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Biofortification of iron in rice

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Abstract

Agricultural cultivars with higher concentrations of micronutrients may help the iron deficiency that affects more than two billion people (biofortification). Fe content in rice seeds may be increased by a variety of transgenic methods, which we discuss in detail in this work. Twofold increase in Fe content in converted seeds emerged from this method's implementation.

Keywords: Biofortification, iron, zinc, transgenic rice

Introduction

Micronutrient iron (Fe) is essential for all living things, plants included. Many individuals die each year from an iron shortage that affects two billion people throughout the globe, according to Stoltzfus. Organization for the Prevention of Malaria Malnutrition is the sixth leading cause of death and disability in underdeveloped nations, according to the (WHO 2002) [80].

Plants and animals alike need iron in order to maintain appropriate blood flow (Fe). An estimated 0.8 million people die each year as a result of micronutrient malnutrition, which affects two billion people throughout the globe. (Stoltzfus *et al*. 1998) [67]. It is estimated that anemia is the sixth leading cause of death and disability globally (WHO 2002) [80].

Iron deficiency affects as many as two billion people worldwide, with the majority living in the United States. Iron deficiency may lead to anaemia, heart attacks, and other cardiovascular problems. There is an abundance of fertilizer in the soil, but it is useless due to its low solubility, which is particularly problematic in alkaline soils. Agricultural and fruit production suffer from Fe shortage in 30 percent of the world's land, according to estimates. Soils with low Fe levels and symptoms like yellowing in early leaves (chlorisis) may have had a part in it, as well. If the symptoms are severe, any or all of these therapies may be beneficial: dietary modifications, micronutrient supplements, prescription medicines or even surgery. Some people are not able to benefit from this treatment due of geographical and financial limitations. It is difficult to fortify food with iron since the more soluble and absorbable iron compounds (like FeSo4) modify the taste and color of fortified meals while the less absorbable iron compounds (like Fe4 (P2O7) 3).

At the beginning, rice was farmed in China because it was an excellent source of energy. As a result, wheat has more protein. In 6-7 percent of the samples, the protein has been identified. Rice is a low-fat meal option (2-2.5 percent). More than 60 percent of the world's population relies on it as their primary source of nutrition.

Because of this, biofortification of rice provides a viable solution to iron deficiency anemia in countries where rice is farmed as the primary crop (WHO 2002; Juliano 1993) [80, 31]. In terms of nutritional content, brown rice is unmatched. It is common for individuals to substitute polished rice with mineral-depleted endosperm tissue (Grusak and Cakmak 2005) [15]. Biofortification may be used to improve the iron content of puffed grain.

People with micronutrient deficiencies may benefit from the use of supplements, dietary fortification, and biofortification methods. Inadequate intakes of micronutrients have been significantly reduced thanks to widespread use of nutritional supplements and food fortification strategies. Micronutrient supplements and fortified meals may not be readily available in rural areas, making these treatments less effective. Instead, rural areas may lack buying power, market access, and health care accessibility. Many projects were unable to be finished as a result of rising costs and other challenges. There is no post-harvest processing or specialized equipment required for this form of biofortification (i.e., improving the bioavailability of essential components in edible sections of agricultural plants by traditional breeding or genetic engineering). Due to its low cost and longevity, biofortification serves as a benefit to both individuals and governments (Mayer *et al*. 2008) [47]. It may be possible to remedy micronutrient deficiencies by supplementing and fortifying diet. For those unwilling to change their diet due to cultural, religious, or economic reasons, biofortification may be a big help.

On the other hand, traditional plant breeding is focused on finding and choosing parent lines that share desirable traits with both parent species. A good example of this is the iron bean, a factory-bred plant with a high iron content and bioavailability that also produces a significant quantity of iron. There are additional advances in biotechnology such as marker-assisted selection. The precision and speed with which promising lines in the daughter plants may be found has been improved.

More and more scientists are exploring the potential advantages of genetic alteration thanks to recent advancements in genetic engineering techniques. It is possible to remove, modify, or add segments of the plant genome using genetic engineering in order to enhance the adaptability of the plant. An in-depth knowledge of iron absorption is essential for successful genetic engineering.

Nutrition, vitamins, and medical supplies are supplied to both rural and urban inhabitants by the government and non-governmental organizations (NGOs). If you do not have the money to keep it up and running, it is not going to be a long-term success either. Since micronutrient deficiency in the food supply may be remedied quickly and cheaply via biofortification, it has gained popularity as a solution.

