# Biofortification for Nutrient Content and Aroma Enrichment in Rice (*Oryza sativa* L.)

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## Abstract

Biofortification is the process of enhancing the content and density of vitamins and minerals that are improving nutritional quality in a staple crop through conventional plant breeding or agronomic practices or using transgenic approaches. Rice (*Oryza sativa* L.) ranks second in most widely consumed cereals in the world and used as a staple food for more than 60% of the world population. Micronutrients are essential for plant growth and development as well as for animal and human health. In the last two decades, the concept of hidden hunger arises in which one-sixth of the world's population suffers from hunger that is a deficiency of micronutrients, vitamins, and nutrients. Rice grain has large genetic variability in the concentration of micronutrients; hence, it is included in the biofortification program, and breeding of new rice cultivars with an enhanced level of grain micronutrients is one of the most sustainable and cost-effective strategies for preventing hidden hunger. To overcome the major problem of hidden hunger and looking to the future, the agricultural community has a fundamental responsibility to produce crop enriched with minerals and vitamins to secure national health, and there is a need to increase the nutritional quality of food through various methods like supplementation, fortifications, etc. Aroma acts as a supporting trait for nutrition; therefore, it needs to be considered in biofortification. It has been shown to enhance food appetite in hungry and in satiated states, so it may be used to stimulate meal initiation and appetite in people

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that are malnourished. Here the process and progress of biofortification with micronutrients have been briefly described, and future prospects to alleviate widespread micronutrient deficiencies in the human population are discussed.

**Keywords**

Biofortification · Minerals · Vitamins · Transgenic · Aroma · HarvestPlus

## 3.1 Introduction

### 3.1.1 Rice

Rice is the staple food of around three billion people, most of them in Asia, which accounts for 90% of global rice consumption. Rice constitutes a major source of nutrition and contributes a significant share of dietary energy in a number of Asian countries. Among 23 species of genus *Oryza, Oryza sativa* L. is cultivated in Asia and *O. glaberrima* Steud. in West Africa (Vaughan et al. 2003). *O. sativa* L. has been further differentiated into *indica* and *japonica* subspecies (Khush 2000). Rice has immense diversity, and it is estimated that more than 100,000 varieties of rice exist in the world (Khush 1997). India has an ancient heritage of rice cultivation and has over 70,000 cultivars of rice germplasm (Siddiq 1992).

Rice is a rich source of proteins, starch, carbohydrates, vitamins, and minerals, which increases the importance of consumption of rice and thus included in a balanced diet. Over two billion people in Asia derive 80% of their energy from rice (Juliano 1985). In addition to common widely consumed white rice, there is some special colored rice like red, brown, and black. These colored rice are due to the deposition of anthocyanin pigment in the outer coat of rice grain (Chaudhary 2003). Black rice is especially rich in anthocyanin, protein, phytochemicals, and vitamins and known for its antioxidant activity as antioxidants are crucial for strengthening the immune system and enhancing the memory (Pengkumsri et al. 2015). According to Ahuja et al. (2007) red and black rice has a high amount of zinc (Zn), iron (Fe), and minerals. These qualities like high mineral content, antioxidant activity, starch quality, and the glycemic index have made rice unique among all the cereals. India is a home for various rice varieties that have medicinal properties and fits in the description of healthy food in terms of modern as well as old concepts (Chaudhari et al. 2018).

Scented rice constitutes small but a special group of rice and considered as of the best quality. Indian sub-continent flourishes with hundreds of indigenous aromatic cultivars and landraces, and the diversity of scented rice of India is highest in the world (Singh and Singh 2003). Scented rice is further classified as basmati and non-basmati type. Basmati type is a long-slender grain; it exhibits kernel length above 6.6 mm, L/B ratio of more than 3, and high kernel elongation after cooking (ratio above 1.8). These unique features of basmati are said to be due to the culmination of centuries of selection and cultivation by farmers that are well preserved and maintained in their purest form as traditional basmati varieties (Siddiq et al. 2012). In addition to basmati varieties, many indigenous non-basmati scented rice varieties are also locally cultivated. In a compendium published by Singh and Singh (2003), the authors mentioned that the diversity of scented rice from India is the largest in the world. The majority of the indigenous scented rice cultivars are small and medium-grained (Singh et al. 2000).

### 3.1.2 Importance of Micronutrients

Micronutrients are essential for plant growth and development as well as for human and animal health. Manganese (Mn), iron (Fe), and zinc (Zn) are of special interest among the micronutrients as they are essential for all higher organisms (Bashir et al. 2013). In the last two decades, the concept of hidden hunger arises in which one-sixth of the world's population suffers from hunger that is a deficiency of micronutrients, vitamins, and nutrients. This hidden hunger is due to the quality of food available, and it is related to the fact that many developing countries rely on low nutrient (intake of vitamins and minerals are too low) staple crops that fail to sustain good health and development. Micronutrient malnutrition is unacceptable and has a severe impact on the health of an individual resulting in poor health, low workability, and decreased earning potential (Bailey et al. 2015). Vitamin and mineral malnutrition affect children less than 5 years of age and women of reproductive age. Deficiency of vitamin A, iron, zinc, iodine, and folate is most common in many countries, which ultimately results in anemia, blindness, increased susceptibility to various diseases, lower IQ, and mortality (Nestel et al. 2006). Anemia is most common worldwide which occurs due to deficiency of iron and affects 38% pregnant woman, 29% non-pregnant women, and 43% pre-school children (Organization 2005).

