

## BIOFORTIFICATION OF IRON, ZINC, AND IODINE IN CROPS USING TRANSGENIC TECHNIQUES

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**Abstract :** Malnutrition is a very big issue in poor nations, particularly in Asia and Africa, where millions of preschool-aged children with pregnant women suffer from it. Because they eat a carbohydrate-rich but micronutrient-scant plant-based diet, poor individuals are more vulnerable to malnutrition and hidden hunger. The spread of high-yielding varieties but low-micronutrient cultivars exacerbated malnutrition. Supplementation & food fortification of staple foods with minerals are two ways that can be used to track the issue of proper nutrition safety. However, fortified and dietary supplements are also not possible or cost-effective for iodine, particularly iron. To treat micronutrient deficiencies, genetic biofortification of crops has recently developed as a self-targeted and non-recurrent strategy. Because there wasn't enough genetic variation in the crossable gene pools, most traditional breeding methods were limited. Furthermore, it lacks the micronutrient and iodine accumulation-related modulation of target gene expression. At this point, genetic engineering-based food biofortification appears to be a potential strategy to solve hidden hunger, particularly in areas where breeding is difficult due to a scarcity of genetic diversity. If there is insufficient genetic variability and fixable major gene effects, genetic engineering will be a viable option for enhancing micronutrients at targeted levels. Transgenic technologies are one method that can be used to improve genotypes by changing specific metabolic pathways with different genes.

**Keywords:** iron; zinc; biofortification; transgenic techniques

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## INTRODUCTION

Micronutrient malnutrition will become more prevalent as the world's population grows, and it currently affects over three billion people globally (Carvalho et al. 2013; Hoekenga et al. 2014). In people living in low-income countries, malnutrition lacking, occasionally known as "hidden hunger," enhancement the risk of infectious illness & death from diarrhea, measles, malaria, and pneumonia (World Health Organization, 2000). Malnutrition has severe and long-lasting repercussions, which can even be passed down from generation to generation (Haseen et al. 2004). Malnutrition during pregnancy raises the chance of death and stunted growth, resulting in low birth weight and jeopardizing the child's life. Malnutrition continues to be a serious public health issue in developing and impoverished countries (BBS/UNICEF, 2005; Mannar et al. 2004). People's diets are low diverse now than they were 30 years ago, resulting in micronutrient deficiencies, mainly iron (Fe), zinc (Zn), and iodine (I) (Gene et al. 2005; Gene et al. 2009). Iron and Zinc are critical trace elements for a range of metabolic functions (Underwood. 1977; Prasad. 1978). Deficiencies in zinc and iron were ranked 5<sup>th</sup> and 6<sup>th</sup>, respectively, among the top 10<sup>th</sup> risk factors for disease burden over the world (Kumar et al. 2011). Since blood loss during menstruation & childbirth, micronutrient deficiencies are common in children and even more so in women (Singh et al. 2009). Furthermore, in underdeveloped nations, micronutrient insufficiency is exaggerated by a scant understanding and affordability of various and balanced meals, dietary patterns, and a high frequency of infectious diseases (Graham et al. 2001; White et al. 2009; Borrill et al. 2014; Kumar et al. 2015). Various strategies such as food diversity, pharmacological supplementation, and fortification have been emphasized to fight the nutritional deficit, particularly mineral inadequacy.

### Current Malnutrition-Prevention Strategies

The greatest strategy to prevent and even eliminate micronutrient deficiency is to eat a well-balanced diet rich in micronutrients. Supplementation, dietary fortification, homestead food production, and crop biofortification are all conventional but effective ways to manage malnutrition and micronutrient deficiencies. The very most practical technique for preventing vitamin deficiency in people is to fortify the diet with micronutrients. Food fortification, as an approach for filling dietary gaps, has the added benefit of being able to give nutrients to vast portions of the population without necessitating drastic changes in eating habits. Some countries have already begun to implement vitamin A fortification of butter, margarine, and sugar, as well as iodine fortification of salt, vitamin-fortified milk, and vitamin B-fortified cereals, all of which have a lengthy history. Food fortification that is mandated rather than optional is much more effective and has been used successfully for decades around the world. Fortification of milk and oil is required in the 14<sup>th</sup> and 27<sup>th</sup> nations, respectively. In 134 nations, salt fortification with iodine and fluoride is also required.