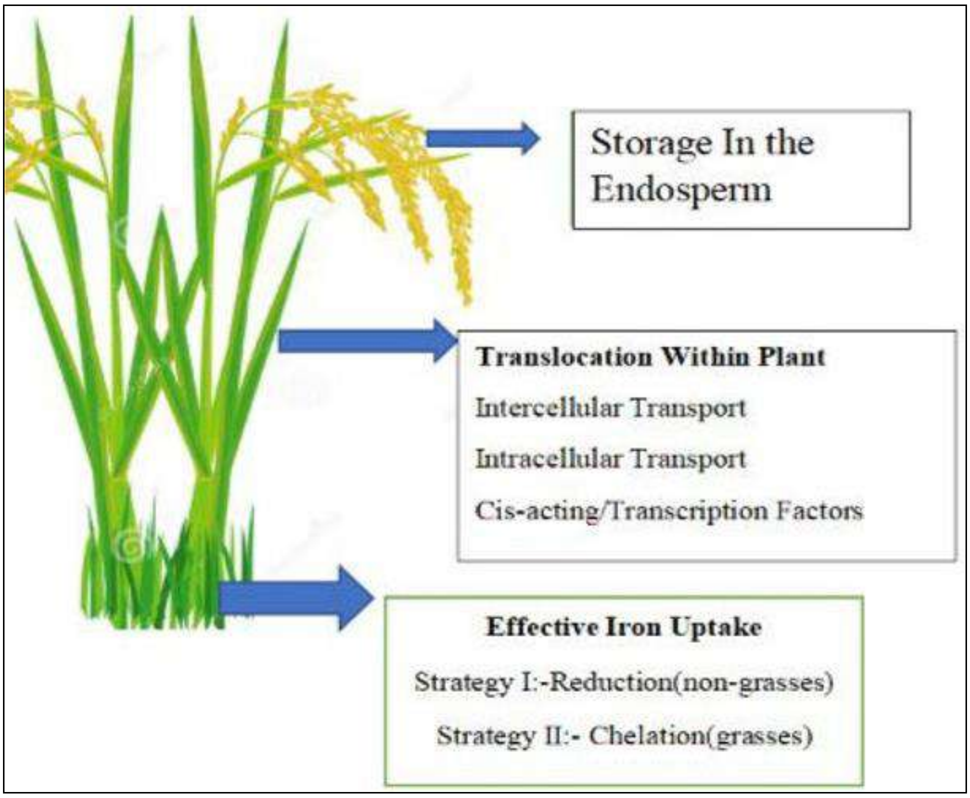


Fig 2: Iron biofortification strategies

"Approach 1: Increasing Fe accumulation in seeds by expressing the Fe storage protein, ferritin gene, SoyferH1 and SoyferH2, using endosperm-specific promoters."

Increasing the ferritin gene's expression may raise the Fe content of rice seeds. Growth-promoting substances particular to the endosperm 4,000 iron atoms may be stored in a single ferritin molecule, which is a protein (Theil 2003) [74]. Fertilizer may be more readily absorbed by the human digestive system with the aid of soybean ferritin (Lonnerdal 2009). By analyzing how the human body absorbs Fe from the supplement ferritin, it is feasible to understand how it does so. Humans need ferritin in their diets to prevent iron shortage symptoms from developing (Theil *et al*. 2011, 2012) [75, 76].

It was found that the rice endosperm-specific, 1.3kb OsGluB1 promoter was exploited to drive increased expression of the SoyferH1 gene in transgenic rice plants A two fold increase in Fe accumulation was seen in the transformants, compared to the control group. The endosperm's Fe content was also increased by twofold. Multiple studies have indicated that rice endosperm may significantly enhance yield, such as the 2.2-fold increase in brown seeds of transgenic japonica cv. Taipei 309, the 3.7% increase in polished IR68144, and the 2.1% increase in polished rice cv. Pusa Sugandhi II (Luca *et al*. 2002). Those findings are supported by Paul's and his team's study (Paul *et al*. 2012) [57].

If two endosperm-specific promoters were used, SoyferH1's Fe concentration was boosted by a factor of two in the seeds.

Ferritin expression was not substantially increased in rice seeds when many promoters were used, compared to the experiment described above when just one endosperm-specific promoter was used (Qu *et al*. 2005) [60].

