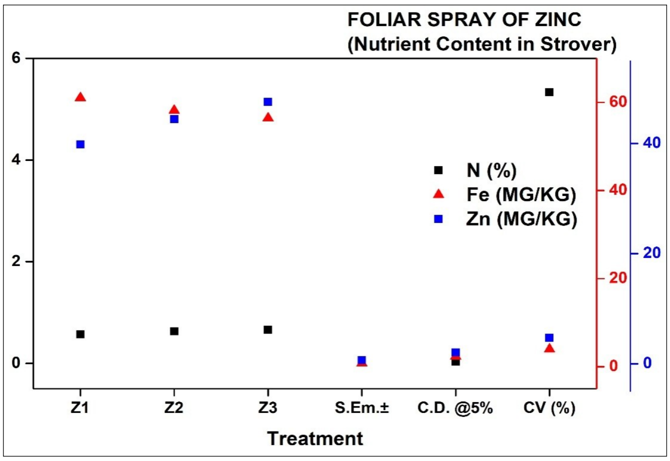

Fig. 7d. Effect of Foliar spray of zinc form analysing nutrient content in millet stover. Treatments Details: Z_1- Control (No spray); Z_2-$ZnSO_4$ at 0.5 % at 25 DAS; Z_3: $ZnSO_4$ at 0.5 % at 25 & 50 DAS; S.Em.± C.D. at 5 %; C.V. % (72).

Conclusion

The current discourse underscores the paramount importance of addressing micronutrient deficiencies through biofortification, with a particular emphasis on agronomic approaches, as a means to augment crop productivity and ameliorate global nutrition. Micronutrient deficiencies, notably zinc (Zn), iron (Fe) and selenium (Se), pose a significant global challenge, impacting both crop development and human health.

Agronomic biofortification, which encompasses the application of micronutrient fertilizers, foliar sprays, seed treatments, or soil inoculation, has emerged as a cost-effective and expedient method to enhance the uptake and accumulation of micronutrients in edible plant parts. This approach holds immense potential for addressing the pervasive issue of micronutrient deficiencies, thereby contributing to improved crop productivity and human nutrition.

The review clarifies the essential roles of Zn, Fe and Se's in plant metabolism and growth. These micronutrients are essential to many physiological functions in plants and deficiencies in them can cause a variety of symptoms that negatively impact plant development and output. This discourse offers a thorough examination of the ways in which diverse application techniques, including as seed treatments, foliar sprays and soil application, might improve the micronutrient content of a variety of crops, including wheat, rice, and baby corn.

Despite the challenges and limitations associated with agronomic biofortification, such as soil and climatic variability, nutrient interactions, and potential environmental and health risks, the potential of this approach to improve crop nutrition and human health is substantial. The nationwide program in Finland, where the fortification of fertilizers with Se has led to a marked increase in Se levels in cereals and a reduction in Se deficiencies among the Finnish population, serves as a successful example.

Further research and development are necessary to optimize methods, evaluate efficacy and safety, and promote the dissemination and utilization of biofortified crops. This review provides valuable insights into the gaps and potential improvements in agronomic biofortification techniques, emphasizing the need for continued efforts to address micronutrient deficiencies and enhance global nutrition.

In conclusion, agronomic biofortification represents a promising strategy to combat micronutrient deficiencies, improve crop productivity, and enhance human nutrition. However, it is imperative to continue research and development efforts to optimize application methods, assess the efficacy and safety of biofortified crops, and promote their widespread adoption. This will require a concerted effort from researchers, policymakers and stakeholders in the agricultural sector to realize the full potential of agronomic biofortification in addressing global nutrition challenges.

Authors' contributions

HS created the figures, contributed to the literature survey and drafting and performed sequence alignment. MK updated the tabular information, participated in the literature survey and drafting and worked on sequence alignment. AS contributed to the literature survey and drafting of the manuscript. MG was involved in the literature review and manuscript drafting. SS participated in the literature survey and contributed to manuscript writing. BG assisted in literature collection and supported the drafting process. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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