### 3.1.3 Weapons to Fight against Hidden Hunger and Micronutrients Malnutrition

To overcome the major problem of hidden hunger and looking to the future, the agricultural community has a fundamental responsibility to produce minerals and vitamins rich food to secure national health, and there is a need to increase the nutritional quality of food through various methods like supplementation, dietary diversification, commercial fortification, etc. In supplementation, pharmaceutical manufacturers produce tablets, capsules, and injections supplemented with a high concentration of vitamins and minerals to fill the short-term gap. Dietary diversification includes the cultivation of multifarious staple food crops such as vegetables and fruits with an elevated level of nutrient content that can be produced for better consumer behavior. Commercial fortification is the practice of increasing the content of essential micronutrients in food and fortifies food with essential nutrients at the time of food processing.

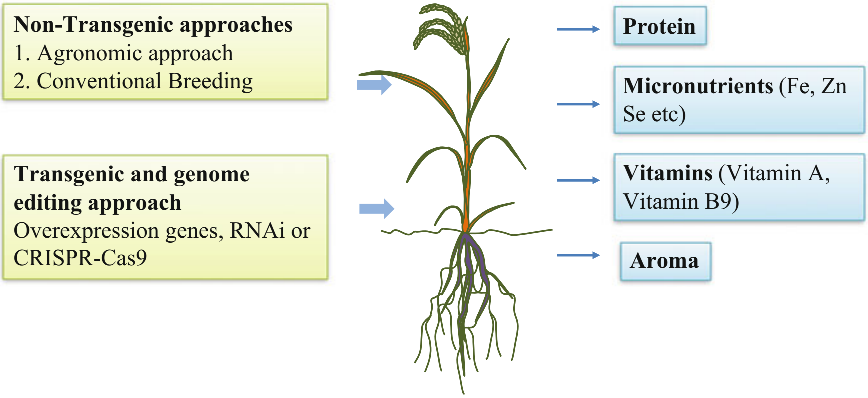

**Fig. 3.1** Different approaches being used for the biofortification in rice. The major biofortification aspects include improvement in protein, micronutrient like Fe, Zn, and Se, vitamins like *A* and B9, and aromatic compounds

These approaches cannot efficiently solve the problem of malnutrition because they only have a temporary role in malnutrition and have expensive costs. These methods also fail to reach the poorest areas of the general people who are at a high risk of micronutrient deficiency.

### 3.1.3.1 Biofortification

A recent approach with high potential to overcome many conventional strategies is the biofortification of staple crops. Biofortification is the process of enhancing the content and density of vitamins and minerals that are improving nutritional quality in a staple crop through conventional plant breeding or agronomic practices or transgenic approaches (Fig. 3.1) (Bouis 2018). Biofortification is a promising and sustainable strategy to target the above-mentioned nutrient deficiencies, especially in a hidden hunger. One of the first biofortified crops under the initiative was rice. Later several varieties of staple crops were developed with an increased level of specific micronutrients (Haas et al. 2005). Biofortification has two key advantages that it is an effective and cheaper alternative to traditional way as these methods are difficult to afford by a large proportion of the world's population especially those who have limited resources and low income and second, it is a feasible means of reaching underserved rural populations that only have limited access to market and healthcare facilities. Once implemented, this strategy will lower the number of micronutrients deficient population, which depends on supplementation and fortification programs (Bouis and Welch 2010).

### 3.1.4 Need for Biofortification in Rice

In comparison to other cereals, rice is eaten every day by poor malnourished people as a staple food; even the addition of a small amount of micronutrients in the diet is beneficial to improve the health and development of an individual. Rice is a major source of carbohydrates and has other nutritional values. Rice grain has large genetic variability in the concentration of micronutrient; hence, it is included in biofortification program, and breeding of new rice cultivars with an enhanced level of grain micronutrients is one of the most sustainable and cost-effective strategies for preventing hidden hunger (Babu et al. 2013). The rice grain is made up of the outer hull, pericarp layer, and inner starchy endosperm with the embryo. The rice is subjected to various operations like harvesting, transport, processing by drying, or milling from the time it was harvested to convert it into white rice with superior cooking quality (Roy et al. 2011). In the first stage of rice milling, the hull is removed, converting it into brown rice, which consists of bran and endosperm. In the next stage, during polishing, the bran layer is removed, which yields the most commonly consumed white rice. Milling is essential as it decreases cooking time, but over milling can result in high breakage and loss of nutrients. The consumers always prefer well-milled rice, but proteins, minerals, and vitamins are high in outer layers, and removal of these during processing can cause considerable loss of nutrients, hence cannot be ignored (Abbas et al. 2011). Losses of macro and micronutrients have been studied in rice grain. Loss of 29% of protein, 67% of iron, approximately 80% of thiamin and other nutrients like lysine, riboflavin has been reported upon milling (Ramberg and McAnalley 2002). The lower content of lysine is the major amino deficiency (Mandić et al. 2009). The current concern in developing new biofortified rice varieties signalizes a need for genotyping differences in polishing losses and how they relate to the distribution of mineral elements in the rice grain.

### 3.1.5 Indian Scenario of Malnutrition

Hidden hunger and micronutrient malnutrition is a major problem in India. Intake of an improper daily diet, which has fewer amounts of micronutrients (<50% RDA), is observed in over 70% of the Indian population (Vijayaraghavan et al. 2002).