Ferritin injections alone did not raise the Fe content of brown seeds in transgenic rice, as previously found (Masuda *et al*. 2013) [46]. In transgenic plant leaves, Fe deficient symptoms were seen with the introduction of a single gene, ferritin (Qu *et al*. 2005) [60].

Increasing rice grain Fe concentrations only by ferritin overexpression may not be effective. Rice's endosperm can only transmit a limited amount of Fe to the grain. Because it contains more iron than other rice tissues including the husk, husk, and the aleurone layer, the endosperm is used to manufacture rice bran. Fe concentrations in brown seeds and husks of Tsukinohikari rice were both higher than those found in polished seeds (endosperm), however only 0.5 percent of the plant's total Fe content was found in the polished endosperm. The work of Masuda and his associates (2008). However, even while rice plants are capable of absorbing some iron, it may not be in a form that is beneficial. This shows that rice plants may have some type of complicated mechanism in place to control the absorption of Fe by their endosperms. As a result of this, the Fe concentration in polished seeds may not be considerably higher. Fe biofortification of rice seeds requires not only more seed Fe, but also greater soil Fe absorption and enhanced plant body Fe translocation.

These treatments (numbers 2 to 7) are considered an approach for increasing the amount of Fe in rice endosperm.

“Approach 2: Overexpression of the nicotianamine synthase gene NAS improves Fe transport inside the plant body.”

“Rice's Fe retention, transport, and homeostasis atomic cycles have recently been explained (Bashir *et al*. 2010) [5], (Kobayashi *et al*. 2012). Increased levels of iron and zinc-chelating cation NA in rice were brought about by the expansion of the SAMS and SAMS characteristics in rice (Higuchi *et al*. 1994) [19]. There is a transfer of Fe and other metals across all higher plant cells through NA (Hell and Stephan 2003). There are several similarities between the tomato chloronerva freaks and the tomato chloronerva freaks that lack NA. The paper (Rudolph *et al*. 1985) [61] claims that (Takahashi *et al*. 2003) [72] were responsible for creating NA-lacking transgenic tobacco plants. Quality of grain NA aminotransferase HvNAAT-A grain NA aminotransferase (Rudolph *et al*. 1985) [61]. (NAAT). Fe and Zn levels were observed to be significantly reduced in the leaves and blooms of both plants due to a malfunction in the inner metal vehicle of the juvenile leaves. When HvNAS1 was overexpressed in tobacco plants, the amounts of Fe and Zn in their leaves, flowers, and seeds increased.

In rice, researchers discovered three NAS properties. Fe's ability to travel long distances is transmitted in the cells concerned (Inoue *et al*. 2003) [24]. Rice Phyto siderophores and long-distance Fe transport have been shown to be affected by the activity of NAS and NA in this study. (Higuchi *et al*. 2001) [22] used CaMV35S, an advertisement from the cauliflower mosaic virus, to generate HvNAS1 in rice. This transgenic rice sprout has a threefold increase in nitrate (NA) content. We hypothesized that increased NAS expression in plants would lead to an increase in the iron content of seed coats. As a consequence, we were able to produce OsActin1 or 35S promoter-driven HvNAS1 transgenic rice (Masuda *et al*. 2009) [44]. HvNAS1 expression and NA levels increased 5- to 10-fold when we employed HvNAS1-overexpressing transgenic rice. FOUR times more Fe and twice as much Zn were found in T1 polished seeds from transgenic plants. When seeds were polished in T2, Fe content rose significantly. Fertility of polished rice seeds was increased by a ratio of 3–4 when the NAS gene was overexpressed, as reported by (Lee *et al*. 2011). Plants may be able to increase Fe transport via the phloem by boosting NAS expression. Studies by (Lee *et al*.2009) [37-39] and (Johnson *et al*. 2011) [30] have been published on this subject. Lee et colleagues found that mice fed enhanced rice seed with an OsNAS3 enhancer tag had reduced anaemia caused by Fe deficiency.

