Biofortification of Rice (*Oryza sativa* L.)

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Sushil Kumar Singh, Jyoti Prakash Sahoo, Sandhani Saikia,
Pranaya Pradhan, Ambika Prasad Mishra, Laxmipreeya Behera,
Devraj Lenka, and Narayan Panda

Abstract

As a result of increased population, rising per capita incomes, and urbanisation, global agricultural production is increasing, and food demand is expected to continue growing over several decades. Approximately 60% of total calories consumed in developing countries come straight from cereals, with values reaching 80% in developing countries. Rice is the essential source of calories for humans amongst grains. Over half of the world's population is fed on rice. More than 2 billion of them suffer from "hidden hunger," as they do not consume enough nutrients or micronutrients in their regular diet. As part of a complete food

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S. K. Singh
DBT North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

J. P. Sahoo (✉) · L. Behera
Department of Agricultural Biotechnology, Odisha University of Agriculture and Technology, Bhubaneswar, India

S. Saikia
Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

P. Pradhan
Department of Nematology, Odisha University of Agriculture and Technology, Bhubaneswar, India

A. P. Mishra
Faculty of Agriculture, Sri Sri University, Cuttack, India

D. Lenka
Department of Plant Breeding and Genetics, Odisha University of Agriculture and Technology, Bhubaneswar, India

N. Panda
Department of Soil Science and Agricultural Chemistry, Odisha University of Agriculture and Technology, Bhubaneswar, India

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systems approach, biofortification is an effective technique for nutrition enrichment, which refers to developing a micronutrient-rich diet by utilising traditional breeding practices and sophisticated biotechnological tools. To enhance the profile of rice grain for biofortification-related properties, researchers must first understand the genetics of critical biofortification characteristics. The polishing procedure removes essential nutrients from white milled rice grains. As a result, seed-specific critical nutrient absorption is necessary. Significant increases in iron and zinc and many other essential minerals and provitamins are acquired in rice grain using the biofortification strategy. Most indica and japonica rice types have been biofortified over the world, giving them the titles of “high-iron rice,” “low-phytate rice,” “high-zinc rice,” and “high-carotenoid rice” or “golden rice”. Different techniques of rice biofortification, as well as their effects, have been explored in this chapter.

Keywords

Rice · Biofortification · Hidden hunger · Micronutrients · Bioavailability

6.1 Introduction

Oryza sativa L., commonly known as rice, is the most demanding crop worldwide as a staple food. Enriching it with essential nutrients, which are otherwise absent, would solve nutrient deficiencies to a great extent. To achieve successful biofortification, the mechanism of the particular nutrient uptake and the genes involved have to be elucidated and studied. Essentially those varieties are targeted for biofortification with highly dense micronutrient-rich traits which already have highly preferable agronomic characteristics in the genomic background. Supplements or industrially fortified food can deliver a high level of essential micronutrients to human body. Even if the biofortified rice cannot suddenly increase the concentration in the human body, it can increase the daily sufficiency of micronutrient absorption throughout human life cycle (Bouis et al., 2011).

Minerals can be defined as elements present in the food that human body requires for its healthy growth and development. Out of the 16 essential minerals, 11 are either present abundantly in the traditional food sources, or their deficiency is seen in sporadic cases. Five of these crucial minerals, such as iodine (I), iron (Fe), zinc (Zn), calcium (Ca), and selenium (Se), are present in a limited concentration in traditional food sources. Their deficiencies can lead to severe health disorders. Diseases result from a lack of these minerals when a staple food such as ground grains (with low biomineral content) dominates the diet (Christou and Twyman 2004). Developing countries face a significant crisis in mineral deficiency, lacking in availability of fresh and hygienic foods (Gómez-Galera et al. 2010). However, lacking nutrients like calcium is a common health concern even in the developed countries. Providing access to a more nutrient-rich and diverse diet is challenging in developing and less developed countries. Therefore, biofortification can be a sustainable way to eliminate deficiency diseases in these countries (Bouis and Saltzman 2017).

Table 6.1 Details of economic status and hunger index of major rice-growing countries worldwide

Country	Population (crores) (2019–2020)	Rice production[a] (million metric tonnes) (2019–2020)	GDP per capita (2019–2020) (USD)	GDP growth rate (2019–2020) (% annual change)	Hunger index[b] (2019–2020)
China	139.77	148.5	10,261.68	6.1	<5
India	136.64	116.42	2099.60	4.2	27.27
Indonesia	27.06	36.7	4135.57	5.0	19.1
Bangladesh	16.3	34.91	1855.74	8.2	20.4
Vietnam	9.65	27.77	2715.28	7.0	13.6
Thailand	6.96	20.34	7806.74	2.4	10.2
Philippines	10.81	11.73	3485.08	6.0	19.0
Brazil	21.1	7.14	8717.19	1.1	<5

*Data was obtained from
[a] https://www.statista.com/statistics/255945/top-countries-of-destination-for-us-rice-exports-2011/
[b] https://www.globalhungerindex.org/ranking.html

China assembled parboiled rice about 148.5 million metric tonnes in the 2018/2019 crop year, more than almost any other country. In that crop year, by producing 116.42 million metric tonnes of parboiled rice, India came second. In the 2018/2019, the total production of parboiled rice in volume was 495.9 million metric tonnes worldwide. The largest rice-consuming countries are China, India, and Indonesia, respectively. The consumption of rice in 2018/2019 China was 143.79 million metric tons, and per capita intake in the world has remained remarkably stable since 2000, averaging about 53.9 kg per year (https://www.statista.com/statistics/255945/top-countries-of-destination-for-us-rice-export-2011/) (Table 6.1). Hidden starvation, caused by insufficient essential nutrients, is a major problem affecting approximately two billion people worldwide. Iron deficiency leads to anaemia accounting for 30%–40% (Global Burden of Disease 2015, Collaborators in Disease and Injury and its prevalence, 2016). Children and women are most likely to be infected with IDA. IDA has been shown to impair physical development, decrease immunity, and increase the likelihood of maternal and perinatal death.

The breeding target for Fe to meet the approximate average Harvest Plus requirement of 30% for women and children in polished rice is 13 mg/g or a five to six fold increase in grain iron in ordinary rice. Although wheat contains the dry weight of 59 mg/g of iron, which is twice the amount found in other cereals (Bouis et al. 2011), it is challenging to develop a more nutritious diet that can alleviate micronutrient deficiencies in developing and least developed countries. With rice consumed by half the world's population, genetically engineered rice grown explicitly to counteract "hidden hunger" is perhaps the best promising staple crop. Brown rice, the healthiest type of processed rice, is made by hulling raw rice (field harvested paddy). Unpolished brown rice is rich in iron, zinc, copper, calcium, and phosphorous, as well as vitamins such as thiamine-B1, riboflavin-B2, niacin-B3, pantothenic acid-B5, pyridoxine-B6, biotin-B7, folic acid-B9, and tocopherol-E, but is low in vitamins A, C, and D (Ghosh et al. 2019).

