Agronomic Biofortification of Paddy through Nitrogen, Zinc and Iron Fertilization: A Review

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\begin{abstract}
Globally, micronutrient malnutrition alone affecting more than two billion people, mostly among resource-poor families in developing countries, with Zn, Fe, I and vitamin A deficiencies most prevalent. Approximately, five million children dies micronutrient malnutrition every year. Currently, micronutrient malnutrition is considered to be the most serious threat and global challenge to human kind and it is avoidable. Among different micronutrients, zinc and iron deficiency is a well-documented problem in food crops due to which crop yields and nutritional quality decreases. Generally, the regions in the world with Zn-deficient soils are also characterized by widespread Zn deficiency in humans. Current trend indicate that nearly half of world population suffers from Zn and Fe deficiency. Cereal crops mainly rice which play an important role in satisfying daily calorie intake in developing world, but they are inherently very low in Zn and Fe concentrations in grain. It provides 21\% of energy and 15\% of protein requirements but does not provide essential micronutrients i.e. Zn and Fe to eliminate their deficiencies. So, the enrichment of rice with N, Zn and Fe fertilization can solve the problem of Zn and Fe deficiencies, which are two amongst the most serious nutritional problems affecting human beings. Among the strategies being discussed as major solution to Zn and Fe deficiency with the use different modes of fertilizers, agronomic biofortification appears to be a most sustainable and cost-effective approach useful in improving Zn and Fe concentrations in grain. Scientific evidences show this is technically feasible without compromising agronomic productivity.
\end{abstract}

\section{Introduction}

Rice (\textit{Oryza sativa} L.) is a self pollinated, short day plant belonging to the family \textit{Poaceae} and it is the cereal crop with the second highest worldwide production. Rice is the dominant staple food for more than half of the world’s population (Wang \textit{et al.}, 2005). It is grown in more than 100 countries, predominantly in Asia. Rice provides 21\% of energy and 15\% of protein requirements of human populations globally (Depar \textit{et al.}, 2011). It provides 23\%, more than that provided by wheat and corn, of all the calories
consumed by the world’s population, and even provides 50-80% of the energy intake of the people in developing countries but it does not provide enough essential micronutrients to eliminate zinc (Zn) and iron (Fe) deficiency (IRRI, 2006). Rice, however, is a poor source of many essential mineral nutrients, especially Zn and Fe for human nutrition. The polished rice contains on an average only 12 mg kg$^{-1}$ Zn and 2 mg kg$^{-1}$ Fe whereas the recommended dietary intake of Zn for people is 12-15 mg and that of Fe is 10-15 mg per day (Welch and Graham, 2004). Currently, malnutrition of Zn and Fe afflicts more than 50% of the world’s population. Heavy and monotonous consumption of rice with low concentrations of Fe and Zn has been considered a major reason for Fe and Zn malnutrition (Graham et al., 2001). Therefore, a slight increase in its nutritive value would be highly beneficial for alleviation of Fe and Zn malnutrition and for human health. Poor grain nutritive value of Zn and Fe in cereals is an important reason for widespread micronutrient malnutrition among the populations eating rice, wheat or maize as staple food.

**New approaches**

Recently, food based approach ‘biofortification’ has been recognized as an efficient mean to reduce micronutrient and protein malnutrition. Biofortification is a scientific method for improving the nutritional value of foods already consumed by those suffering from hidden hunger. It involves the development of functional staple food crops that are selectively bred to enhance specific nutritional qualities, such as the levels of biologically available Zn and Fe. Biofortification complements other interventions and is a means to provide micronutrients to the most vulnerable people in a comparatively inexpensive and cost-effective way, using an agricultural intervention that is sustainable (Pfeiffer and McClafferty, 2007). Broadley et al., (2006) revealed that agronomic biofortification strategy has been suggested as a promising way for enriching cereal grains in UK. Likewise, agronomic biofortification is economically sustainable and practically adoptable solution to overcome the Zn deficiency issue in rice (Qamar et al., 2017). Cakmak (2008) reported that severe human health problem in mind; science as well as the agrochemical industry together with the farmers is forced to find practicable and cost-effective solutions to improve the Zn status of human beings in the areas affected by Zn deficiency. He also reported that low dietary intake of Zn and Fe appears to be the major reason for the widespread prevalence of Zn and Fe deficiencies in human populations.