When rice was overexpressed with HvNAS1, the levels of NA and DMA were much greater than in control rice. NAAT and DMAS synthase (DMAS) are used by plants like rice to synthesize DMA (Takahashi *et al*. 1999; Bashir *et al*. 2006; Inoue *et al*. 2008) [70, 5, 25]. As a consequence of AtNAS1 overexpression coupled with Pvferritin endosperm expression, the levels of OsSAMS2, OsSAMS1, OsNAS1, OsNAS3, and OsDMAS1 in rice roots were enhanced (Wang *et al*. 2013) [79]. Rice may be creating more DMA as a consequence of the increased NAS and DMAS expression in rice. It was iron-deficient soil that boosted DMA concentrations in rice xylem sap (Kakei *et al*. 2009) [31]. As a result of this process, DMA may transport Fe to other tissues through xylem. Femininity is possible when DMA is used in conjunction with other fertility-enhancing methods.

Expressed in the root stele and epidermis, OsYSL15 is an iron transporter that helps the plant carry Fe (III)-DMA around (Inoue *et al*. 2009) [26]. In reproductive tissues and lamina joints, the Fe (III)-DMA transporter OsYSL18 was found. Fe (III)-DMA transporters known as OsYSL16 may be found in the root epidermis and vascular bundles of the whole plant, according to (Kakei *et al*.2012) [32]. Using rice phloem sap as an example, researchers have discovered that DMA is essential for the long-distance transfer of Fe (III). A new study suggests that DMA and NA may play a role in the transfer of Fe from rice roots to rice seeds. Fertilization of rice crops with excessive NA or DMA may increase the transfer of Fe and Zn into the grain. DMA levels in rice are high, which may help increase root DMA synthesis and hence soil iron absorption. Fe and Zn may be more easily accessible to plants if the NAS gene expression is increased. DMA concentrations may rise as a consequence of rice grain NA synthesis and subsequent accumulation, however this is not a definitive outcome (Masuda *et al*. 2009) [44]. Root DMA secretion might be promoted as a result of increased DMA concentration in rice, which could lead to an increase in the soil's ability to absorb iron. Rice grain Fe and Zn contents may increase as NA and DMA expression increases.

“Approach 3: By expressing the Fe (II)-NA transporter gene OsYSL2 under the control of the OsSUT1 promoter, we were able to increase Fe influx to seeds.”

It is possible to boost crop iron concentrations by the reduction of membrane transporters (Schroeder *et al*. 2013) [63]. Using the OsYSL2 3 gene, the Fe (II)-NA transporter gene expression in rice seeds was increased. According to Koike *et al*. 2004, the OsYSL2 gene is expressed in the cell walls of rice leaf phloem cells as well as the cells of flower vascular bundles and developing seeds. There was an 18 percent decrease in brown seed Fe concentrations and a 39% decrease in polished seed Fe concentrations in OsYSL2 knockdown rice (Ishimaru *et al*. 2010). To put it another way, OsYSL2 is critical in the transport of Fe to rice seeds.

Due to the increase in OsYSL2, we hypothesized that rice seed Fe concentrations would increase. Root ferrochrome levels were increased by CaMV35S promoter-controlled overexpression of OsYSL2, despite the fact that seeds had a lower ferrochrome content. Overexpression of OsYSL2 in rice may interfere with Fe translocation.

Flag leaf and rachis phloem and OsSUT1 sucrose transporters were discovered (Scofield *et al*. 2007) [81]. OsSUT1 was found to be expressed at a greater level as the seedlings and panicles expanded (Aoki *et al*. 2003) [2]. However, the sucrose absorption and seed germination rates of the antisense OsSUT1 rice were dramatically decreased. (OsSUT1 transfers sucrose from the phloem to the seed and was discovered by Ishimaru and his coworkers in 2001) [27]. It is possible that the OsSUT1 promoter may be used to control rice seed metal levels. As a result, whereas Cd concentrations were reduced by 50% in brown seeds containing a promoter for OsHMA2, Zn concentrations increased by 20%.

It was decided that using the OsSUT1 promoter in rice seeds would be an effective way to boost Fe (II)-NA translocation in these seeds. As it turned out, this was an effective strategy. Up to four times as much Fe could be incorporated into polished rice seeds by applying the OsSUT1 promoter. A group of assailants led by It shishimaru (Ishimaru *et al*. 2010). Increasing rice seed Fe content by inducing OsYSL2 expression in rice seeds has been recognized as one of the most effective approaches.