On the other hand, the average consumer prefers white rice grains because of their softness, lightness, ease of digestion, better consuming properties, and less time for cooking. The bran layer and the substrate, embryo, and a small part of the endosperm are removed from polished (milled) white rice (Champagne et al. 2004). Milled rice has poor nutritional quality in comparison with brown rice, with the reduced iron content lowered by 2.14 times to 4.75, and the zinc content lowered by 1.83 times, and essential minerals, fats, fibres, proteins, and vitamins lowered by 1.83 times (Masuda et al. 2009). However, the amounts of mineral reductions can vary between rice varieties and grain polishing processes. Whilst greater awareness and education have improved in the consumption of brown rice, the major rice consumers still prefer polished white rice by considering that the polished white rice was developed as nutritionally improved through biofortification (specific endosperm), leaving scientists to reconsider. Improving essential nutrients to be bioavailable in the edible parts of staple foods through conventional breeding, biotechnology techniques, or agricultural strategies can help alleviate deprivation in places where staple foods are the primary source of micronutrients and calories (Bouis and Saltzman 2017).

6.2 Understanding of Essential Mineral Uptake by Plants

6.2.1 Iron

Even though there is abundant iron available in the soil, most remains unused due to its low solubility. Plants have evolved mechanisms for accessing the insoluble iron from the ground in a highly regulated manner. This mechanism has been divided into two strategies (Marschner et al. 1986). By intensifying the phenolic and proton release (mediated by the enzyme H+ ATPase) into the rhizosphere, nongraminaceous plants bring the soil's pH down. Phenolics efflux zero 1 (PEZ1) transports the phenolics to facilitate and use the precipitated Fe (Takahashi et al. 2011). Iron is converted into its more soluble ferrous form by activating the ferric-chelate reductase expression. The soluble ferrous ion is shifted to the plasma membrane via its iron transporter, IRT1. On the opposite hand, graminaceous plants facilitate iron uptake by exuding MA, which are iron (III) chelators from plant roots, which states strategy II (Takagi et al. 1984). Phytosiderophores (PS) are synthesized and secreted due to the activity of nicotianamine synthase (NAS), nicotianamine aminotransferase (NAAT), and deoxymugineic acid synthase (DMAS) (Shojima et al. 1990). A soluble complex called the Fe (III)-the binding of Fe3+ forms PS with PSs (Fig. 6.1). The YSL proteins (yellow stripe-like proteins) facilitate the inhibition of these complexes from the rhizosphere to the root cells. (Nozoye et al. 2011). Rice uses strategy II, but it can also take up iron via IRT1 from the rhizosphere.

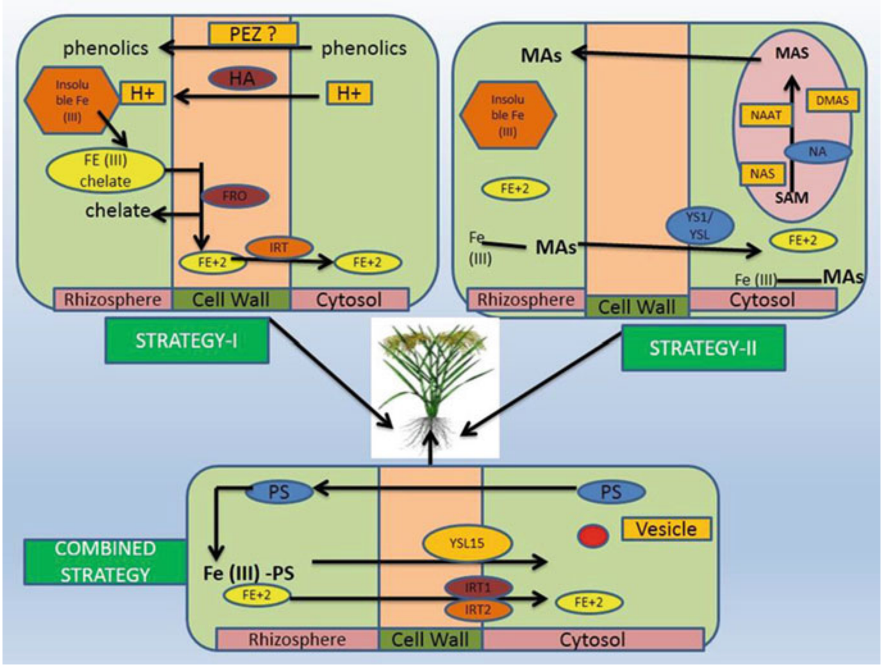

Fig. 6.1 Iron absorption and translocation in rice

6.2.2 Iodine

Iodine makes its way into the plants via two pathways: atmosphere to the plant pathway and soil to the plant pathway. The stomata of the plants and the roots take in iodine from the atmosphere and soil, respectively (Shaw et al. 2007). After iodine has been applied to the plants as IO_3^-, iodate reductase reduces it to I, which responds to iodine absence and presence in the environment (Kato et al. 2013). However, Lawson et al. (2015) reported that plants could readily absorb IO_3^- instead of I^-. Due to the more availability of IO_3^- it in the soil, it has been hypothesized that plants are taken up more efficiently by plants rather than I^- which is relatively more thermodynamically stable. Plants absorb I^- form iodine via chloride transporters and proton pump-actuated ion channels (White and Broadley 2009). In this process, perchlorate, nitrate, thiocyanate, and other anions may cause absorption interference (Voogt and Jackson 2010). The identity of iodine transporters has not been established with certainty, but it has been assumed that they share their activities with other transporters such as Na: K/Co-transporters (Landini et al. 2012).

6.2.3 Zinc

Rice grown on zinc-poor soils yields a low yield, and the nutritional quality is very poor (Welch and Graham 1999). The zinc deficiency eventually leads to weakness in humans, especially with the large population, which depends on the staple food mostly in developing countries (Hussain et al. 2012). Zn is usually taken up as a free ion (Zn^{2+}) and can also be taken up as $ZnOH^+$ at a very high pH. The plant takes up zinc via the transporter-mediated secondary transport across the root plasma membrane. ZIP or zinc iron permeases from the family of metal carriers are primarily responsible for the rapid absorption of zinc from the environment (Palmgren et al. 2008). But they speculated that carrier proteins might also have some role to play in the uptake of zinc; likely Mugineic acid (MA) synthesized from methionine (Met) has a vital role in the pathway of Zn uptake (Suzuki et al. 2006). 2′-Deoxymugineic acid or DMA is one of the first MAs to be excreted through TOM1 into the rhizosphere (Nozoye et al. 2011) transporter of MA. This then binds to the Zn in the soil forming a MA-Zn complex, absorbed via yellow stripe 1-like family transporters (Inoue et al. 2009).