The following are the major malnutrition related issues in India.

- Deficiency of micronutrients among children, adults, pregnant and lactating women.
- Iron deficiency anemia (IDA) is a major issue; about 62% of pre-school children are suffering from vitamin A deficiency leading to an annual 3.3 lakh child mortality. 69.5% pre-school children, 58.7% pregnant women, 63.2% lactating mothers are anemic.
- About 57% of pre-school children and their mothers have subclinical VAD.

- The Zn deficiency has not been properly investigated, due to a lack of suitable biomarkers.

## 3.2 Criteria for Biofortification

1. Effective—Micronutrient enhancement level must have an appreciative impact on human health.
2. Stability—Enriched levels of micronutrients must be relatively stable.
3. High yield—For farmers to accept this new concept, crop productivity must be maintained to the guaranteed level.
4. Efficacious—Bioavailability in biofortified lines must be tested in humans so that to ensure that they improve the micronutrient status of people consuming them.
5. Taste and cooking quality—Taste and cooking quality of biofortified crops should be maintained.
6. Consumer acceptance—Acceptability of biofortified crops by the consumer is the major criteria of biofortification.

## 3.3 Approaches Used for Biofortification in Rice

There are several non-mutually exclusive methods used to develop biofortified crops like agronomic biofortification, conventional plant breeding, and transgenic manipulation, which involves genetic modifications or bio-engineering.

### 3.3.1 Non-Transgenic Approaches

Non-transgenic approaches have less regulatory concerns compared to transgenic approaches. Such approaches involve the following methods to enhance micronutrients and to improve the nutritional quality of crops, especially in rice.

#### 3.3.1.1 Agronomic Biofortification

Agronomic biofortification is the fastest and easiest way of biofortification in crops with essential micronutrients in developing countries where cereals are a staple food. Micronutrient fertilizers containing both N, P, K, and S and micronutrient fertilizers like Zn, Ni, Co, Mo, and Se can have substantial effects on the accumulation of nutrients in edible plant parts (Allaway 1986). Agronomic biofortification involves the application of nutrient-rich fertilizers to soil or foliage to elevate the micronutrients concentration in edible parts of the crop and thus increase the uptake of essential micronutrients by consumers. From the viewpoint of biofortification, the foliar application is much better and requires fewer amounts of fertilizers than soil application. Using agronomic biofortification, selenium fertilization has been successfully implemented in increasing selenium content in rice up to 35.9% (Chen et al. 2002). 58% enhanced zinc content in the wheat grain and 76% increase in wheat flour has also been reported (Zhang et al. 2012). This approach is the only way to reach the poorest of the poor rural populations, which cannot afford to improve the components of their diet by incorporating animal products, and it is a win-win approach for developing countries where cereals are a major staple food. However, there are some limitations of agronomic biofortification, like limited access, short-term approach, non-availability in abundance, expenses involved, and failure to reach all individuals, which limit its use and success.

#### 3.3.1.2 Conventional and Marker Assisted Plant Breeding

Plants show genetic variation in micronutrients. Some plants show the high concentration of these essential micronutrients, which allow their use through breeding programs to improve the level of minerals and vitamins in other crops. Plant breeding has been adopted by the farmers for the last hundreds of years. Plant breeders search germplasm bank or seed for the existing population of crops, which are naturally high yielding and high in micronutrients content. These selected varieties were used for breeding. In conventional plant breeding, one of the parents in an initial cross has a high level of target micronutrients, and crossing of these parents results in the progeny with characteristics of both parents (Garcia-Casal et al. 2016). Rice is the best example of conventional plant breeding. In the last decade, biofortification through conventional breeding was focused on increasing the level of three most important micronutrients: zinc, iron, and vitamin A (Ortiz-Monasterio et al. 2007). Besides these, breeders have also developed hybrids with increased content of other micronutrients. Progeny with a high level of micronutrients and high yield was developed by crossing rice variety, which contains a high level of iron and zinc with high yielding rice variety (Khush et al. 2012). This approach has several significant disadvantages when compared with transgenic approaches such as this strategy rely of limited genetic variation present in the gene pool. In some cases, these limitations can be overcome by crossing to distinctly related crops. Conventional plant breeding is prevalent at present as it is cheaper, quicker, and less controversial than genetically engineered crops. Table 3.1 gives an account of the

**Table 3.1** Rice varieties improved for nutritional quality through conventional breeding

| Variety released | Nutrient targeted | Country | Reference/source |
|---|---|---|---|
| CR Dhan 310, hybrid (*O.nivara* × IR 64) | Protein | India, Columbia | Mahender et al. (2016) and Mahmoud et al. (2007) |
| BRRI Dhan 62, BRRI Dhan 72, BRRI Dhan 64, DRR Dhan 45 (IET 23832) | Zinc | Bangladesh | HarvestPlus |
| IR68144-3B-2-2-3 | Iron | India, Philippines | Gregorio et al. (2000) |
| Jalmagna | Iron and Zinc | India | Gregorio et al. (2000) |
| BRRI Dhan 62, BRRI Dhan 72, BRRI Dhan 64 | Zinc<br>Iron | Bangladesh | HarvestPlus |

nutrient rice hybrid rice varieties developed through a conventional breeding program.