Around 83 countries are now using fortification legislation to supplement basic foods with micronutrients. However, there are certain disadvantages to current food fortification procedures. Food fortification is typically only possible in countries that have developed, well-monitored, and well-regulated food and pharmaceutical processing industries. Another significant disadvantage of supplementation and fortification systems would be that they accrue recurring costs year after year, and their performance is dependent on funding. This strategy works especially well in countries where the poorest people supplement their diets with small amounts of processed goods. Unfortunately, consumption of industrially processed foods is small in the poorest developing countries, where the majority of the poor, particularly the farming population, rely on their products for nourishment. As a result, the reach of these food fortification programmers' may be limited, particularly in developing countries' rural areas, where the majority of the poor live. In India, obligatory fortification began in

1953 with the fortification of hydrogenated vegetable oil with vitamins A and D (Liu et al. 2014). To manage Goiter, salt fortification with iodization was used in 1998.

West Bengal was the first to fortify wheat flour in 2000, followed by the Andaman and Nicobar Islands. Micronutrient deficits are treated in industrialized nations by supplementation and fortification (Naqvi et al. 2009). In poorer nations, however, extensive implementation of dietary diversification and food fortification/supplementation programs is hampered by low-income and poor market access for the target population (Timmer et al. 2003; Bohra et al. 2016). Unfortunately, none of these measures based on socio-economic development are effective in combating micronutrient deficiency. Furthermore, the success of the aforementioned tactics necessitates societal behavioral changes that rely on literacy, communication, social marketing, and repeated investments (Kumar et al. 2015). Numerous investigators' global experience has confirmed that, rather than a single more expensive technique, a combination of several less expensive approaches is required to prevent nutritional shortage (Bohra et al. 2016). Plant scientists are concentrating their efforts to develop methods to apply fertilizers and/or use plant breeding strategies to increase the concentrations and/or bioavailability of mineral elements in the edible portion of crop plants to address the occurrence of micronutrient deficiencies in human populations (White et al. 2009; Cakmak et al. 2008; Cakmak et al. 2004; Graham et al. 2007; Kumar et al. 2016 and Pfeiffer et al. 2007). Biofortification methods are classified as 'agronomic' (fertilizer-based) and 'genetic' (breeding based).

Biofortification is a method of increasing the micronutrient content of agricultural produce by targeting and modulating mineral nutrient movement pathways (root uptake, transport, remobilization, storage, and enhanced bioavailability), 'pulling' nutrients from the soil and 'pushing' them to economically important parts of the plant in bioavailable forms. Agronomic biofortification, traditional plant breeding, and genetic engineering are the three basic methodologies for biofortifying food crops that have been used to date. The main focus of agronomical methods is on optimizing mineral fertilizer application and/or improving mineral element solubilization and mobilization in the soil (White et al. 2009). By lowering 'anti-nutrient' concentrations, the latter two techniques aim to generate and/or improve plant cultivars with greater micronutrient accumulation capability and increased levels of bioavailability. To overcome the shortcomings of supplementation/fortification, "biofortification" (breeding for higher mineral and vitamin content) of staple foods is a potential, practical, and effective approach for supplying nutrient-dense food to rural populations.

This strategy can complement existing initiatives by providing a long-term and less expensive means of eliminating under-nourished populations that rely on supplementation and commercial fortification for nutrition. The biofortification strategy entails one-time fixed costs for creating breeding procedures, breeding nutritional quality features into present crop varieties, and adapting these varieties to a variety of conditions. After nutritious varieties have been disseminated, this technique will require minimal recurrent investments. Furthermore, the expenditures do not rise in proportion to the number of people, and the advantages may be distributed globally, particularly to underdeveloped countries. Finally, there will be no yield penalty if you breed for higher trace mineral density in the consumed plant portions (Graham et al. 2001; Kumar et al. 2015). The two approaches to biofortifying crops with minerals such as iron and zinc include conventional and molecular breeding, as well as genetic engineering techniques (Pfeiffer et al. 1999; Johns et al. 2006; Kumar et al. 2018; Tiwari et al. 2009). Because polygene with small effects influences the uptake and accumulation of micronutrients in edible portions of plants, traditional breeding-based biofortification efforts have had only limited effectiveness (Naqvi et al. 2009).