6.2.4 Calcium

Calcium is an essential mineral needed for growth, protection from pathogens, and development of the plant. It promotes proper plant cell elongation and is a vital participant for enzymatic and hormonal processes. Plant calcium uptake is mediated in roots by the expressed plasma membrane channels. Scientists classified permeable calcium channels into DACCs (depolarisation-activated channels) and HACCs (hyperpolarized-activated channels) based on their electrophysiological properties (Miedema et al. 2001). Calcium can be taken up through the ischemic pathway as well as the ischemic pathway. However, a higher concentration of calcium causes cell toxicity. Therefore, to maintain a sub-micromolar concentration of calcium in the cytosol, the cell retains the ability to generate a calcium signalling stroke pathway. White (2001) suggested that through the cortex from the epidermis, Ca moves apoplectically till it reaches the endodermis Casparian strip, which is mainly made of suberin and lignin. This strip composition makes it impermeable for the movement of water and solutes (Schreiber et al. 1999). Upon arriving at this layer, Ca move with the help of channels to enter the endodermal cell cytosol. With the help of Ca^{2+}-ATPases or Ca^{2+}/H^+ antiporters, it is exported to the stele apoplast to be finally loaded into the xylem. After that, the calcium is ultimately distributed to the leaf cells through the shoot (White and Broadley 2003).

6.2.5 Selenium

Incorporation of selenium into two amino acids selenomethionine and selenocysteine is found in cells. The most common form of Se available in soil for uptake by plants is selenate (SeO_4^{2-}), which is more water soluble than that of selenite. Selenium is found in various forms in both acidic and alkaline soils. Selenate is the standard form found in alkaline soils, and acidic soils contain selenite. Both differ in their motility rates and absorption capacity and are subsequently broken down into seleno compounds (Li et al. 2008). The root cell membrane contains selenium uptake transporters. For selenite uptake, the phosphate transport mechanism is responsible (Li et al. 2008), and selenite is transported on the other hand by sulphate transporters (Dong et al. 2003). The nutritional state of the plant, both in and out, is mainly responsible for using transporters for selenite or sulphate uptake (White et al. 2004). Based on the cumulative capacity of plants on Se, they have been classified into over-accumulators, secondary accumulators, and non-compounds (Bodnar et al. 2012). Plants that can accumulate more than 1000 mg of Se/Kg DW and thrive well in selenium-rich soils are called hyperaccumulators. Plants get Se to the extent that the plant shows no sign of toxicity (100–1000 mg of Se/kg DW), and they are called secondary accumulators. Plants that accumulate less than 100 mg citrate/kg of their dry weight are termed non-accumulators. They show retarded growth or fail to survive.

6.3 Transgenic Efforts for the Development of Golden rice

Children who have blindness have been a result of severe vitamin D deficiency. Developing countries face deficiency diseases due to the lack of proper nutrients in their diet. Physiological traits such as cellular differentiation, growth, reproduction, and vision, to name a few, depend on the role played by carotenoids (Wurtzel et al. 2012) as they are known to help tackle several ROS-generated diseases, namely, cancers and neurological and cardiovascular diseases along with eye disorders (Bai et al. 2011). β-Carotene biosynthetic pathways in rice have been the target for the creation of "golden rice." Golden rice enriched with β-carotene was formulated in supplementing provitamin A, which naturally enhances immortality caused by Vitamin a deficiency (VAD). Phytoene synthase (PSY) of daffodil (*Narcissus pseudonarcissus*) and phytoene desaturase (crtI) of pathogenic bacteria (*Erwinia uredovora*) were chosen to be introduced and expressed in the endosperm of IR64 and BR29, which are Asian rice varieties under the endosperm-specific promoter (Datta et al. 2007) to produce golden rice. The β-carotene synthesis pathway is already present in rice.

However, it is only expressed in the leaves and not in the grains. This pathway can be reactivated for β-carotene synthesis to take place in the grains by incorporating the genes mentioned above. β-Carotene (carotenoids with at least one unsubstituted β-ionone ring) possesses provitamin A activity. Rabbani et al. (1998) stated that tissues that had a high level of lipid accumulation could act as a lipophilic sink, which can drive the formation of carotenoids. But rice had a non-carotenogenic endosperm which has low lipid concentration. This leads to the nonavailability of carotenoid deposition in the rice endosperm. There were also doubts on the presence of precursors of the carotenoid biosynthesis pathway in the grains of golden rice. Due to many questions arising on the success of the golden rice project, a lengthy research phase was conducted to answer the questions. Ye et al. (2000) reported that the breakthrough achieved by Prof. Peter Beyer and Prof. Ingo Potrykus led to the discovery that incorporation of only two transgenes was necessary for golden rice to see the light of the day.

Plant phytoene synthase (PSY) was the first transgene to form phytoene from geranylgeranyl-diphosphate (GGPP), which is found endogenously. Bacterial carotenoids (CRTI) are encoded by a second gene that adds four double bonds that lead to conjugation. PSY and CRTI, in combination, form a red compound called lycopene, which has been observed and established in tomatoes. However, in any rice, the transformant accumulation of lycopene has never been observed. Lutein and zeaxanthin (oxygenated carotenoids) have instead been found in association with α- and β-carotene. It was revealed by observing the carotenoid pattern in the grain endosperm that the pathway went beyond the point that was supposed to be the peak of the transgene's action. The wild-type rice endosperm produces lycopene cyclases (LCDs) and α- and β-carotene hydroxylases (HYDs) downstream. However, PSY and the plant carotene desaturases are not present in the same. This results in the use of lycopene produced by PSY and CRTI as substrates for the enzymes mentioned above, leading to the formation of the noticeable products (Fig. 6.2).

6.3.1 Golden Rice: First Attempt

Ye et al. (2000) proved in the early development years that rice grains could produce β-carotene. It has been quite evident that there was a need for only transgenes (PSY and CRTI) for β-carotene production. There was, however, no need for lycopene synthase. Although initial studies were carried out in japonica cultivar, indica varieties were also included later (Hoa et al. 2003). Many ways were developed in rice seeds to improvise with the production of carotenoids to the permissible levels. The first generation of golden rice or GR1 was transformed with two transgenes (from daffodils and bacteria) and placed under an endosperm-specific gt1 promoter. Field carotenoid levels amounted to four times to that of the proposed model.