## 3.3.2 Transgenic Approach

In some crops, where the target nutrient does not naturally exist at the required levels, and crops that are very difficult to breed, transgenic approach is a feasible way to produce biofortified crops with desired agronomic traits and nutrients.

### 3.3.2.1 Genetic Engineering Via Overexpression of Genes, RNAi, CRISPR/Cas9

In contrast to plant breeding nowadays, the availability of modern techniques to identify and characterize desirable gene function has been a driving force in recent biofortification efforts to transfer these heritable traits between completely unrelated species through genetic engineering. This was made achievable by the rapid development of high throughput whole-genome sequencing techniques, metabolite profiling, physical mapping, and global gene expression analysis in a variety of plant species. The methodology involves molecular techniques or genetic modifications to transfer specific traits or introduce a gene from novel sources for desirable traits to a recipient organism. This approach has benefits like a rapid and direct application by introduction into popular varieties, unlimited access to the gene of interest, and targeted expression in tissues of interest. Genetic modification has two advantages over conventional plant breeding that it allows the transfer of specific genes and takes a short duration to produce a crop with a trait of interest expressed in a stable way.

Several transgenic experiments have been successfully done in many agriculturally important crops to target proteins and micronutrient accumulation in specific tissues. For the efficient manipulation using transgenic technology appropriate integration of omics scale data is useful (Deshmukh et al. 2014; Chaudhary et al. 2019b, c). In-depth understanding about the transgene effect ensures the success of such program aiming for the release of transgenic product for the cultivation. Golden rice is a popular example of genetically modified biofortified crops (Ye et al. 2000). Rice, with high zinc and iron content has been developed through a transgenic approach (Trijatmiko et al. 2016). Currently, significant progress is being made to develop transgenic plants with an increased level of iron and zinc and also increased phyto-availability of mineral elements in soil, their uptake, translocation to the shoot, and accumulation in inedible parts (Zhu et al. 2007; Rana et al. 2019) (Fig. 3.2). These transgenic varieties have tremendous nutritional potential, but limited progress for release has been made so far due to constraints of intellectual property and approval through national biosafety and regulatory processes. In this regard, genome editing approaches more particularly the CRISPR/Cas mediated genome editing looks promising to achieve desirable changes bypassing the regulatory concerns (Vats et al. 2019; Mushtaq et al. 2020). Numerous studies using different CRISPR/Cas9 based tools have been performed in rice (Vats et al. 2019). Most of the initial studies

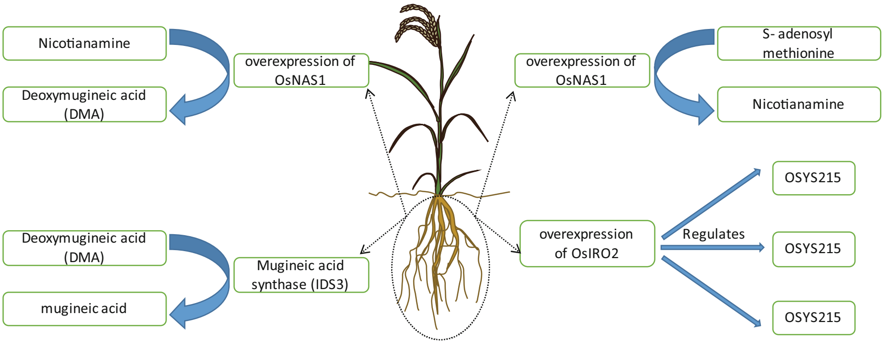

**Fig. 3.2** Set of genes regulating molecular mechanism involved in the iron uptake in rice. These are the major four genes which has been widely targeted through transgenic and other approaches for the iron biofortification in rice

have performed with genes where sufficient information about molecular mechanism is known. Up to now few studies aiming for biofortification through genome editing have been performed in rice. Such studies are discussed here in subsequent sections. Similarly, traditional approach like mutation breeding is more cheaper and convenient way to get desired trait improvement (Chaudhary et al. 2019a, d). However, mutation breeding is more depends on chance to get the desired modification (Bansal et al. 2019; Kumawat et al. 2019). In case of biofortification related traits governed by single gene, chances are much higher to get desired improvements. Another limitation with mutation breeding is the chances getting improvements in the traits governed by positive regulation are much lesser than the traits governed by negative regulation. Recently, mutants selected from the set of 87,000 EMS induced rice mutants have been screened for the improved iron and zinc content in the grains. The study showed a wide range of variation for different elements (Sevanthi et al. 2018). There are numerous studies where mutants with improved iron and zinc have been identified. For instance, a study by Cheng et al. (2007) identified a rice mutant having sequence variation in nicotianamine aminotransferase which found to stimulated the Fe(II) acquisition system and resulted into increased iron accumulation in rice. Similarly, another study where several mutants having high iron and zinc content in polished grains have been identified by screening a set of mutant lines derived from IR-64 variety (Jeng et al. 2012).