Combination of Approaches 1–3

Gene addition to Japonica 309 rice using CaMV35S promoter-AtNAS1 and Globulin promoter-Pvferritin was accomplished by combining the first and second steps, according to Wirth *et al*. Gp-Afpytase gene cassette decreased rice seed levels of phytate and increased iron bioavailability. Fe concentrations in hydroponic cultures with a range of Fe nutritional circumstances rose up to six times using this rice, known as NFP rice, according to the research. Fe-biofortified rice was generated by combining these three methods. As of this writing, rice lines (Japanica. Tsukinohikari; Fer NAS YSL2 lines) have been produced utilizing the OsGluB1 and OsGluB1 promoter–SoyferH2 gene cassettes respectively (Masuda *et al*. 2012) [45].

For T2 seeds, a non-transgenic line increased Fe content six fold, while a transgenic Fer-NAS-YSL2 or OsActin1 promoter-HvNAS1 line tripled the amount. It was tested in a rice field to see whether Fer-NAS-YSL2 lines could produce more Fe-enriched rice seeds. As an isolated paddy zone, T2 lines were planted to achieve this. No yield loss was seen when T3 polished paddy field seeds were compared to non-transgenic seeds, despite having a Fe concentration that was almost four times greater. Compared to non-transgenic seedlings, transgenic seedlings accumulated Zn at a rate 1.6 times higher. There may be more success in achieving fe balance with the addition of additional fe balance-related genes. Growing crops in greenhouses and paddy fields with Fe biofortification is more effective than doing so with individual genes in soil.

It was shown that the inserted genes ferritin, HvNAS1, and OsYSL2 worked in concert to boost Fe levels in the FerNAS-YSL2 rice crop. An increase in tissue levels of NA and DMA was seen after overexpression of the NAS gene. This made it possible to efficiently synthesize iron-containing peptides such as Fe (II)–DMA and Fe (III)–DMA. Increased vascular iron (Fe) transport was also seen in plants that overexpressed N-acetylsalicylic acid (NAS). In phloem sap, the OsYSL2 transporter was successfully used by the OsSUT1 promoter to transfer Fe (II)–NA to its final destination. Ferrous nitrate may be transported into cells by OsYSL2 produced by endosperm cells via the OsGlb1 promoter. Another example of this is the OsGlb1 promoter and ferritin, which is generated by the OsGlb1 promoter. According to research conducted in seed endosperm cells, the transcription factor OsGluB1 is responsible for iron accumulation (Fe). Fer-NAS-YSL2 rice polished seeds had a greater Fe content than rice polished using a single transgenic method (Masuda *et al*. 2012) [45].

Iron deficiency is a common cause of anemia in Myanmar (MOH 2003) [49]. Burma has one of the world's highest per capita rice consumption rates, at 578 grams a day (Maclean *et al*. 2002) [42]. Strengthening the grain by adding Fe to the rice is an excellent idea. The Fer-NAS-YSL2 gene was successfully introduced into the Myanmar rice variety Paw San Yin with the aid of this study (Aung *et al*. 2013) [4]. For Myanmar's population, a 3.4-fold increase in Fe content in polished seeds was achieved using this transgenic rice.

"Approach 4: Introducing the phytosiderophore synthase gene IDS3 to improve Fe absorption and translocation"

Soil-retention by Graminaceous Plants is made possible by the Phenotypic Siderophores of the Mucineic Acid Family (MAs), a naturally occurring iron (III) chelator (Takagi 1976; Mihashi and Mori 1989) [69, 48]. It has been revealed that a fundamental component of MAs has been uncovered. It is possible to produce DMA from NA or other MAs utilizing DMA from grains that include IDS2 and IDS3 clones (Nakanishi *et al*. 2000);(Kobayashi *et al*. 2001) [51, 35]. During plant establishment in the rhizosphere, MAs are released, which chelate insoluble iron. Graminaceae are plants that produce grain (III). One of the carriers moves Fe (III)-Mas edifices to take root (yS1 or YSL). Remember that OsYSL15 used the TOM1 carrier of mugineic corrosive family phytosiderophores to deliver DMA to the Fe (III)-DMA edifices (Curie *et al*. 2001) [9]. For further information, please see (Nozoye and colleagues, 2011) [53]. It is been shown by a number of researchers (Lee, *et al*., 2009) (Inoue *et al*., 2009) [26, 37-39].