6.3.2 Golden Rice 2

Golden rice 1 gave us the possibility to produce β-carotene in rice endosperm. It also helped us see that to tackle vitamin A deficiency, β-carotene should be produced in higher amounts. Since only two transgenes have been involved in the production of β-carotene, it was simple to understand that by manipulating the enzymatic activities of the products of the two genes, one can achieve higher β-carotene. Normal pathways usually have specific rate-limiting steps which control the entire path. Overcoming the rate-limiting step simply enhances the concentration of the rate-limiting enzyme or by shifting to a more active enzyme. Different PSY sources were examined to see that the maize and rice genes were more efficient (Paine et al. 2005).

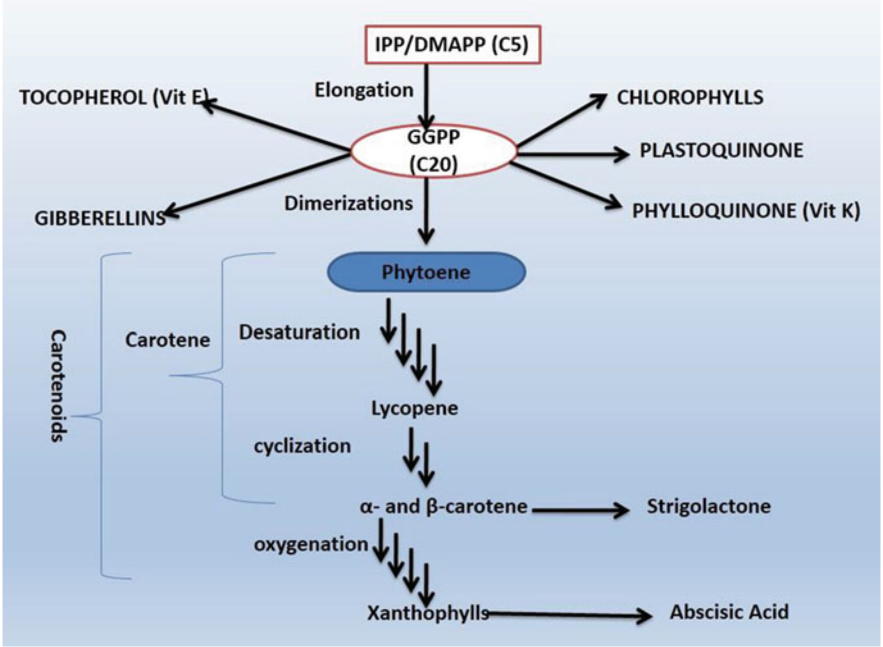

Fig. 6.2 Overview of carotenoid synthesis pathway: isopentenyl-diphosphate (IPP) and dimethylallyl-diphosphate (isomer of IPP-DMAPP), C5 compound is the starting molecule for this pathway. On elongation by C5 units, geranylgeranyl-diphosphate (GGPP) (C20 compound) is formed, a precursor for many biosynthetic compounds. Phytoene, the first carotenoid in the pathway, is formed due to the dimerisation of GGPP. After that, after a series of desaturation events, lycopene, a coloured compound, is included. Further cyclisation events lead to the production of β- and ε-ionone rings. Additional oxygenation of the rings eventually produces xanthophylls (Figure courtesy: www.goldenrice.org)

Fig. 6.3 Comparative visualisation of wild type, golden rice 1, and golden rice 2. (image courtesy: goldenrice.org)

Golden rice 2 (Fig. 6.3) was generated to synthesize approximately 37 μg/g of carotenoids. Amongst this, only 31 μg/g had β -carotene was significantly higher compared to 1.6 μg/g found in the first-generation golden rice (Al-Babili and Beyer 2005).

6.3.3 Case Study 1: Thiamine Biofortification of Rice

Three rice tpk or thiamine phosphokinase variants were analysed in the promoter region. This was done to check if the endosperm-specific *cis*-elements were present or absent in the promoter. Higo et al. (1999) reported that motifs such as AACA, ACGT, prolamin, and TATA box were present in a 300 bp region upstream of the start site of the tpk3 promoter. The CRISPR-Cas9 approach was the method of choice due to its simplicity in the gene-editing scenario. The promoter region of the tpk3 gene does not reflect an essential motif, GCN4. Adding this motif can be possible if editing is done at a selected position. A gRNA sequence of 20 bp containing NGG as an adjacent protospacer motif or PAM is chosen to be the target site. Heigwer et al. (2014) mentioned that this site had been specifically selected as the target site because off-site targets are absent in this region.

Tools like E-CRISP and Cas-OFFinder have been used for this purpose. Cas9 cleaves a part in the DNA 3 to 4 nucleotides upstream of PAM. The introduction of GCN4 box at the site of editing a very stable transformation with Cas9 and gRNA is essential. Homologous recombination promoting oligonucleotide has been used for co-transformation for the introduction of the GCN4 box (Zhang and Huang 2012). The box absence has been synonymous with the expression of tpk3 in rice, which is quite negligible. With the introduction of the edited gene, enhanced propagation of tpk3 is predicted. Higher knowledge of this pathway is needed for opportune enhancing to be performed for higher thiamine manufacturing in rice. A summary of the thiamine biosynthesis pathway in rice is listed in Fig. 6.4. The box absence has been synonymous with negligible expression of tpk3 in rice. With the introduction of the edited gene, enhanced production of tpk3 is expected. A better understanding of this pathway is needed for proper editing for higher thiamine production in rice. A summary of the thiamine biosynthesis pathway in rice is indexed in Fig. 6.4.

6.3.4 Case Study 2: Biofortification of High-Zinc Rice

Zinc is an essential nutrient for the proper absorption of iron in the body. Plants typically take up zinc from the soil. Several genetic approaches have been made to enrich the plants to increase the uptake of zinc from the rhizosphere. Zinc translocation and mobilisation involve a lot of genes. Overexpressing these genes for increased bioavailability may lead to an essential way by enhancing Zn content in rice grains. In rice, overexpressing NA synthase genes via the 35S enhancer element has contributed to its manifold increase. Transgenic rice harbours barley nicotianamine synthase gene HvNAS1, which showed a threefold higher zinc accumulation under the influence of rice actin1 promoter. OsIRT (ZIP family protein