## 3.4 Biofortification in Rice

### 3.4.1 Protein

It is estimated that milled rice grain contains 6–7% of protein, representing second abundant constituent in rice (Juliano 1985). It is one of the cereals which are naturally gluten-free with highly digestible protein, therefore ideal for people with celiac disease, babies, and older people. On the basis of solubility, rice proteins are classified into four classes, glutelin (alkali/acid-soluble), a prolamin (alcohol soluble), globulin (salt soluble), and albumin (water-soluble). Despite being the staple food of the world, rice has the lowest protein content as compared to other cereals, so it is essential to produce rice with higher protein content. An inbreed "Frontiere" rice cultivar with high protein was developed by Utomoa Professor and his team at LSU (*Louisiana State* University) and was released in 2015. Another protein-rich rice varieties CR Dhan 310 and CR Dhan 3119 Mukul was developed by ICAR-NRRI, by improving the popular high yielding variety Naveen through conventional breeding and released in 2016 (Mahender et al. 2016). Both varieties have 11% protein content, which is 53% higher than its original parents. Mahmoud et al. (2007) reported that interspecific hybrid between IR64 and *Oryza nivara* showed 12.4% the higher protein. Rice proteins are deficient in some essential amino acids (Lee et al. 2001). Therefore, rice with increased levels of glycinins, lysine, tryptophan, and sulfur-rich storage seed protein such as cysteine has been produced by transgenic approach (Wu et al. 2003; Katsube et al. 1999; Tozawa et al. 2001; Hagan et al. 2003). More recently, the WBLRP lysine-rich protein from winged bean seeds (WBLRP) was transferred into rice (Gao et al. 2001; Liu 2002) and 12% of total soluble seed protein content was observed. Two transgenic rice lines HFL1 and HFL2 (high free lysine) were obtained by a transgenic approach that showed a 25-fold increase in lysine content (Yang et al. 2016).

## 3.4.2 Minerals

Minerals like zinc, iron, selenium, manganese, and copper play an important role in plants and animals. It is estimated that half of the world population is affected by a mineral deficiency, and it is the greatest health concern in the human population (Pfeiffer and McClafferty 2007). It is estimated more than two billion people of the world suffer from minerals deficiency (IFAD W. FAO 2013). Therefore, the crop plants need to biofortified with these minerals.

### 3.4.2.1 Zinc

In comparison to other cereals, rice (*Oryza sativa* L.) contains low Zn concentration (10–22 mg/kg) with less bioavailability (Welch 1993; Myers et al. 2014). Therefore, it is necessary to increase Zn concentration in rice. Pooniya and Shivay (2013) reported that the application of Zn (as zinc sulfate heptahydrate) in Zn deficient soil significantly increased grain yield of rice as well as Zn concentration in rice grain. Zn content in rice can be enhanced through foliar or soil application of fertilizers or $ZnSO_4$ (zinc sulfate). They further reported that combined application of fertilizer with green manures and Zn increases Zn content in grains and yield in basmati rice. Agronomic biofortification of rice by the triple foliar spray of Fe, Zn, and pesticide is a cost-effective strategy to increase Fe and Zn content in rice (Zhang et al. 2018). Biofortification with Zn fertilizer in rice can increase zinc content (Welch 1986). It is reported that Zn content in rice was increased by overexpression of

OsIRT1 (Lee et al. 2009) and the introduction of mugineic acid family phytosiderophores (MAs) synthesis genes from barley (Masuda et al. 2008). “BRRI dhan-62” is the world’s first Zn enriched rice variety developed by Bangladesh Rice Research Institute (BRRI) in 2013 and contains 19–20 parts per million (ppm) zinc. The variety has been developed by conventional breeding by crossing with local variety. DRR Dhan 45 (IET 23832)’ is India’s first zinc enriched (22.6 ppm) and high yielding rice variety produced by ICAR-IIRR and released in 2015. It was developed by a cross between IR 77080-B-34-3 and IR 73707-45-3-2-3.

### 3.4.2.2 Iron

Iron (Fe) deficiency is one of the most serious micronutrient deficiency problems in the human population. Iron content in plants can be enhanced by changing soil conditions such as pH, moisture content, and aeration and through fertilizer application. Wei et al. (2012) and Yuan et al. (2013) reported that Fe fertilizer through foliar application increases iron uptake and efficient translocation into the rice as compared to soil fertilizer. Macronutrient content in the soil also plays a crucial role in the enhancement of iron content in plants. For crop improvement, landraces and wild relatives are considered as an important source of donor genes (Hoisington et al. 1999). So, with this goal, IRRI evaluated 939 rice genotypes for the variability of iron content in grains. The range in Fe concentration was from 7.5 to 24.4 mg $kg^{-}$(Graham et al. 1999). This suggests that sufficient potential of genetic diversity exists to increase the concentration of Fe in the grain. Biofortified high iron rice was developed by conventional breeding at IRRI that has 4–5 times more iron than commercially available rice (Haas et al. 2005). Besides this, through the transgenic approach, the ferritin gene from *Phaseolus vulgaris* in rice endosperm under the control of the glutelin promoter was transferred and recorded twofold increase in the iron content (Lucca et al. 2001). Through*Agrobacterium*-mediated transformation Goto et al. (1999) transferred the soybean ferritin gene, SoyferH1, into the endosperm of rice under the control of endosperm-specific GluB-1 promoter; the transgenic rice showed three-fold more iron content than untransformed rice. Qu et al. (2005) generated transgenic rice by introducing soybean ferritin gene SoyferH-1in the endosperm under the control of the rice seed storage glutelin gene promoter, GluB-1, and the rice seed storage globulin gene promoter, Glb-1, and by introducing the SoyferH-1gene under the control of Glb-1promoter alone. They observed three-fold more iron content than non-transformed rice. Further, for the production of iron-biofortified rice was produced by Masuda et al. (2012) using combined three transgenic approaches. First, through the expression of the Fe storage protein ferritin under the control of endosperm-specific promoters to enhance Fe storage in grains. Second, by overproduction of the metal chelator nicotianamine to enhanced Fe translocation. Third, through the expression of Fe (II)–NA transporter OsYSL2 (metal nicotianamine transporter) under the control of an endosperm-specific promoter and sucrose transporter promoter to enhance Fe flux into the rice endosperm. They observed 4.4-fold increases in Fe concentration when cultivated in the field and six-fold in the greenhouse condition and concluded that for iron biofortification, the introduction of multiple iron homeostasis genes is more effective than the introduction of an individual gene. Tan et al. (2015) generated transgenic rice by overexpression of MxIRT1 (*Malus xiaoginenses iron-regulated transporter 1*) gene from apple trees. The transgenic rice exhibited three-fold higher levels of iron and zinc content.