Several methods are used to solve micronutrients deficiencies such as micronutrient supplementation, food fortification, soil application, coating seed with Fe, soaking of seed in micronutrient solution, dipping roots of seedlings in solutions or suspensions, bio-fortification and fertifortification (Nestel et al., 2006). Nitrogen (N) application is the main segment in agronomic biofortification in rice and Gregorio et al., (2000) have reported that N level is an important factor which determines grain mineral content. However, the effects of N on Zn and Fe concentration in rice grains are not unambiguous, though N fertilization on increasing nutrient concentration in other crops had been proved to be effective (Wangstrand et al., 2006). Soil application is the most common method and prophylactic in nature whereas, the foliar application with Fe (Fertifortificaton) are therapeutic in nature. Fertifortification is a technique to increase Zn and Fe content in rice grains and brown rice. Bio-fortification and commercial fertifortification, though is a slow process and show low efficiency of nutrient enrichment, but still these are highly complementary.
Among different techniques to enhance Zn and Fe content in rice, agronomic fertifortification may have important spin-off effects for increasing farm productivity enriched with nutrients in developing countries in an environmentally beneficial way.

Zinc and Fe deficiencies that weaken immune system function and may impair growth and development afflict more than 50% of world population and are widespread in the world, especially in developing countries, where it is estimated that 40-45% of school-age children are anaemic and that about 50% of this anaemia results from Fe deficiency. Zinc and Fe deficiency in human is a serious threat not only to the health of individuals but also to the economy of developing nations. Overcoming malnutrition related disorders in the world has been identified as the top priority by a panel of distinguished economists. Micronutrient malnutrition have affected lives of billions as evident by 5 billion suffering from Fe and 2.7 billion suffering from Zn deficiency all over the world (ACC/SCN, 2004). In India, 47% children suffer from protein energy malnutrition (PEM), 74% children under three years of age, 43% preschool children, 90% adolescent girls and 50% women are having clinical Fe deficiency and 27% of total population in India is affected by Zn deficiency related disorders such as poor immune system, diarrhoea, poor physical and mental growth (WHO, 2007). Zn deficiency claims about 4.4% of the total child deaths in the world whereas anaemia is the major reason of post natal death of mothers as well as infants.

Among different techniques to enhance Zn and Fe content in rice, ferti-fortification may have effect on increasing farm productivity, enrichment of rice grains with Zn and Fe. Research on ferti-fortification of rice with Zn and Fe has already been started in many developing countries but the extent of Zn and Fe enrichment in parmal rice and basmati rice at different stages of growth and its absorption in grain needs further investigation.