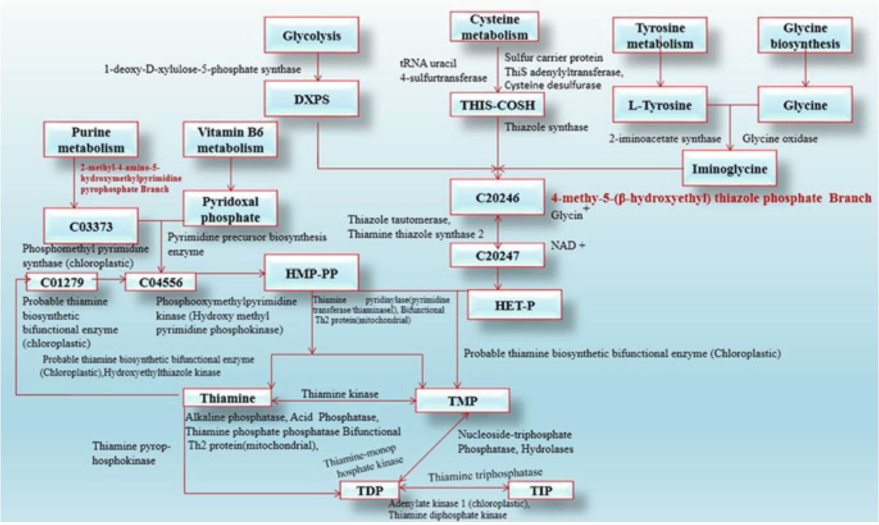

Fig. 6.4 Summary of thiamine biosynthesis pathway (Courtesy Minhas et al. 2018)

in rice) can be overexpressed for a higher concentration of zinc to accumulate in rice. OsZIP1, OsZIP2, OsZIP3, and OsZIP4 have been connected with Zn homeostasis (Ishimaru et al. 2007). Lee et al. 2009 concluded that in GE rice, high amount of Fe and Zn in rice grains resulted from the overexpression of OsIRT and MxIRT genes. Boonyaves et al. (2016) reported that the polished grains of GM rice accumulated at the highest concentration of Fe and Zn as a group of four genes (AtIRT1, Pvferritin, AtNAS1, and Afphytase) were channelled to rice. Many reports have also been published where there was overexpression of rice OsNAS genes, leading to a high accumulation of zinc in grains (Johnson et al. 2011). RNAi silencing was also used to increase zinc concentration in GM rice. Phytic acid metabolism pathway's MIPS gene was silent via this mechanism, and this increased the concentration of zinc, calcium, iron, and magnesium in rice grains. Ali et al. (2013) suppressed IPK1, another gene from the same pathway, to report an increased zinc concentration. Paul et al. (2012) stated that in PSII rice, the ferritin gene (Osfer2), was overexpressed to show a 1.37-fold increase of zinc (Fig. 6.5).

6.3.5 Case Study 3: RNAi Technology Low-Phytate Rice

Overall in most cereals, approximately 80% of the total phytic acid is accumulated at the aleurone layer of the grains except for maize. Phytate, a cumulation of salts, accumulates as phytic acid accumulates. Since phytate contains six negatively charged ions, including Fe^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+}, and lowering their bioavailability. Rice mutant varieties (low phytic acid (LPA) phenotype) have been developed in a series of endeavours to truncate phytic acid levels (Hambidge 2000). Despite their

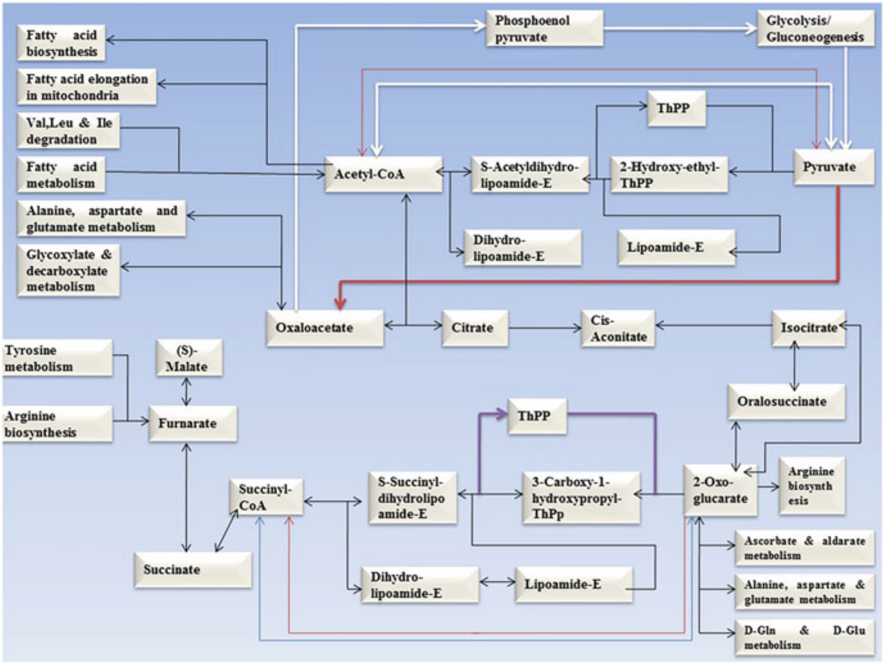

Fig. 6.5 TCA cycle in zinc-fortified rice variety (source: Boonyaves et al. 2016)

efficacy, these mutant lines obstructed crop yield and overall performance (Roda et al. 2020). Transgenic crops were developed as an alternative approach by suppressing the phytic acid biosynthetic pathway (Fig. 6.6) utilising RNA interference (RNAi) (Fig. 6.7).

6.4 Different Approaches for Improvement of Nutraceutical Properties in Rice Grain

For successful biofortification to occur, a proper understanding of nutrient uptake from the soil by the plants is necessary. In these consecutive years, progress has elucidated the pathways associated with various nutrient uptakes by plants from the ground. Hao et al. (2005) stated that rice crops rich in iron accumulate high amounts of Fe and Zn in the grain's endosperm tissues than iron-deficient plants. Molecular genetics has been able to divulge the process of zinc uptake by plants from the soil in various crops, including wheat and rice (Yang et al. 1994). Grotz and Guerinot (2006) have explained the critical process of chelation, distribution patterns, and iron, copper, and zinc transport mechanisms.