Because of its strong MA synthesizing capabilities, grain root is one of the safest graminaceous plants for Fe insufficiency. HvNAS1, IDS2, and IDS 3 are three of the characteristics. As a result of these and other studies (Higuchi *et al*.1999), (Takahashi *et al*. 1999) [70, 21], Nakanishi *et al*. Rice only emits DMA as a chemical by product. Rice is viewed as a better option when it comes to dealing with a lack of Fe (Kobayashi *et al*. 2001) [35]. Because of the combination of qualities contained in the MA mix that help in the absorption and transportation of Fe, a new technique of Fe biofortification has been developed as a fourth option. The HvNAS1 and HvNAAT-A/B transgenic rice lines, which possess the mungiineic corrosive, were created using gene components from the grain genome (Higuchi *et al*. 2001; Kobayashi *et al*. 2001; Masuda *et al*. 2008; Suzuki *et al*. 2008) [43, 35, 23,]. Transgenic lines for paddy field agriculture were evaluated in Fe-rich and calcareous soils. Fertilizer-deficient soils yielded seeds from IDS3 rice with 1.4 and 1.3 times better maturity than non-transgenic rice the hue of these seeds was clear and earthy. When planted in soils with restricted Fe accessibility, IDS3 rice lines produced 1.3 percent more earthy-colored rice seeds. DMA is no longer necessary to make MA in rice because Fe (III) edifices are more stable in MA than DMA at moderately acidic pH levels, therefore rice may benefit from IDS3's creation of MA. Thus, the grain genomes of these transformants had areas that regulated the flow of promoters for MA biosynthesis quality. The roots and leaves of these rice plants are used to generate a record via manure exhaustion by these rice marketers (Higuchi *et al*. 2001; Kobayashi *et al*. 2001; Takahashi *et al*. 2001) [71, 35, 23]. When there are more Fe-centered traits available, they are likely to be expressed.

"Approach 5: Overexpression of the Fe transporter gene OsIRT1 or OsYSL15 increased Fe absorption from soil."

Rice seed typically contains low levels of Fe (Grusak and Cakmak 2005) [15]. Reduced Fe concentrations are a consequence of endosperm milling and sanding. Those who live in rural areas tend to eat more rice than those who live in cities (Juliano, 1993) [31]. Fe concentrations in polished rice grains should thus be increased. "In China and Southeast Asia, the typical individual consumes 300 to 600 grams of rice per day (Maclean *et al*. 2002) [42]. Mature females need between 15 and 18 milligrams of Fe a day (Food and Nutrition Board 2001) [10].

People who eat 600 grams of rice per day need to take 7 to 14 milligrams of iron in each gram of polished rice. This would provide one-fifth of the body's daily Fe requirements. According to Pfeiffer and McClafferty (2007) [58], a target level of Fe biofortification is proposed at this level. The paddy fields produced a broad variety of indica rice varieties between 1% and 2% Fe (Aung *et al*., unpublished data). To achieve the criteria, the concentration of Fe must be increased four to ten times. As a result, this aim was not achieved when just procedures 1-4 were used. Procedures 1-3 resulted in a six-fold improvement in performance. Then then, this may not be enough in other cases. Three novel strategies for increasing seed Fe biofortification have emerged as a result of this research over expressing an iron transporter is a last method for enhancing iron absorption.

In previous experiments, researchers found that seeds with overexpressed Fe transporters had larger levels of Fe in them. OsIRT1 was produced via an ubiquitin promoter in Lee *et al*. 2009 [37-39] transgenic rice.

While the Fe content of brown seeds rose by 13%, the Fe content of leaf tissue rose significantly. In order to boost the iron content of rice grains, OsIRT1 may be used. A 1.3-fold increase in Fe concentration was found in earthy-colored rice seeds compared to non-transgenic rice when OsYSL15 was expressed using the OsActin1 promoter, according to Lee and his colleagues(2009) [37-39]. Researchers from (Gómez Galera *et al*. 2012) [12] created transgenic rice that expresses the HvYS1 grain Fe (III)-MA carrier quality. When compared to non-transgenic leaves, transgenic leaves had 1.5 times the amount of flavour.

Abundance HvYS1 articulation boosted rhizosphere Fe intake in a recent research, according to the results of that investigation. (Lee *et al*. 2009) [37-39] and (Gómez-Galera *et al*. 2009) found that overexpression of OsIRT1, OsYSL15, or HvYS1 enhanced Fe levels in leaves but had no impact on Fe levels in seeds. When OsIRT1 was overexpressed, Lee *et al*. 2009 [37-39] found that there were less "turners" in the cells they studied. Rice's metal homeostasis may be disturbed by the constant expression of OsIRT1; hence, they recommended that OsIRT1 expression be limited to select promoter regions. By alone, application of Approach 5 had no effect on seedling levels of ferrous iron (Fe). In addition to the procedure detailed here, other approaches to raising Fe levels are possible.