**Effect of N on production system in rice crop**

**Yield and yield contributing parameters**

Nitrogen is the most important component of plant which constitutes about 1-5% by weight. Nitrogen is mostly absorbed by plant as nitrate (NO$_3^-$) and ammonium (NH$_4^+$) ions. Nitrogen is an integral part of pyroll ring in chlorophyll, which is primary absorber of light energy needed for photosynthesis (Havlin et al., 2011). It plays an important role in optimum vegetative growth, dry matter accumulation and partitioning of proteins in the sink/grains. Singh and Walia (2000) conducted a field experiment and found that application of 120 and 150 kg ha$^{-1}$ gave similar grain yield in DSR and TPR but significant superior to 90 kg ha$^{-1}$. Similarly, Sathiya et al., (2008) found that application of 175 kg N ha$^{-1}$ resulted in higher yield attributes and grain yield compare to 100 and 125 kg N ha$^{-1}$. Shivay and Singh (2002) at IARI, New Delhi also reported that each unit increase in nitrogen level led to significant increase in growth, yield attributing characters and yield of transplanted basmati rice. At PAU, Kumar et al., (2015) reported that at 125% of RDN, 10.41 per cent higher grain yield and dry matter produced as compared to 100% RDN which recorded only 6.54 per cent higher grain yield.Two field experiments were conducted by Lampayan et al., (2010) in Central Luzon at the village of Dapdap in Tarlac Province and at the experimental station of the national soil and Water Resources Research and Development Center, Bureau of Soils and Water management at San Ildefonso in Bulcan province and revealed that yield of aerobic rice obtained with application of 90 kg N ha$^{-1}$.
was at par with 120 and 150 kg N ha\textsuperscript{-1} but significantly higher than yield obtained with 60 kg N ha\textsuperscript{-1} and control. All yield attributing characters increased significantly with the increase in levels of nitrogen from 40 to 100 kg ha\textsuperscript{-1} (Prasad et al., 2003), whereas, an application of 200 kg N ha\textsuperscript{-1} significantly increased the plant height (127.3 cm), total number of tillers per hill (16.3), dry matter production (16.0 t ha\textsuperscript{-1}), grain and straw yields (6.5 and 9.5 t ha\textsuperscript{-1}) of hybrid rice. While 100 kg N ha\textsuperscript{-1} resulted in highest benefit: cost ratio of crop (Meena et al., 2002). Similarly, Singh et al., (2007) reported that tiller density and panicle length increased significantly with N application up to 120 kg N ha\textsuperscript{-1}. Gill and Walia (2014) reported that significantly higher grain and straw yield of basmati rice was obtained with 125 percent of recommended dose of nitrogen (90 kg urea ha\textsuperscript{-1}) over control, 75 and 100 per cent of recommended dose of nitrogen. Sharma et al., (2014) conducted an experiment with different Basmati rice varieties to find out the effect of nitrogen levels on yield components of basmati rice cultivars and they found that nitrogen had significant positive effect and was equally superior in terms of tillers hill\textsuperscript{-1}, grains panicle\textsuperscript{-1} and straw yield. Highest number of panicle m\textsuperscript{-2} was recorded with 160 kg N ha\textsuperscript{-1} however; differences in filled grain /panicle between 120 and 160 kg N ha\textsuperscript{-1} were statistically similar.

Milling quality parameters of rice

Higher levels of N (60, 90 kg ha\textsuperscript{-1}) decreased the head rice recovery by 3-8 per cent (Rao et al., 1993). Singh et al., (1997) reported that N fertilization resulted significant increase in rice recovery, protein content in grain, length and breadth of kernel as well as sensory aroma in cooked rice with increase in the N dose upto 100 kg ha\textsuperscript{-1}. Cagampang et al., (1996) reported that higher protein rice was more resistant to abrasive milling than low protein rice. Dixit and Gupta (2000) reported that quality parameters like hulling percentage, milling percentage, protein and amylase content increased due to use of FYM and NPK fertilizers. Highest carbohydrate (84.90 q ha\textsuperscript{-1}) and protein (7.72 q ha\textsuperscript{-1}) yield of the rice-rice system was obtained when 50% N was substituted through FYM. Rao et al., (2006) reported that the conjunctive use of 25% N through FYM over and above 100% recommended dose of N through fertilizer and 60 kg ZnSO\textsubscript{4} ha\textsuperscript{-1} resulted in best quality parameters viz. protein. Mishra et al., (2006) reported that the milling percentage, kernel breadth before and after cooking were significantly higher due to application of organic sources of nitrogen. Kumar et al., (2015) conducted an experiment at PAU and reported that brown rice, white rice and head rice recovery significantly improved with increased doses of N, Zn application and foliar Fe.