Biofortification programs have accommodated the idea of making the nutrients bioavailable in the plant system. It has been proposed to be more important than

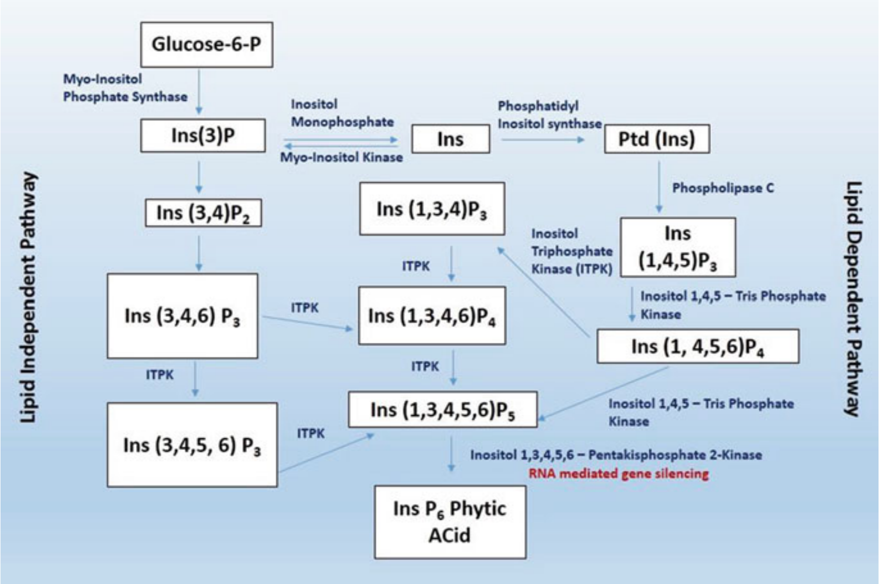

Fig. 6.6 Phytic acid biosynthesis pathway (source: Suzuki et al. 2007)

simply increasing the density of the nutrients. Phytic acid has been found to restrict micronutrient absorption (Nagashima et al. 2014). Thereby, there have been attempts to decrease phytic acid concentration in the plant system through enzymatic pathway modification. However, many antinutrients like phytic acid and polyphenols are required by the plants to build stress resistance and increase vigour (Yang et al. 2007a, b, c). They are also crucial for human health, considering their involvement in reducing heart diseases (Ferguson and Harris 1999). Hence, Welch and Graham (2004) rightly pointed out that antinutrient concentration manipulation via breeding approaches should be done carefully, keeping in mind the side effects associated with it. Various methods have been described for increasing the nutritional value of different crops. Some of them are listed below.

6.5 Breeding Approach

It has been very well documented in genetic diversity found on micronutrients in rice and other food crops (Yang et al. 2007a, b, c). Genetic diversity has been the primary target for developing nutritionally superior varieties by various scientists and breeders (Zapata-Caldas et al. 2009). Many programs create excellent varieties of rice, wheat, potato, bean, etc., with a higher amount of Fe, Zn, vitamin A, etc. (Pfeiffer and McClafferty 2007). In a program (in search for new donors) initiated by IRRI (International Rice Research Institute) in collaboration with the University of Adelaide, Australia, 7000 varieties have been evaluated for zinc and iron

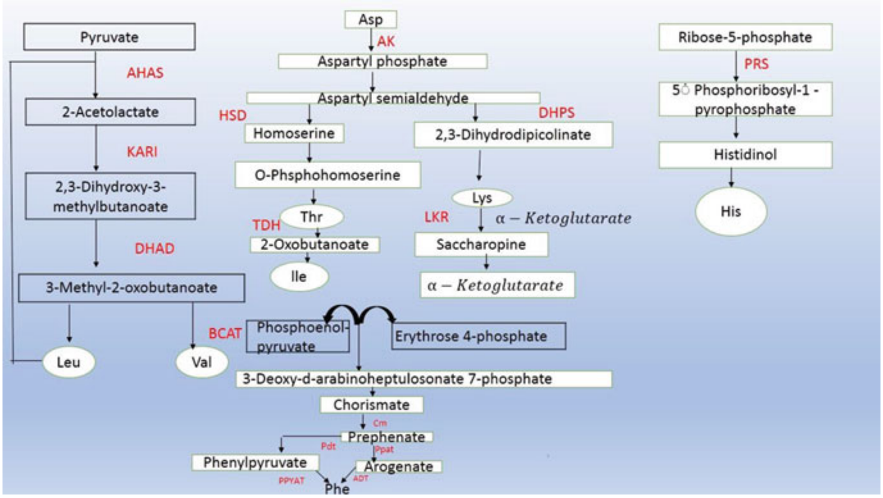

Fig. 6.7 Leading amino acids using biosynthesis for the formation of leucine, valine, isoleucine, threonine, methionine, lysine, histidine, phenylalanine, and tryptophan. Abbreviations: AK (Asp kinase), HSD (homoserine dehydrogenase), DHDPS (dihydrodipicolinate synthase), LKR (lysine ketoglutaric acid reductase), TS (Thr synthase), CGS (cystathionine synthase), TDH (Thr dehydratase), PRS (ribose-phosphate diphosphokinase), MGL (Met synthase), SAMS (S-adenosylmethionine synthase), AHAS (acetohydroxyacid synthase), KARI (ketol acid reductoisomerase), DHAD (dihydroxy-acid dehydratase), BCAT (branched-chain aminotransferase), DAHPS (3-deoxy-D-arabinoheptulosonate 7-phosphate synthase), CM (chorismate mutase), AS (anthranilate synthase), PDT (prephenate dehydratase), PPAT (prephenate aminotransferase), PPYAT (phenylpyruvate aminotransferase), ADT (arogenate dehydratase) (source: Wang et al. 2017)

concentration in the rice grains. Later on, Khush et al. (2012) reported rice grains with a higher zinc and iron concentration. A significant variation in iron concentration has been written for many staple crops like rice, wheat, maize, bean, cassava, etc. (Frossard et al. 2000).

Tiwari et al. (2009) suggested developing nutrient-rich cultivars of different crops, and selective breeding is used as a tool. IR 68144-3B-2-2-3 (IR72 X Zawa Bonday) is identified as an expanded Indian breeding line. Similarly, certain wheat varieties have been found to survive zinc-deficient soil despite maintaining high zinc concentration (Peleg et al. 2008). Through many scientific discoveries and research, it has been found that any food crop can be made nutritionally potent through simple breeding techniques. However, breeding techniques can only be successful if micronutrients are available in the soil for the plant to take in. In the past two decades, above 20 QTL mapping studies have occurred to study the genetic basis of rice protein matter (Mousavi et al. 2013). In addition, to our knowledge, more than 80 stable and reliable QTL GPC have been identified and mapped to all twelve rice chromosomes, with most identified on chromosomes 1, 2, 6, 7, 10, and 11 (Table 6.2).