Furthermore, the effectiveness of this approach is heavily reliant on the gene pool's natural variation. Genetic engineering will be a viable option for enhancing micronutrients at targeted levels

if there is insufficient genetic variability and fixable major gene effects (Bhullar et al. 2013; Dunwell et al. 2014). Despite the enormous efforts made through traditional plant breeding programs, utilizing the field of genetics (quantitative genetics, heterosis, transgressive segregants, mutational breeding, marker-assisted breeding, QTL mapping, and so on) to entrap natural genetic variations for micronutrients and vitamin accumulation, there is still a long way to go: the dream of nourishing the world's population. This is primarily due to several flaws and threats to conventional breeding, including the need for sufficient genetic variations for a trait in the species, which may not be available for many economically important crops, the need for genes targeting the trait in sexually compatible plants, the long time required for breeding to introduce a single as well as multiple traits (pyramiding traits) into locally adapted elite varieties without the risk of linkage drag, and the lack of genes targeting the trait in sexually compatible plants (*e.g.* cereal seeds, tubers, etc.), as well as the soil's reliance on phyto-availability of mineral nutrients. Furthermore, the inverse link between grain yield and grain mineral concentration, has made it difficult for traditional breeding approaches to overcome such trade-offs (Garvin et al. 2006; Shi et al. 2013; Fan et al. 2008; Oury et al. 2006 and Wenefrida et al. 2013).

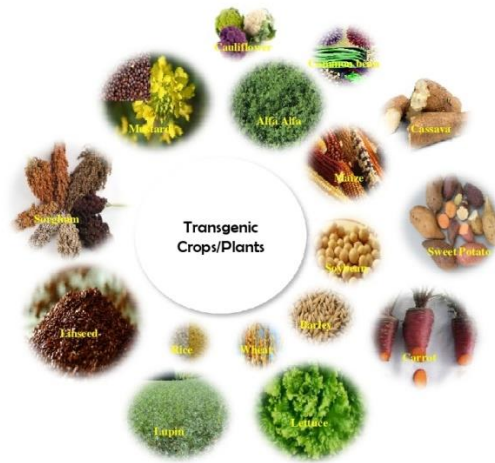
Genetic engineering to make transgenic has also been used to transfer genes directly into elite genotypes as a modern weapon to combat the mineral deficit. Transgenic technologies are methods that can be used to improve genotypes by altering certain metabolic pathways. These technologies provide the door for the modification of proteins (Wenefrida et al. 2013), vitamins, carbs, lipids, minerals, and other metabolites, which will be discussed in the sections that follow. However, two factors should ideally be considered while developing transgenic for nutrient biofortification: (a) selection of a widely adapted genotype of an economically important crop; (b) nutrient accumulation in the edible section of the crop plant without compromising plant physiology, development, or economic yield. Biofortification based on conventional breeding is a well-accepted strategy for improving micronutrients in crops. Using conventional breeding, a large range of crops has been targeted for fortification (Garg et al. 2018; BPB, 2014). When there is an insufficient genotypic variation for the desired feature within the species (for example, provitamin A in rice), or when the crop is not amenable to conventional breeding, genetic engineering techniques provide a feasible alternative to traditional breeding tactics (due to a lack of sexuality; *e.g.* banana).

New gene-editing techniques such as transcription activator-like effectors nucleases (*TALENs*) and *CRISPR/Cas9*, as well as greater availability of fully sequenced genomes in staple crops, have opened up new possibilities for this biofortification strategy (Ricroch et al. 2017). Transgenic can be used to redistribute micronutrients between tissues, improve the efficiency of biochemical pathways in edible tissues, reconstruct selected pathways (for example, in the field of system biology), increase micronutrient bioavailability by removing antinutrients, and transfer multiple genes in a single plant (Carvalho et al. 2013; Naqvi et al. 2009). As a result, bridging the gap between plant breeders and molecular biologists is critical for harnessing the power of genetic alteration for agricultural plant biofortification (Figure 1).