Zn and Fe concentration in paddy grains

An experiment was conducted in China on two rice varieties i.e. indica ‘Zhenong 952’ and the japonica ‘Bing 98110’ by Zhang et al., (2008), to study the effect of N fertilizer on Fe and Zn concentration in rice grains. Two rice cultivars fertilized with four rates of urea (0, 0.50, 1.00 and 1.50 g N pot\textsuperscript{-1}) and he found that the optimum application of N alone on rice crops could increase the concentration of Fe in the polished rice. Hu-lin et al., (2007) studied the effects of N fertilizer application on the concentrations of Fe and Zn in shoot of rice and the quality of brown rice in two rice varieties i.e. IR64 and IR68144 and they found that in the treatments with N fertilizer application, the concentrations of Fe and Zn in most parts of rice shoot increased compared with control (no N fertilizer application), they also reported that the concentrations of those microelements in brown rice increased at first and then decreased with further increase of N.
fertilizer application, reaching the highest at 160 kg ha\(^{-1}\), at which the Fe and Zn concentrations in brown rice increased by 28.96% and 16.0% for IR64, and by 22.16% and 20.21% for IR68144 compared with control, respectively. Kumar \textit{et al.}, (2016) found in his study of two consecutive years of 2013 and 2014 reported that concentration of Zn in grains and brown rice significantly increased at higher dose of nitrogen by 25.83 and 27.78 per cent and 25.57 and 27.61 per cent, respectively. Kutman \textit{et al.}, (2010) reported that grain concentrations of Zn and Fe can be enhanced by increasing the nitrogen (N) supply, and Zn and N applications have a synergistic effect on grain Zn concentration of durum wheat. Cakmak \textit{et al.}, (2010) found that nitrogen nutrition of plants appears to be a critical component for an effective biofortification of food crops with Zn and Fe due to several physiological and molecular mechanisms which are under the influence of N nutritional status. Waters \textit{et al.}, (2009) reported that the plant N status is an important factor in enrichment of cereal grains with Fe. Increasing molecular evidence is available showing that remobilization from vegetative tissue and translocation into seed of N and Fe (as well as Zn) is maintained by the similar genetic mechanisms. Kumar \textit{et al.}, (2016) reported that at higher dose of nitrogen application @ 125% RDN elevated the grain Fe concentration in grain as well as in brown rice.

In the case of Zn, increasing N supplies not only significantly enhanced Zn uptake and root-to-shoot Zn translocation in wheat, but also increased Zn retranslocation from flag leaves into grains. Thus, these studies indicate that N management represents a promising agronomic strategy to improve micronutrient contents in cereal grains. Dash \textit{et al.}, (2010) conducted an experiment to study the effect of organic and inorganic sources of nitrogen on Zn and Fe content and their uptake and he found that incorporation of chemical fertilizer enhanced the contents of micronutrients in plants and their uptake in plant and in grain and straw at harvest in comparison to rest of the N sources. Ram \textit{et al.}, (2013) conducted an experiment in BHU on two rice varieties i.e. NDR-359 and HUBR 2-1 with two sources of fertilizer application i.e. 100% recommended dose of fertilizer (RFD) of NPK through inorganic source and 75% RFD through inorganic and rest 25% through FYM and he found that amongst varieties, var. Zn and Fe content were significantly increased in HUBR 2-1. Fertilizer source as application of 75% RFD through inorganic and rest through FYM recorded significantly higher Zn and Fe content of grain than 100% RFD through inorganic source.

**Effect of soil and foliar application of Zn on yield and Zn concentration in grains**

Zinc is one of the most important micronutrients in biological systems, and plays critical role in protein synthesis and metabolism. Several of Zn-binding proteins are transcription factors necessary for gene regulation and necessary for more than a half of enzymes and proteins involved in ion transport. Any decrease in Zn concentration in human body may result in number of cellular dysfunctions, including a high susceptibility to infectious diseases, retardation of mental development, and stunted growth of children, he also reported that zinc deficiency is considered to be one of major causes of children death in the world. In wheat, foliar Zn spray, especially at later growth stages (e.g., early milk stage and dough stage), was very effective in increasing Zn concentration of both whole grain and also in the endosperm fraction, while soil Zn applications remained less effective (Cakmak \textit{et al.}, 2010). Jan \textit{et al.}, (2016) said that foliar application of Zn is much more efficient in grain Zn accumulation than the soil application. Deposition of
protein, iron and zinc in rice grains depends on the plethora of interrelated metabolic pathways involved in uptake of N, Fe and Zn from soil, their transport to source tissues such as culms and leaves and mobilization and/or remobilization to developing grains.