Table 6.2 List of QTLs identified for biofortification traits in rice (adopted from Sharma et al. 2020)

Purpose	Population type	Cross	Number of QTLs	Chromosome	Phenotypic variability explained
Amino acid	RILs	Zhenshan 97 × Nanyangzhan	2 QTLs	1, 7	4.05–33.3
	RILs	Zhenshan 97 × Minghui 63	10 (His) + 8 (Arg)	1, 2, 3, 6, 7, 10, 11, 12 (His); 2, 3, 5, 6, 7, 10, 11, 12	12–35 (His); 16–33 (Arg)
	RILs	Zhenshan 97 × Minghui 63	12	1, 11	3.4–48.8
	RILs	Zhenshan 97B × Delong 208	3 QTLs	1, 7, 9	4.2–31.7
	RILs	Dasanbyeo × TR22183	6	3	10.2–12.4
Protein	RILs	Zhenshan 97 × Minghui 63)	2	6, 7	6.0–13.0
	DH	Caiapo × IRGC 103544	4	1, 2, 6, 11	4.8–15.0
	DH	Gui630 × Accession 02428	5	1, 4, 5, 6, 7	6.9–35.0
	BC3F1	V20A × Accession 103,544	1	8	9.0–10.0
	RILs	Moritawase × Koshihikari	3	2, 6, 9	2.3–16.3
	BIL	Kasalath × Koshihikari)	2	6, 10	14.3–14.8
	RIL	Chuan × Nanyangzhan	2	6, 7	2.69–4.50
	RILs	Xieqingzao B × Milyang 46		3, 4, 5, 6, 10	3.9–19.3
	RILs	Zhenshan 97 × Minghui 63	9	2, 3, 5, 6, 7, 10, 11, 12	1.60–9.26
	DH	Samyang × Nagdong	3	1, 11	6.92–22.98
	RILs	Asominori) × IR24)	10	1, 3, 4, 6, 7, 8, 9, 10, 12	8.53–23.70
	RILs	Zhenshan 97B × Delong 208	2	1, 7	7.2–25.9
	DH	Cheongcheong × Nagdong	1	2	39–41
	DH	CJ06) × TN1	1	10	12.3–15.8
	DH	Cheongcheong × Nagdong	3	8,9,10	39–40
	RILs	M201 × JY293	5	1, 2, 3, 4	6.74–13.50
	DH	Cheongcheong × Nagdong	1	7	14

(continued)

Table 6.2 (continued)

Purpose	Population type	Cross	Number of QTLs	Chromosome	Phenotypic variability explained
Zinc and iron	DH	IR64 × Azucena	Zn-2; GFe-3	9	Zn (1, 12); Fe (2, 8, 12)
	RILs	Zhengshan 97 × Minghui 63	GZn-3; GFe-2	Zn (5, 7, 11); Fe (1, 9)	Zn (5.3–18.61); Fe (11.11–25.81)
	RILs	Bala × Azucena	GZn-4; GFe-4	Zn (6, 7, 10); Fe (1, 3, 4, 7)	Zn (11.2–14.8); Fe (9.7–21.4)
	DH	ZYQ8 x JX17	GZn-2	4, 6	Zn (10.83–12.38)
	RILs	Madhukar) × Swarna	GZn-6; GFe-7	Zn (3, 7, 12); Fe (1, 5, 7, 12)	Zn (29–35); Fe (69–71)
	F2	PAU201) x Palman 579	GZn-3; GFe- 8	Zn (2, 10); Fe (2, 3, 7, 10, 12)	Zn (4.7–19.1); Fe (2.4–26.8)
	RILs	Swarna X Moroberekan	GFe-1	1	Fe (39)
	F4	PAU201 x Palman	GZn-1; GFe-5	Zn (6); Fe (5, 7, 9)	Zn (25); Fe (34.6–95.2)

6.6 Agronomic Approach

Cakmak (2008) observed that with the varied and worldwide use of high-yielding varieties of food crops, the soil had been stripped of its fertility. The soil deficient in micronutrients cannot help in attaining the biofortification of crops through conventional breeding techniques. In such a scenario, micronutrient fertilisation of such ground is needed for the plants to show any response. There have been reports of nutrient concentration improvement in crops after adopting fertilisation practice. For increasing productivity and at the same time increasing micronutrient density in grains, zinc fertilizer was applied to the rice, pea, and cowpea (Hu and Lutkenhaus 2003; Li and Vasanthan 2003; Fawzi et al. 1993). However, the method of microelement application makes a significant impact on the accumulation of the concerned micronutrient in the parts of the plants that can be consumed.

Missana et al. (2009) conducted an experiment, where it was observed that, if zinc is sprayed on wheat leaves during its early dough or milk stage, the impact is much more than that of fertilizer application to the soil zinc concentration by many folds. One of the most critical consequences of foliar application of zinc in rice is that phosphorus accumulation in the grains is reduced, thereby reducing the amount of phytic acid. It has been observed that iron fertilizers are not effective enough to make a difference (Missana et al. 2009). Even if iron is applied in the foliar form to the plants, it is quickly converted to the insoluble form, which becomes unavailable for the plants to use (Frossard et al. 2000). Suggestions were made to use organic iron as micronutrient fertilizers. It has been reported that, if iron is chelated with other compounds like Ethylene diamine tetra acetic acid (EDTA), Diethylenetriamine pentaacetate (DTPA), or ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) (EDDHA).

Waters et al. (2009) noticed that the same genetic process controls nitrogen and iron transportation in the vegetative parts of the crop plant. Hence, to make Fe biofortification a success, special care should be taken for enriching nitrogen in crops. It has been reported by many researchers like Gunes et al. 2007 that intercropping of cereals with dicot plants facilitates the enhancement of iron and nitrogen amount in plants as a result of interspecific root interactions. Selenium fertilizers like Na_2SeO_4 and K_2SeO_4 have proved effective during foliar application and immediately increased selenium in plants (Stroud et al. 2010). However, Yang et al. (2007a, b, c) reported that agronomic approaches were very limiting considering factors like soil-chemical interaction, making the micronutrients less available to the plants, reducing transportation efficiency, reducing the roots' concentration, etc (Miedema et al. 2008).

6.7 Biotechnological Approach

Biotechnology has provided very modern and permanent approaches for biofortifying micronutrients in cereal crops. Researchers like Raboy (2002) and Tucker (2003) have stated that a higher concentration of vitamins and nutrients can be built up by modifying the plant's genetic makeup. The synthesis of antinutrient compounds can be reduced. Transgenics has played an essential part in the biofortification of micronutrients in crops. Agrobacterium-mediated transformation was used to transfer the iron storage protein gene, ferritin, from the French bean to the rice crop (var. Kitaake) (Lucca et al. 2001), increasing the iron concentration.

A synergistic effect was noticed on the storage and uptake of iron concentration in rice endosperm upon evaluation of two transgenes, i.e. NAS (AtNAS1) from *Arabidopsis thaliana* and ferritin (Pvferritin) from *Phaseolus vulgaris* in rice. Phytosiderophore biosynthetic pathway in rice has been modified by introducing nicotinamide aminotransferase gene via transgenic approach. This resulted in transgenic rice with high resistance to growth in iron-deficient soils and maximum yield than those in controls (Takahashi et al. 2001). White and Broadley (2005) reported that these transgenic approaches could be used to increase material accumulation. On the other hand, stimulating agents reduce the concentration of antinutrients.