## **MATERIALS AND METHODS**

### **Transgenic Approaches for Improvement of Iron, Zinc, and Iodine Concentration in Crops/Plants**

Plants cannot create minerals, thus they must obtain Fe and Zn from the rhizosphere and surrounding environment (Morrissey et al. 2009). Various crops have been genetically modified to increase mineral content, particularly Fe and Zn. Transgenic techniques to boost Fe and Zn content in crops have primarily focused on modulating transporter expression in order to improve plant uptake and utilization efficiency (Kerkeb et al. 2008) Figure 1.



**Figure 1.** Iron, Zinc, and Iodine Biofortification in Crops/Plants Using Transgenic Technique

Phytic acid, for example, is an anti-nutritional component that should be reduced. In contrast to Fe and Zn, transgenic methods. In the sections that follow, we'll go through some of the Fe and Zn genes and transgenic techniques used in important grains. (Table 1)

**Table 1.** Increased Iron, Zinc, and Iodine In Biofortified Transgenic Crops

Sl.No.	Crops	Nutrient	Gene/Protein	Reference
1.	Rice	Fe, Zn	<i>HvNAS1</i>	Masuda et al., 2009
		Fe, Zn	<i>fumigates phytase</i>	Lucca et al., 2001
		Fe	<i>OsYSL2</i>	Ishimaru et al., 2010
		Fe	<i>OsIDEF1</i>	Kobayashi et al., 2009
		Fe	<i>Ferritin</i>	Wirth et al., 2009
		Fe	<i>Lactoferrin</i>	Theil et al., 1997
		Fe	<i>Lactoferrin</i>	Suzuki et al., 2001
		Fe	<i>Metallothionein-like protein</i>	Lucca et al., 2001
		Fe	<i>OsNAC5</i>	Ricachenevsky et al., 2013
2.	Wheat	Zn	<i>OshMA1</i>	Lee et al., 2007
		Zn, Fe	<i>OsNAS2</i>	Singh et al., 2017
		Zn, Fe	<i>phy A</i>	Abid et al., 2017
		Zn, Fe	<i>phy A</i>	Brinch-Pedersen et al., 2000
		Fe	<i>TaVIT2</i>	Connorton et al., 2017
3.	Soybean	Fe	<i>GmFerritin</i>	Borg et al., 2012; Xiaoyan et al., 2012
		Zn	<i>NAM-B1</i>	Connorton et al., 2017
4.	Barley	Fe, Zn	<i>Phytase</i>	Gao et al., 2007
5.	Maize	Fe	<i>AtZIP1</i>	Ramesh et al., 2004
6.	Maize	Fe	<i>Ferritin and lactoferrin</i>	Drakakaki et al., 2005
		Fe	<i>brz and dgl</i>	Rogers, and Guerinot, 2002
7.	Tobacco	Fe	<i>AtNAS1</i>	Douchkov et al., 2005
	Tobacco	Iodine	<i>HMT, S3H, and SAMT</i>	Halka et al., 2019
8.	Arabidopsis	Fe	<i>AtNRAMP3 and AtNRAMP4</i>	Lanquar et al., 2005
	Arabidopsis	Iodine	<i>hNIS</i>	Landini et al., 2012

## RESULTS AND DISCUSSION

### Iron (Fe)

Rice, wheat, and maize are targeted in large biofortification programs to address micronutrient inadequacies since they provide more than half of the caloric need globally (Brinch-Pedersen et al. 2007). Lactoferrin (a Fe-chelating glycoprotein) and ferritin were used in experiments to raise the Fe content of the endosperm (Kanyshkova et al. 2001). Lactoferrin is abundant in human milk (1-2 g/l) (LF). Nandi et al. 2002, created transgenic rice grains with the human LF gene under the control of the rice glutelin-1 promoter to boost Fe content for future use in a newborn formula. Heterologous protein expression was much higher than the control, reaching 0.5 percent of grain weight, and bioavailability was validated using a human Caco-2 bioassay. Lee et al. 2010, found that the hLF gene was expressed in transgenic japonica rice and that it accounted for about 1.5 percent of total soluble protein. Ferritin, a localized protein found in plant plastids, is a significant non-toxic Fe storage form that can release Fe as needed for metabolic processes. Ferritin is a widespread protein that stores around 4,500 Fe atoms in an accessible state (Darbani et al. 2013). As a result, increased Fe accumulation via ferritin gene expression regulated by endosperm-specific promoters is critical for Fe biofortification.