#### 3.4.2.3 Selenium

Selenium is an essential element for humans and plays an important role in many metabolic pathways, production of selenoproteins, thyroid hormone metabolism, and immune functions (Malagoli et al. 2015). Selenium deficiency leads to many diseases such as Keshan disease, cardiovascular diseases, hyperthyroidism, enhanced susceptibility to infections, and cancer (Malagoli et al. 2015; Brown and Arthur 2001). Selenium content in rice is usually low, so it limits the nutritional requirement of populations that depend on rice consumption for their dietary selenium intake (Williams et al. 2009). Mangueze et al. (2018) reported that foliar Zn and Se application increases the accumulation of these minerals in the IR grains. Boldrin et al. (2013) reported that soil selenate application was more effective for Se accumulation in grain than selenite. Foliar application of both selenite and selenate enhances grain yield. Both soil and foliar Se application could be useful for increasing Se content in rice. Premarathna et al. (2012) reported that broadcast application of $SeO_4^{2-}$ (selenate ion) enriched urea granules to flood water at the heading stage in rice was extremely effective as an agronomic biofortification strategy.

### 3.4.3 Vitamins

Vitamins are essential micronutrients; they cannot provide energy but play an important role in many metabolic processes. The human body cannot synthesize all vitamins, so it is necessary to get them from the diet. Rice is a poor source of vitamins. So far, through the transgenic approach, rice with vitamin A and vitamin B9 has been produced.

#### 3.4.3.1 Vitamin A

Vitamin A deficiency can result in night blindness and xerophthalmia (Sommer 1982). Rice plants produce β-carotene, the precursor of vitamin A in green tissues, but rice grains are devoid of β-carotene. Since rice did not have any cultivar with carotenoids in the grain (Beyer 2010), transgenic approaches have been used for increasing vitamin A content in rice.

**Golden Rice** Golden rice was developed by Prof. Ingo Potrykus of Swiss federal institute of technology and Prof. Peter Beyer of the University of Freiburg. For the development of Golden rice, two genes encode the enzymes phytoene synthase (PSY) from daffodil and phytoene desaturase (CRTI) from the soil bacterium *Erwinia uredovora* naturally involved in carotene biosynthesis were inserted into

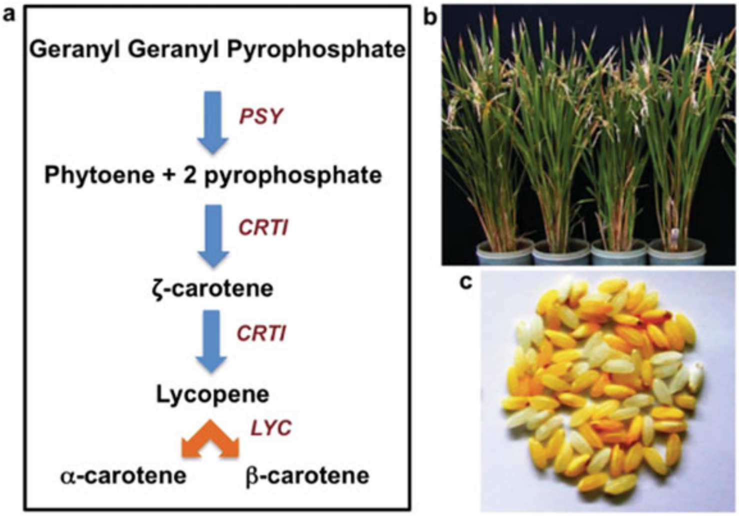

**Fig. 3.3** Development of golden rice by introducing carotenoid biosynthesis pathway through transgenic approach, (**a**) Carotenoid biosynthesis pathway with genes involved in different steps; (**b**) Uniform morphology of transgenic and control rice plants; (**c**) similarly polished rice grains from golden rice (yellow and orange) mixed with control (white). The figure is reproduced from Majumder et al. (2019) available with Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)

the rice genome under the control of endosperm-specific promoter to restart the carotenoid biosynthetic pathway that is normally inactive in rice leads to the production and accumulation of β-carotene in the grains (Fig. 3.3). The new version of golden rice GR2 (31 μg/g and more β-carotene) was produced by Syngenta scientists (Paine et al. 2005), which contains a PSY gene from maize.