The Clustered Regularly Interspaced Palindromic Repeats (CRISPR) genome editing tool for a specific change within the genome was recently developed, giving scientists the opportunity to precisely target genes or intergenic regions. Rice has benefited from this technology in yield and stress tolerance (Mishra et al. 2018). Using a CRISPR to knockdown OsVIT2 to increase grain Fe, similar to the reported

T-DNA insertion attempting to silence a gene in different rice cultivars (Bashir et al. 2013), could be a key illustration. Alterations of genes' expression resulted in iron homeostasis by a simple edit to the regulatory element of iron homeostatic genes, which results in iron-enriched rice and wheat could withal benefit from this method (Liu et al. 2004).

6.8 Challenges for the Public Release of Golden Rice

In 1999, the first proof-of-concept β-carotene fortified rice was created. After many modifications made to the prototype golden rice, the inventors gave away the technology and any future version of it for the improvised world to reap the benefits. However, to date, advantages due to this technology have not yet reached the malnourished people in the world. There are varied reasons for the delay. The majority of these reasons are listed below:

1. Golden rice contains genes that have been sourced from other organisms, thereby making it a genetically modified crop. GM crops are those crops that include at least one gene from another species. Different national governments strictly regulate GMO crops. Officials are appointed that keep data of the altered genome structure, with the potential of causing allergies compared with the known allergy-causing agents in the databases, relation, and comparison with the standard genomes of the same crop or any such data that can certify that the GMO is non-invasive and is safe for cultivation and human consumption. Only when the regulatory bodies give such clearances to the applicant after being judged safe, the GMOs can be cultivated and consumed in that country.
2. Money and time invested in creating golden rice could have been better utilized for looking at solutions already available for (VAD). Some of the readily available solutions are vitamin A tablet food fortification to educate people of the weaknesses caused by the lack of proper nutrition. Instead of focusing on the magic of golden rice, many people believe that, concentrating more on other readily available options would be the key to eradicating VAD. There have been apprehensions amongst the farming community about the cultivation of GE golden rice. It may lead to contaminating other non-GE wild-type rice varieties (via cross-pollination). According to a publication by Greenpeace Southeast Asia, GE golden rice can contaminate non-GE rice, thereby negatively affecting traditional and organic farming, which in turn would affect the rural livelihood scenario. If GE golden rice shows adverse effects, then the contamination would be the main bottleneck in countries having rice as a primary or staple food (Cakmak and Kutman 2018).
3. Some section of the society also feels that it is irresponsible for the scientific community to impose the golden rice technology on people who are unwilling to take it for various reasons like going against their religious beliefs or cultural heritage. IRRI or the International Rice Research Institute has also addressed biodiversity loss and maintains that these claims are baseless and non-scientific. The farming community is worried about the introduction of golden rice, pushing

the farmers to enter industrial monoculture production. However, scientists also reported that the practice of monoculture would lead to adverse loss of biodiversity in the local ecosystem (Ishimaru et al. 2011).

6.9 Economic and Social Constraints for the Biofortified Rice

When food crops are enhanced with added nutrition, using modern biotechnological techniques to make the nutrients more readily available to the human population is referred to as biofortification. Even though a diet rich in various types of food is recommended for daily micronutrient intake, the poor section of the society depends on the cheap and reliable source of calories, *i.e.* rice and wheat. Biofortification promises to deliver micronutrients to the impoverished community as a cost-benefit method for its single investment in breeding. The process of biofortification will rely primarily on public funding. Therefore, many factors will come into play, including the R&D resources dedicated to this effort. If private financing is to be considered, then returns would have to be generated either in the form of hybrids or intellectual property rights that prohibits farmers from selling the seeds of their produce (Landberg et al. 2009).

Biofortification does not fall under this model as it has been generated for public use worldwide. Biofortification also faces a challenging limitation. High production cost, i.e. equipment, technology, patenting, etc., is a cause of concern that can hamper this method to go global. Powell (2007) stated that the profit margin for private investors is shallow in the case of biofortified crops. Scarce public funds aggravate the situation. Society's involvement during the designing process also plays a significant role in making any biofortified crop a reality. The existence of a deficiency makes the research process more rational or sensible. If society cooperates, then acceptance becomes a lot easier. There is a cultural or local limitation to golden rice's success, i.e. the value of pure white rice in various cultures and traditions (Thurber and Fahey 2009), the ways of feeding children, and openness towards newer discoveries. Another major constraint is the lack of education which controls the socio-economic status. Consumers should make an informed decision about golden rice, for which education in the necessary field is of utmost importance. The consumers should be in a state to examine critically (Kettenburg et al. 2018). Empowering women and children through education and capacity building would help make golden rice more acceptable. Kettenburg et al. (2018) stated that a complete evaluation of golden rice would need to be conducted on monetary terms to realize its economic efficiency finally. Biofortification can very well help in eradicating malnutrition. However, scientists need to move out and disseminate the knowledge to the masses so that an informed decision can be taken.

6.10 Conclusion

Biofortification is a sustainable agricultural method having minimum cost and has a positive impact on facilitating the well-being of the world's most sizably voluminous undernourished people. Biofortification strategies predicate on crop breeding targeted genetic modification, and the application of mineral fertilizers has an abundance of promise for addressing human mineral malnutrition (Saini et al. 2020). Endeavouring to develop biofortified victuals crops with higher nutritional content, such as Fe, Zn, Se, and provitamin A, ascertains that sufficiency of these and other micronutrients is accessible in the diets of developed and developing countries. In achieving this goal, international initiatives, like CGIAR (Consortium of International Agricultural Research Centers), the Centres Collaboration with HarvestPlus, and national initiatives, serve as pillars. These activities have resulted in crops that can increase both the amounts and bioavailability of essential minerals in human diets, particularly in class grain crops such as rice, wheat, maize, cassava, beans, and sweet potatoes. On the other hand, biofortification of crops is a daunting task. Plant breeders, nutritionists, genetic engineers, and molecular biologists must converge to make this happen. Breeding approaches are generalized and easy to adopt and have been adapted to sustainably improve victorious nutritional qualities. Molecular breeding approaches, which have much higher success rates as genetically fortified crop plants, are facing difficulties due to consumer acceptance and the costly and high regulatory approval processes used between different countries. The use of biofortified crops will create a desirable future as they can kick off malnutrition regarding the micronutrients amongst the poor people worldwide, especially people of developing countries.

Conflict of Interest The authors declare that they have no conflict of interest.

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