According to research, overexpression of ferritin in a variety of crops increased Fe content and bioavailability. Goto et al. 1999; Lucca et al. 2001; Drakakaki et al. 2000; Borg et al. 2012; Drakakaki et al. 2005; Lucca et al. 2002; Vasconcelos et al. 2003; Qu et al. 2005; Aluru et al. 2011) produced rice transformants of SoyferH1 under the endosperm-specific GluB1 rice promoter to boost Fe accumulation in the endosperm of brown rice seeds, and showed a threefold increase in grain Fe content compared to non-transformed lines. Under the control of the maize ubiquitin promoter, soybean ferritin cDNA was also transferred in wheat and rice (Drakakaki et al. 2000). Due to the substantial role of leaves as a sink, the resulting transformants had higher Fe content in leaves than seeds. That extra ferritin sequesters Fe in the leaves, decreasing Fe mobilization to the seeds, is also a possibility. Qu et al. 2005, used a strong endosperm-specific globulin promoter to introduce soybean ferritin into rice, resulting in a 13-fold increase in ferritin protein expression above Goto et al. 1999. The Fe content, on the other hand, had just a modest enhancement (30%). These findings revealed that, in addition to increased Fe storage, higher Fe transport from the soil and greater translocation within the plant system are required (Masuda et al. 2012).

Genetic engineering proved to be a promising technique for Fe biofortification in cereals in rice trials. In comparison to rice, however, very little work has been done to enhance the Fe content of wheat and maize grains, particularly in the endosperm. Borg et al. 2012, cloned and analyzed wheat ferritin genes (TaFer1-A) and demonstrated that ferritin overexpression in the endosperm of wheat can enhance Fe concentration. The TaFer1-A gene was over-expressed in the endosperm under the control of the HMW glutenin 1DX5 promoter, resulting in a 50-85 percent increase in wheat grain content. (Table 1)

- 1) Iron-binding protein gene insertions:** The first transgenic approach involves putting an iron-binding protein gene (lactoferrin) under the control of an endosperm-specific promoter in the seed that is consumed by humans. This research focuses on plants or crops that store iron in their seeds. For example, several attempts have been undertaken to increase the iron content in rice grains. Rice has several advantages, including low allergenicity and the absence of toxic compounds that interfere with gene expression (Suzuki et al. 2003).

Researchers were able to successfully express human lactoferrin in de-husked rice using a potent endosperm-specific promoter. It resulted in a 120 percent increase in iron content, which was appropriate for supplementing neonates; however, because one molecule of lactoferrin can only bind to two ferric ions, it was still insufficient to meet the daily needs of adults (Suzuki et al.

2001). Soybean ferritin boosted the amount of iron in wheat by 1.5 and 1.9 times, respectively (Borg et al. 2012; Xiaoyan et al. 2012).

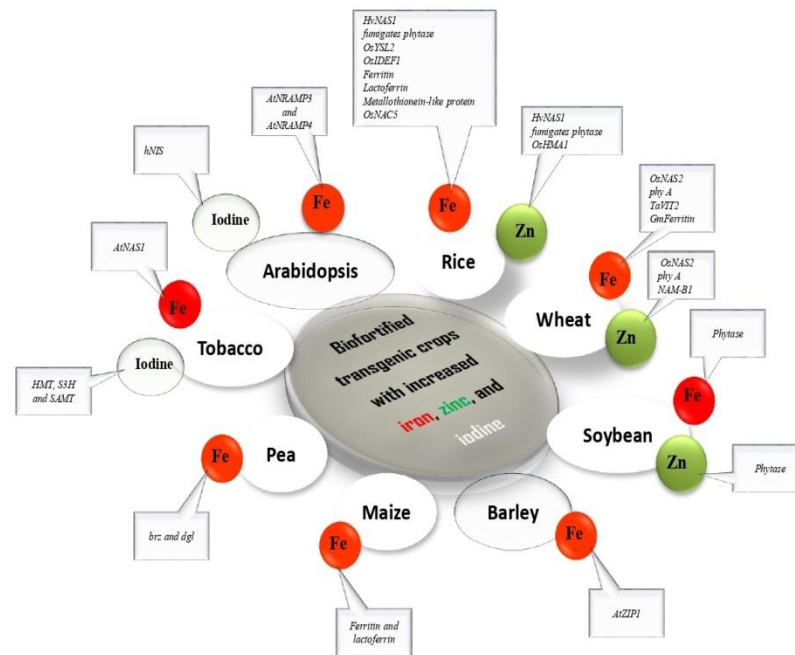
- 2) An iron-chelator gene was inserted:** Nicotianamine (NA) is a key iron chelator for Fe homeostasis and assimilation (Curie et al. 2009). The ferritin gene, when co-expressed with the nicotianamine synthase (NAS) gene, resulted in a 6-fold increase in Fe content in rice (Table 1), which is higher than the single-gene technique (Wirth et al. 2009). Another study found that transgenic rice seeds with *OsNAS3* expression had a 7-fold increase in Fe concentration.