#### 3.4.3.2 Vitamin B9 (Folates)

Vitamin B9 is an essential water-soluble vitamin and synthesized de novo in bacteria, fungi, and plants and but the human body does not synthesize and store vitamin B9. It plays an important role in growth and several metabolic processes. Through the transgenic approach, folate concentration in rice has been increased by overexpression of genes encoding *Arabidopsis* GTP-cyclohydrolase I (GTPCHI) and aminodeoxy- chorismate synthase (ADCS) (Storozhenko et al. 2007). They reported that folate concentration increases up to 100 times (38.3 nmol/g) over wild-type rice. Vitamins are unstable molecules; they can degrade easily. So, it is important to stabilize folate in rice. Blancquaert et al. (2015) used two strategies to stabilize folate upon storage, by complexing folates with folate binding proteins and by extending the tail of the folate molecules. They found up to 150-fold higher folate concentration than those in wild-type rice.

### 3.4.4 Oil Quality and Flavonoids

Rice bran is an important oil source, contains antioxidants such as flavonoids, γ-oryzanol, tocopherols and tocotrienols and phytosterol (Park et al. 2017; Sharif et al. 2014) and fatty acids such as palmitic acid, oleic acid, and linoleic acid (Taira et al. 1988) but lacks high levels of α-linolenic acid, which is beneficial for health. α-Linolenic acid content in rice has been increased by introducing the soybean microsomal omega-3 fatty acid desaturase cDNA, the enzyme of which converts linoleic acid to α-linolenic acid under the control of maize ubiquitin-1 promoter (Anai et al. 2003). Transgenic rice seed oil showed enhanced levels of α-linolenic acid content. Oleic acid is oxidatively stable in comparison to linoleic acid (Lopez-Huertas 2010). So, to produce high oleic/low linoleic in rice bran oil, Abe et al. (2018) attempted to disrupt the OsFAD2-1 (fatty acid desaturase) gene, which catalyzes the conversion of oleic acid to linoleic acid by CRISPR/Cas9-mediated targeted mutagenesis. The content of oleic acid increased up to more than twice over wild type. *Japonica* rice varieties possess more antioxidant compounds compared *to indica types* (Goufo and Trindade 2014). Flavonoid content in rice has been increased by expressing maize C1 and R-S regulatory genes (Shin et al. 2006) and phenylalanine ammonia-lyase and chalcone synthase (CHS) genes (Ogo et al. 2013).

### 3.4.5 Aroma and Nutrition

Proper diet is important to the health; hence aroma and taste are essential for proper diet because these are the key determining factor in acceptance and palatability of food. The aroma in the food determines the taste and has many functions such as appetite enhancement, salivation (Rogers and Hill 1989; Engelen et al. 2003; Yeomans 2006), and pleasantness during eating, stimulation, and release of insulin (Johnson and Wildman 1983) and gastric acid (Feldman and Richardson 1986). Besides these, it protects from foul and hazardous food. The aroma in food has been shown to enhance food appetite in hungry as well as in satiated states, so it may be used to stimulate meal initiation and appetite in people that are malnourished (Zoon et al. 2016). It acts as a supporting trait for nutrition; therefore, it needs to be considered in biofortification. Flavor is a combination of smell, taste, and appearance, and it is estimated that 75–95% of flavors of the food we taste actually come from what is smelled (Spence 2015). Taste buds in humans can identify five basic tastes, sweet, sour, bitter, salty, and umami and other remaining tastes are actually identified by the nose through aroma (Choi and Han 2015). Most of the aromatic molecules are volatile such as ether, alcohol, amines, ester, aldehyde, and essential oil, etc. A very minute amount of these molecules can change the flavor of food, and can directly affect the business of food industries.

#### 3.4.5.1 How Is Aroma Perceived?

Richard Axel from Howard Hughes Medical Institute at Columbia University, New York, and from Buck Fred Hutchinson Cancer Research Center in Seattle, Washington. They discovered a gene family consisting of 1000 different genes (3% of human genes) that code for the different olfactory receptors. Compared with the other sensory receptor genes, these are the largest number of genes for one particular system; the olfactory system has been shown to have an important influence. The aroma perception takes place in two ways, orthonasal and retronasal (Landis et al. 2005). The first way, orthonasal is the detection of an aromatic molecule through the nose by sniffing. And the second way, retronasal, is the detection of aromatic molecules that are released from food during eating and drinking. During eating, aromatic molecules are pumped into the nasopharynx, then detected by the same olfactory receptors which are responsible for orthonasal olfaction (Mozell et al. 1969; Halpern 2003). Aromatic molecules are detected by the G protein receptors, which are present on the surface of olfactory cells of the olfactory epithelium. After binding, the receptor triggers a sequence of signals in an olfactory system. The olfactory epithelium passes the signal to the olfactory bulb, which is situated in the brain. Through the olfactory bulb, signals are transferred to the cortex, and response is generated. The proper perception of aroma in the brain is still not fully understood.

#### 3.4.5.2 Aromatic Rice

The aroma is one of the most precious characteristics of rice. Nowadays consumers prefer fragrant rice due to their characteristic and pleasant odor. The aroma is rated the highest desired trait, followed by taste and elongation after cooking by Indian consumers (Bhattacharjee et al. 2002). Asian consumers from the USA consider appearance and aroma as the most important acceptance factors of cooked rice (Meullenet et al. 2001). Hence the demand for aromatic rice is increasing in both domestic and international markets. Several reports show that aromatic rice varieties exhibited higher nutrient content (Gregorio et al. 2000; Renuka et al. 2016).