Foliar sprays of Zn at anthesis and milking stage along with soil application of Zn at the time of transplanting produced higher grain yield as compared to soil application alone and foliar spray alone (Kumar et al., 2015). Phattarakul et al., (2012) conducted an experiment in India and China on zinc application through different methods and he found that the Zn fertilizer treatments had generally little effect on rice grain yield but as average of 17 trials, soil Zn application increased grain yield by about 5 % and soil Zn application had also little effect on grain Zn but foliar Zn application consistently increased Zn concentrations in all trials. In case of un-husked rice, foliar Zn application increased Zn concentration by about 66 %, but soil Zn application had only little effect. The combined application of foliar + soil Zn is beneficial for both grain yield and grain Zn intake (Gomez-Coronado et al., 2016). Rehman (2012) found that soil and plant Zn contents increased when ZnSO\text{4} was applied at tillering or panicle initiation than applied at transplanting or without Zn supply under flooded, alternate wetting and drying and direct seeded aerobic condition. An increase in grain Zn over basal was 2.5, 2.8 and 2.3 times in flooded, alternate wetting and drying and direct seeded aerobic systems, respectively, when soil Zn fertilization was applied at panicle initiation. Significant increases in grain yield, straw and grain Zn contents were observed with foliar application of Zn as Zn-EDTA and ZnSO\text{4}, but the highest increase was observed with Zn-EDTA application. Kumar et al., (2016) in his experiments found that when the zinc was applied through soil + foliar, there was increase of grain Zn concentration by 36.2 per cent as compared to no Zn application. Generally, large increases in grain Zn occur when it is foliarly applied at later stages of plant development. Barua and Saikia (2018) conducted an experiment and found the higher Zn concentration in grain and brown rice in the treatment of Zn through soil + foliar applied. Wu et al., (2010) found that higher translocation of Zn from flag leaves to grains occurred when Zn had been applied at booting or anthesis stage in a nutrient solution when genotypes with high or low grain Zn were used. Foliar application of Zn (0.5 %w/v ZnSO\text{4}) at panicle initiation was effective in increasing whole grain Zn contents 2-fold.

The effect of Zn on yield and yield components of rice and it was also found that Zn alone increased the yield significantly over NPK. The agronomic biofortification with the application of Zn fertilizers to soils and/or leaves can have big short- term improvements in both human health and crop productivity depending on the severity of soil Zn deficiency. Barua and Saikia (2018) reported highest grain yield when the Zn was applied soil + foliar @ 0.5% followed by Soil + seed priming. Foliar application of Zn sulfate has been often shown superior over Zn-EDTA and over a soil application within an on-going HARVEST PLUS program (www.harvestplus.org) in various developing countries (Cakmak, 2008). Cakmak (2010) reported that soil- and foliar-applied ZnSO\text{4} significantly enhanced grain Zn concentration in wheat and rice and he found that largest increases in grain Zn concentration were found in the case of combined application of soil and foliar Zn fertilizers that caused more than a 3-fold increase in grain Zn.

An experiment was conducted at Chiang Mai University, Thailand by Boonchuay et al., (2013) to study the effect of eight foliar Zn treatments of 0.5% zinc sulfate (ZnSO\text{4},7H\text{2}O) which were applied to the rice plant at different growth stages and he found that foliar Zn increased paddy Zn concentration
only when applied after flowering, with larger increases when applications were repeated. The largest increases of up to ten-fold were in the husk, and smaller increases in brown rice Zn. Phattarakul et al., (2012) who showed that a foliar Zn spray applied at late growth to rice grown under field conditions caused a greater increase in grain Zn than a foliar Zn spray before flowering stage.