Anemic mice fed these transgenic rice seeds recovered from iron deficiency and anemia after four weeks, whereas mice fed non-transgenic rice seeds did not. *HvNAS1* overexpression in transgenic rice caused by the *OsActin1* promoter/35S promoter resulted in a 5- to 10-fold increase in endogenous NA levels in the shoots and seeds, as well as a 3-fold increase in Fe content in T1 seeds (Masuda et al. 2009). Similarly, transgenic tobacco with overexpression of nicotinamide synthases (*AtNAS1*) showed increased Fe levels (Douchkov et al. 2005). In comparison to most current wheat varieties, biofortified pearl millet has a 2-fold increase in iron. Around 35 million people who eat biofortified pearl millet have had a 5-10% improvement in iron absorption (Cercamondi et al. 2013; Sahu, 2017).

- 3) Increased production of enhancers that improve Fe absorption:** Some foods have been shown to help with iron absorption. This includes vitamins like b-carotene, ascorbic acid, and tocopherol, as well as amino acids derived from proteins after digestion. Ascorbic and citric acids have been found to reduce Fe to a ferrous form and improve absorption in the small intestine. As a result, transgenic approaches for over-expressing ascorbic acid and ferritin can be used (Gropper et al. 2005). Higher cysteine concentrations have also been shown to improve Fe absorption (Layrisse et al. 1984). Rice cysteine concentration increased 10-fold after overexpression of rice metallothionein-like protein. It has the same 12 cysteine residues as metallothionein per mol of protein (Lucca et al. 2001). Iron absorption has been reported to be boosted by golden rice with high levels of b-carotene (Beyer et al. 2002).

### Zinc (Zn)

Over 300 enzymes and 1000 transcription factors require zinc as a cofactor (Palmgren et al. 2008). The natural variation of grain Zn content in cereals is modest (Sharma et al. 2015). As a result, boosting the zinc content of cereal grains is critical for human nutrition and metabolism. Manipulation of the Zn content in cereal grains, on the other hand, may be more difficult than manipulating the Fe content (Brinch-Pedersen et al. 2007). Ozturk et al. 2006 found high connections between protein content, Fe, and Zn content in their study. *Gpc-B1* (*GRAIN PROTEIN CONTENT B1*) is a wheat quantitative trait locus linked to higher grain protein levels as well as higher Zn and Fe levels (Uauy et al. 2006). After introducing the *Gpc-B1* locus from the wild tetraploid wheat (*Triticum turgidum* ssp. *dicoccoides*) into different recombinant chromosome substitution lines into cultivated wheat, an increase of 10-34 percent in grain Zn, Fe, Mn, and protein was observed, indicating the role of *Gpc-B1* in the remobilization of protein, Zn, Fe, and Mn from the leaves to the grains (Distelfeld et al. 2007).