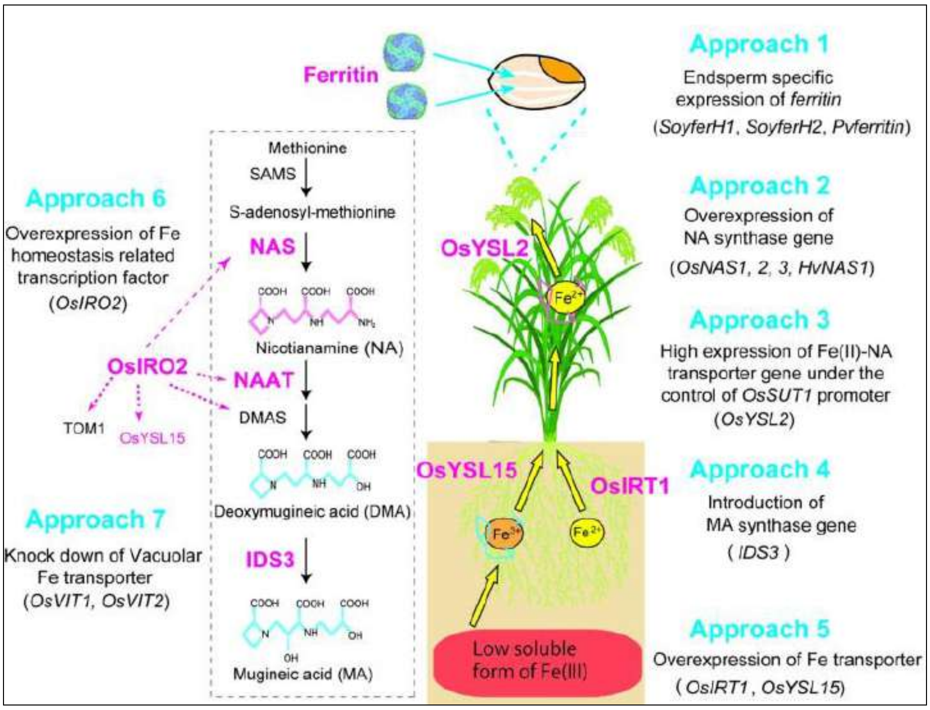
"Approach 6 Overexpression of the Fe homeostasis-related transcription factor increased Fe absorption and translocation. OsIRO2"

Involved in gene expression regulation. The OsIRO2 enzyme, discovered by Ogo *et al*, is activated when rice is depleted of Fe2. NAS1 and NAS2 are two MAs-related Fe uptake genes that are regulated by OsIRO2, for example (Ogo *et al*. 2007; 2011) [55, 56]. In order to enhance rice plants, use Ogo *et al.* CaMV35S. Non-transgenic rice could not overcome Fe shortage as well as rice transgenic for OsIRO2. Fertilizer content in transgenic brown rice seeds increased by twofold in calcareous soils. As a result, using this strategy might lead to increased earnings. With seeds cultivated in low-iron soils, iron (Fe) levels

"Approach 7: Knocking down the vacuolar Fe transporter genes OsVIT1 or OsVIT2 improved Fe transfer from flag leaves to seeds."

(Kim *et al*. 2006) [33] report that the VIT1 Fe and manganese transporter is abundant in Arabidopsis seeds and is substantially expressed in seedlings where it transports Fe and manganese into the vacuole. Brown seeds had a higher Fe content than flag leaves, according to Zhang and colleagues' findings (2012) [82]. In rice flag leaves, the VIT gene is highly expressed, which may explain our results. Astonishingly, the Fe levels in brown and polished rice seeds were 1.3 and 1.8 times higher in OsVIT2-knockdown mutants, as discovered by Bashir and associates (2013) [7]. OsVIT1 and OsVIT2 inactivation increased the amount of Fe that could be transported from source to sink organs, according to studies.

As a second encouraging discovery, larger Cd concentrations were found in the VIT knockdown rice than in the control rice (Zhang *et al*. 2012; Bashir *et al*. 2013) [82, 7]. When dealing with soils that are polluted with Cd, it is best to stay away from this type of sampling. To boost the concentration of Fe in polished seeds, one may combine this procedure with alternatives 5 and 6, although more work is required.