Fe application to increase the yield and nutrient content in grains

Fe concentration in paddy grains

Nutrient transport into the developing grains depends on exchange between xylem and phloem and remobilization from senescent parts of the mother plant. Hell and Stephan (2003) observed that the accumulation of Fe in grains controlled by a number of processes, including root-cell uptake, root-shoot transfer, and the ability of leaf tissues to load Fe into the vascular phloem that is responsible for delivering Fe to developing grains via the phloem sap. They found that fortification of seeds or foliage with Fe for food and feed implies sufficient storage capacities, which could be provided by phytoferritin and constitutive expresses ion of ferritin in tobacco resulted in a 30 per cent increase in leaf Fe content and about 2 fold elevated Fe in rice seeds and seed-specific expression even achieved 3 fold Fe levels in rice seeds under regular Fe nutrition. In a pot experiment on rice crop. Zhang et al., (2009) observed the enrichment of Fe in rice grains by foliar application of Fe containing different compounds. They reported that foliar application of Fe-containing solutions like Fe-amino acid and FeSO₄.7H₂O could improve the nutritive levels of rice grains and there was significant increase in Fe content of rice up to 80 per cent as compared to control.

Three foliar sprays of 1 per cent solution of FeSO₄.7H₂O on rice crop gave a yield of 45 q ha⁻¹ as against 27 q ha⁻¹ obtained with a soil application of 200 kg ha⁻¹ of FeSO₄.7H₂O reported by Sadana and Nayyar (2000). Dhaliwal et al., (2010) conducted an experiment on loamy sand soil to observe the effect of foliar applied FeSO₄.7H₂O on different rice cultivars. They reported that three foliar application of FeSO₄.7H₂O @ 0.5 per cent improved the grain yield in different rice cultivars from 74.4-77.0 q ha⁻¹ as compared to control which ranged from 67.8-71.4 q ha⁻¹. Similar results were obtained by Aciksoz et al., (2011) in an experiment on biofortification of wheat with Fe through soil and foliar application of nitrogen (N) and Fe fertilizers. They observed that the plant N status is an important factor in enrichment of cereal grains with Fe. Molecular evidences showed that remobilization from vegetative tissue and translocation into seed of N and Fe is maintained by the similar genetic mechanisms, resulting in a positive correlation between grain Fe and N concentrations.

Kumar et al., (2016) reported that foliar application @ 0.5% increased the Fe concentration in grain and brown rice both. A field experiment was conducted by Dhaliwal et al., (2009) at research farm, Department of Soil Science, Punjab Agricultural University, Ludhiana on loamy sand soil to observe the biofortification of wheat grains with zinc and Fe. They observed that four foliar sprays of FeSO₄.7H₂O @ 0.5 per cent at different stages of wheat, starting from maximum tillering, flower initiation, milk and to dough stages, resulted in significant increase in Fe concentration in wheat grains from 13.1to 30.3 per cent as compared control. Frossard et al., (2000) observed that foliar fertilization increases crop yield to a greater extent than it increases the Fe content of the grain, it might be the only available fertilization practices that can increase the Fe content of grains. They reported that most of the variation in Fe content in the seed was due to its genetic component and that environmental effects
have a small impact, similarly genetic differences in Fe content of common bean seeds are expressed over different season and in different environmental.

**Fe concentration in brown rice**

Zhang *et al.*, (2009) observed enrichment of Fe in rice grains with different sources of Fe containing compounds by foliar application. They found that foliar application of Fe-containing solutions could improve the nutritive levels of rice grains. Dhalliwal *et al.*, (2010) conducted a study on loamy sand soil to observe the effect of foliar applied FeSO₄.7H₂O on different rice cultivars. They reported that three foliar application of FeSO₄.7H₂O @ 0.5 per cent improved Fe concentration in brown rice. It ranged from 21.3-28.9 mg kg⁻¹ as compared control (17.3-21.2 mg kg⁻¹) for different rice cultivars.