**Figure 2.** Improvement of Iron, Zinc, and Iodine Involve in the Various Genes/Proteins

Overexpression of genes involved in Zn translocation and mobilization resulted in improved Zn bioavailability without a yield penalty, which is an important strategy to improve grain Zn. Many cation transporters have been discovered in rice, but only a few have been studied in terms of substrate selectivity, expression pattern, and cellular localization. Members of the *ZIP* (*ZRT*, *IRT*-related protein) and *CDF* (Cation diffusion facilitator) families are the most common cation transporter families identified as being important in Zn uptake and translocation. In *A. thaliana* root cells, the *ZIP* protein *IRT1* plays an important role in Zn absorption (Korshunova et al. 1999). Introducing 35S enhancer elements to overexpress NA synthase (*NAS*) resulted in 2-3 fold increases in Zn concentration in paddy (Lee et al. 2009). Similarly, polished rice grains from transgenic rice expressing the barley nicotianamine synthase gene *HvNAS1* under the control of the rice *actin1* promoter gathered 2-3 times more Zn (Masuda et al. 2009). Thousands of IR64 and IR69428 transformants are created at IRRI using soybean or rice ferritin and rice nicotianamine synthase (*NAS2*) overexpressed genetic constructs, and the Zn and Fe content in those lines has exceeded the goal level from field trials. As a result of the overexpression of *NAS* genes, nicotianamine is a promising target for Zn biofortification.

Furthermore, biofortifying grains using *NAS* alone or in conjunction with ferritin has a lot of promise in terms of addressing global human mineral deficiency (Lee et al. 2009; Zheng et al. 2010). Sufficient research has been done on several crop species, including wheat; rice, maize, and barley, to better understand the Fe and Zn pathways in grain. Despite numerous obstacles such as the root-shoot barrier and grain filling (Palmgren et al. 2008), wheat researchers use rice-developed technologies and resources to boost Zn content in wheat grain, resulting in improved wheat lines (Borrill et al. 2014). (Table 1)

- 1) Overexpression of the *NAS* gene family:** Nicotianamine is a transition metal chelator found in higher plants that is responsible for the transport of metal cations over short and long distances. The NA synthase (*NAS*) enzyme is involved in the S-adenosylmethionine trimerization process, which produces NA (Takahashi et al. 2003). In a variety of plant species (Table 1), including Arabidopsis, rice, maize, and barley, metal (Zn and Fe) profiles differentially influence genes that encode for *NAS* (Mizuno et al. 2003). Using recombinant DNA technology, the most frequent technique of raising NA concentration in a plant is to overexpress exogenous or endogenous *NAS* genes. Exogenous *HvNAS1* (barley *NAS* gene) overexpression in tobacco and Arabidopsis

resulted in several-fold increases in zinc, iron, and copper (Cu) concentrations in the seeds of both plant species (Kim et al. 2005). Overexpression of *HvNAS1* in rice resulted in a 15-fold increase in nicotianamine concentration (compared to wild type), as well as 1.5 and 2.5-fold increases in zinc and iron concentrations in polished rice grains, according to another study (Masuda et al. 2009).

Constitutive expression of *AtNAS1* (Arabidopsis NAS gene) in combination with endosperm-specific ferritin synthesis produced an increase in Fe and Zn concentration. Furthermore, a considerable amount of Zn and Fe was achieved by expressing the *OsNAS2* (endogenous NAS gene of rice) and Pv Ferritin (bean ferritin) genes in wheat grains (Singh et al. 2017). However, just a few studies on the utilization of endogenous NAS to enhance Plant NA expression for better metal uptake in plants have been published.

The endogenous NAS genes *OsNAS1*, *OsNAS2*, and *OsNAS3* were overexpressed in rice, resulting in considerable increases in NA, Fe, and Zn concentrations in the endosperm of all three transgenic populations (Wirth et al. 2009). The *OsNAS2* overexpressing population showed 20 and 2.7-fold greater NA and Zn concentrations than the wild-type population in different research. In polished rice grains, overexpression of *OsNAS3* led to a 9-fold rise in NA, a 2.2-fold increase in Zn, and a 2.6-fold increase in Fe concentration (Lee et al. 2011).

**2) Overexpression of the NAC gene family:** Plant senescence is influenced by NAC transcription factors. Before dying, a full crop/plant, or a portion of it, remobilizes nutrients to younger tissues or seeds. Increased Zn and Fe remobilization has been associated with senescence in studies. NAM-B1 (a member of the NAC transcription factors) has been discovered to play a key role in the early beginning of senescence in wheat, resulting in higher Zn levels in grains (Connorton et al. 2017). Phytic acid levels in the body are being reduced. Phytic acid (also known as phytate) is an inhibitor and antinutrient molecule that chelates minerals (Zn and Fe) and lowers their bioavailability, making it the leading cause of mineral shortage globally (Zhou and Erdman, 1995).