Source: Dr. David S. Goodsell

Fig 1: Seven transgenic approaches to Fe biofortification of rice

"Alternative Fe transport methods within rice plants with the purpose of increasing Fe concentration in seeds"

Mucineic acid and protocatechuic acid exporter genes may also be overexpressed to increase Fe export. When Fe is lacking, the TOM1 gene is expressed throughout the root's exodermis, not only in a few specific spots (Nozoye *et al*. 2011) [53]. Seed dorsal vascular bundle, pollen, and leaf phloem all contain TOM1 throughout seed development. Increased DMA secretion, Fe and Zn levels, and tolerance to Fe deprivation were all demonstrated to be enhanced by overexpressing TOM1 in rice.

Protocatechuic acid (PCA), a plant-produced acid, absorbs precipitated Fe (Cesco, *et al*. 2010) [8]. Rice has been discovered to have the phenolics efflux transporter PEZ1 for the first time ever by researchers (Ishimaru *et al*., 2011). The plasma membrane of Xenopus laevis oocytes expressing PEZ1 had an accumulation of PCA. In order for apoplasmic precipitated Fe to be used in the stele, PCA content in the xylem sap must be raised by PEZ1. Primarily, PEZ1 was found in the stele root. Fe concentrations in transgenic rice leaves carrying the CaMV35S promoter-PEZ1 gene cassette were three times higher. A two fold increase in Stele Fe concentration was attributed to the high axoplasmic precipitation levels in the roots of this transgenic rice. Increasing the expression of PEZ1 may lead to an increase in the concentration of Fe in seedlings.

A variety of methods, including chelate export through TOM1 or PEZ1, endosperm-specific ferritin gene expression under the promoter of OsSUT1 and OsYSL2 expression under the promoter of NAS, may be used to boost iron levels in seeds.

"Fe biofortification of rice by mining high-Fe rice cultivars or other target genes"

When transgenic methods are used to enhance the quantity of Fe in the plant, the cultivar has a substantial influence. For transgenic techniques, the Fe content of the host rice variety must be taken into mind. It is now feasible to estimate the amount of more Fe that is needed. There are many methods for developing high-Fe rice varieties, including traditional breeding procedures, the use of transgenic technologies, and mining existing rice varieties for high-Fe variants.

To increase rice biofortification, novel target genes or growing rice types with greater amounts of Fe are essential. (Anuradha *et al*. 2012) [1] Detected seven QTL and selection markers associated with rice seed Fe concentration. Genetic markers were found in rice varieties from the Madhukar Swarna indica area that linked genes relevant to Fe homeostasis, including the OsYSL and OsNAS loci and the OsNRAMP1, OsIRT1, and APRT genes.

When Sperotto and colleagues (2010) looked at 25 metal-related genes, they found that YSL2 and NRAMP rice homologues, ZIP, IRT1, VIT1, NAC5, NAS, FRO, and NAC5 rice homologues were among them. They also discovered genes that might increase the rice grain's Fe and Zn content.

Jeng *et al*. (2012) [29] identified mutant polished seeds with elevated Fe or Zn contents while researching NaN3-induced mutant lines. It has been reported by (Ruengphayak *et al*. 2012) [62] that they studied the effects of 1,000 fast neutron-irradiated M4 mutant lines on grain Fe concentrations and identified 76 unique mutant lines that exhibited this effect. New genes for rice biofortification may be discovered via the use of high-Fe mutant rice lines

Breeding traditional or marker-assisted rice seed mineral nutrition has been employed in a variety of studies. Seeds of IR68144 rice produced conventionally showed a two-fold rise in Fe levels (Gregorio *et al*. 2000) [14]. Because the rice IR68144 increases the Fe status of women, it is considered to be "normal." Wheat IR68144 (Haas *et al*. 2005) [17].

Conclusions

It was necessary to introduce genes for ferritin, NAS, OsSUT1, and barley IDS3 into transgenic rice in order to boost ferritin levels. Some persons with iron deficiency anaemia may benefit from this treatment because it reduces the symptoms they experience. Rice's biofortification must be increased to guarantee that it contains sufficient quantities of Fe. OsIRT1 and OsYSL15, as well as the OsVIT1 and OsVIT2 genes, may be up regulated to increase rice seed Fe biofortification. Other research is needed on high-Fe rice varieties and additional target genes. Future study on Fe biofortification will benefit from combining these techniques.

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