A pot experiment was conducted by Hu-lin *et al.*, (2007) to study the effect of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality of two rice varieties namely, IR68144 (Fe-dense) and IR64 (Non Fe-dense). They reported that the Fe concentration in brown rice of IR68144 increased up to 41.34 per cent with combination of nitrogen application in contrast to control. Moreover, the increase extent of Fe concentration in brown rice of IR68144 was much more than that in of IR64 under N application, which confirmed that IR68144 had a higher ability of Fe accumulation in grain than IR64. Jin *et al.*, (2007) observed the effect of Fe and N mixed fertilizer (0.1 per cent FeSO₄.7H₂O f.b. 0.4 per cent amino acid f.b. 0.2 per cent urea) on the content of Fe, Zn, Ca, Mg and protein in brown rice. The results showed that Fe and N mixed fertilizer had distinct effects on the accumulation of Fe, Zn, Ca, and Mg and on content of the crude protein in brown rice. They reported that the Fe concentration in brown rice was increased up to 10.28 mg kg⁻¹ as compared to the control (6.52 mg kg⁻¹) with foliar application of Fe and N mixed fertilizer.

**Fe concentration in polished rice**

Zhang *et al.*, (2009) reported that there was the highest concentration of Fe in polished rice (4.7 mg kg⁻¹) of the japonica “Bing 98110” with foliar spray of FeSO₄ and it increased significantly (88 per cent) as compared to the control. Zhang *et al.*, (2008) studied Fe and zinc biofortification in polished rice and accumulation in rice plant as affected by nitrogen fertilization using two rice varieties namely, Zhenong 952 and japonica Bing 98110. They found that there was the highest concentration of Fe in the polished rice of “Zhenong 952” with the application of N as compared with no N supply. In another study conducted by Gregoria *et al.*, (2000), they reported a wide variation of Fe content in rice grains among rice varieties and also in same varieties. They observed that apart from the effect of G×E interaction, milling process may also affect Fe level in milled rice.

In conclusion, micronutrient malnutrition arising from Zn and Fe deficiency is a continuing and serious public health problem in the present world. Several attempts have been made to overcome this malnutrition through diversification of diets, fortification and supplementation. Although food fortification has played an important role in resolve the problem. Increasing the micronutrient density of staple crops, or biofortification, can improve human nutrition on a global scale. Upto some extent, it can be achieved by agronomic fertilization but it is not a long-term sustainable approach in developing countries because some fertilizers (Fe) are costly and dangerous to the environment. But some evidence suggests that nitrogen (N) nutritional status of plants can have a positive impact on root uptake and the
deposition of Fe and Zn in grain or seed. Foliar applications of micronutrients such as Zn and Fe are more suitable than the soil application, due to the rapid overcoming on deficient, easy to use, reduce the toxicity caused by accumulation and prevent the elements stabilization in the soil. There is increasing evidence showing that foliar or combined soil+foliar application of zinc fertilizers and foliar Fe under field conditions are highly effective and very practical way to maximize uptake and accumulation of zinc and Fe in plants. Zinc-enriched grains are also of great importance for crop productivity resulting in better seedling vigour, denser stands and higher stress tolerance on potentially zinc-deficient soils. Agronomic biofortification strategy appears to be essential in keeping sufficient amount of available zinc in soil solution and maintaining adequate zinc transport to the seeds during reproductive growth stage. Finally, agronomic biofortification is required for optimizing and ensuring the success of genetic biofortification of cereal grains with zinc. In case of greater bioavailability of the grain zinc derived from foliar applications than from soil, agronomic biofortification would be a very attractive and useful strategy in solving zinc and iron deficiency related health problems globally and effectively.

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