Because humans lack the intestinal phytase enzyme, phytic acid forms insoluble complexes in the gastrointestinal tract with metal ions, particularly Zn and Fe, which may not be absorbed or digested (Iqbal et al. 1994; Gibson et al. 2010). Phytate can form compounds with endogenously generated minerals like Zn by blocking their reabsorption into the body. To prevent mineral shortage, the amount of phytic acid in edible parts of staple crops must be reduced to increase mineral absorption. According to research, lower phytate levels in the diet are connected to better zinc (Barbro et al. 1985) and iron (Hallberg. 1981) absorption. In phytase, transgenic wheat lines demonstrated a 4-115 percent increase in bioavailable zinc (Abid et al. 2017).

## Iodine

Iodine is essential for the production of thyroid hormones triiodothyronine and thyroxine, making it a critical mineral for human health. More than 2 billion people worldwide are influenced by insufficient iodine intake (Delange 1994; Zimmermann and Boelaert 2015). Thyroxine and triiodothyronine are two hormones that play a vital role in metabolic control. Iodine deficiency reduces the synthesis of these hormones, resulting in the growth of thyroid tissue, also known as goiter. More than 187 million people worldwide suffer from goiter caused by iodine deficiency (Greer et al. 1968; Vos et al. 2012). Further to that, iodine deficiency during pregnancy may interfere with the neurodevelopment of the offspring, while it affects somatic growth and cognitive skills during childhood (Zimmermann and Boelaert, 2015). Adding iodine to table salt is a common strategy for preventing iodine deficiency when compared to other fortification techniques (Gonzali et al. 2017).

Biofortification of crops with iodine, on the other hand, is a more promising strategy for combating mineral malnutrition because it is more sustainable and cost-effective (Garg et al. 2018). In recent decades, several micronutrients, including iron (Wirth et al. 2009), zinc (Abid et al. 2017), and folate, have been successfully fortified into staple crops (Storozhenko et al. 2007). Notwithstanding, no reports of recombinant DNA-based iodine biofortification in crops have yet been published. This is due to a lack of understanding of iodine physiology in plants. According to some theories, iodine moves across the cell membrane of root cells via anion channels and possibly H<sup>+</sup>/halides transporters. Such transporters, however, have yet to be discovered at the molecular level. Iodine volatilization from above-ground plant parts such as the root and leaf, on the other hand, is well documented in a wide range of species. The action of halide ion methyltransferase (HMT) and halide/thiol methyltransferase (HTMT) causes iodine volatilization (Itoh et al. 2009).

The only study that used genetic techniques to improve iodine concentration was on transgenic Arabidopsis. In this study, the *CaMV 35S* promoter was used to over-express the human sodium-iodide symporter (*hNIS*) gene of thyroid glands, resulting in increased iodine uptake. Further to that, the *HOL-1* gene, which encodes for the HMT enzyme (which causes iodine volatilization), was knocked out, and transgenic plants showed a significant reduction in volatilization when compared to wild-type plants (Landini et al. 2012). (Figure 2)

## CONCLUSION

Micronutrients are essential for human nutrition, especially when it comes to treating malnourished children and women. Micronutrients like Fe, Zn, and Iodine are heavily used by biofortification programmers. Transgenic breeding is a strategic tool that can increase the content of these micronutrients in staple grains by several folds. Because genes for those desired qualities are available, it is conceivable to increase micronutrients in many staple crops. Trait-specific techniques have provided proof of concept for improving micronutrients through transgenic. It may be able to employ a combination of genes to improve micronutrients at the same time. After regulatory issues are resolved, transgenic crops will be widely planted to combat malnutrition. Furthermore, genome editing techniques for plant genes, such as *CRISPR-Cas*, *ZFN*, *TALEN*, and others, have recently shown significant promise in crop development. Crop biofortification should also be made possible with genome editing techniques. Though genome editing-based biofortification is still in its early stages, it should be widely used to accelerate biofortification in cereals and horticulture crops, particularly vegetables. Biofortified crop types with improved nutritional properties, on the other hand, must be tested in clinical studies for bioavailability and impact on end-user health.

## ACKNOWLEDGEMENT

Department of Crop Physiology, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (Uttar Pradesh) India, for our department teachers, thanks a lot for writing this review article and for the suggestions made.

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