##### 2-Acetyl-1-Pyrroline (2AP) as a Potent Aroma Molecule and Other Aroma Volatiles

The aroma is a result of more than 250 volatile and non-volatile compounds. 2-Acetyle-1-pyrroline (2AP) has been considered as primary contributor in imparting unique popcorn-like aroma in rice (Buttery et al. 1982). It has been reported that 2AP also gives “roasted flavor” in different food products viz. popcorn (Schieberle 1991), cooked beef, wheat, and rye bread (Schieberle and Grosch 1985), wetted ground pearl millets (Seitz et al. 1993), etc. It has been reported that in addition to rice, 2AP is found in many other plants (Pandan, bread flower, Soybean etc.), animals, fungi, and bacteria also (Wakte et al. 2017). Besides being naturally found in plants, the 2-AP molecule can be synthesized by Maillard reaction, a nonenzymatic reaction between sugar and the amino acid at high temperature (Fuganti et al. 2007)). It is reported that the concentration of 2AP also depends on storage duration and post-harvest treatment (Widjaja et al. 1996; Hashemi et al. 2013; Goufo et al. 2010). Hien et al. (2006) reported that the concentration of 2-AP in the plant is affected by environmental and genetic factors. There are lots of environmental factors that affect the concentration of aroma in plants viz. water stress (Bradbury et al. 2005), abiotic stress (Goufo et al. 2010).

Compounds like alk-2-enals, alka(E)-2, alkanals, 4-dienals, 2-pentylfurans, 2-phenylethanol and 2-acetyl-1-pyroline have been reported as major contributors in total aroma profiling of rice (Gaur et al. 2016).

### Inactivation of BADH2 Gene Leads to Aroma

Any mutational event in functional BADH2 (*Betaine aldehyde dehydrogenase 2*) results in premature termination in the gene producing a truncated protein that results in nullification of the function of the enzyme BADH2 and leads to the synthesis of 2AP. These mutations include 8 bp deletion and 3 single nucleotide polymorphism sites (SNPs) in the seventh exon of Badh2 gene (Bradbury et al. 2005), 806 bp deletion in exon 4–5 (Shao et al. 2013), 7 bp deletion in exon 2 (Shi et al. 2008), and 2 bp deletion in exon 1 (Kovach et al. 2009); these mutations revealed that the aroma is a result of multiple mutational events in BADH2 causing defective BADH2 protein.

### Aroma and Biofortification

Indian sub-continent is considered as a home for aromatic rice diversity (Bisne and Sarawgi 2008). Major attention was given on the improvement of basmati rice type. The improvement of indigenous aromatic non-basmati rice, which showed outstanding quality like aroma, taste, and kernel elongation after cooking and were somewhat neglected because they lacked market value; therefore it needs to be focused on the improvement of indigenous aromatic non-basmati rice type along with basmati type.

There are several examples of improved aromatic rice varieties that are developed by conventional breeding (Table 3.2). India has become the first country for the development of a hybrid variety of basmati rice, Pusa Rice Hybrid-10 (RH-10). Indian Agricultural Research Institute (IARI) released this variety for commercial seed production; apart from this IARI had also developed high yielding basmati varieties viz. Pusa Basmati 1, Pusa Sugandh 2, and Pusa Sugandh 3. Pusa Basmati 1121 released by ICAR-IARI for commercial cultivation, having pleasant aroma, extra-long slender milled grains (9.00 mm) and high cookedkernel elongation ratio of 2.5 with a cooked kernel length of up to 22 mm, volume expansion more thanfour times, good tasteand easy digestibility (Singh et al. 2018). LSU Agriculture Rice Research Station developed the Jazzman rice variety, first US-bred Jasmine-type aromatic rice by cross breeding between the Arkansas variety Ahrent and the Chinese aromatic rice line 96a-8Jazzman has a strong aroma, extremely translucent grains and soft sweet on cooking.

Lei et al. (2017) reported that exogenous application of mixed micronutrients improves yield, quality, and 2-acetyl-1-pyrroline contents in fragrant rice. In vitro study of detached aromatic rice panicles reported that the application of 2-AP, Zn, and La significantly increases 2-AP concentration, Zn and La might be helpful for aroma improvement in rice. Deshmukh et al. (2016) and Mo et al. (2016) reported that the inoculation of rhizobacteria in the basmati rice-growing area at regular intervals might play a role in the enhancement of aroma.

In addition to the non-transgenic approach, the advance transgenic approaches are also promoted and applied for the improvement of aromatic rice varieties (Shan et al. 2015; Niu et al. 2008; Chen et al. 2012)). It is reported that overexpression of the P5CS gene in aromatic rice cultivars results in a more than

**Table 3.2** List of some improved aromatic rice varieties through conventional breeding

| Sr. no | Name of variety | Parentages | Salient features | Reference/ source |
|---|---|---|---|---|
| 1 | Pawana | Pusa-33 × IR-28 | Semi-dwarf, grains: Long slender, resistant to blast, moderately resistant to leaf scald, tolerant to major pests | http://drdpat.bih.nic.in/ |
| 2 | Himalaya-2 | Sabarmati × Ratna | Semi-dwarf, grains: Long bold resistant to blast, susceptible to glume blotch | http://drdpat.bih.nic.in/ |
| 3 | Type-3 | A selection from basmati of Dehradun | Tall, grains: Long slender, white, very fine | http://drdpat.bih.nic.in/ |
| 4 | Punjab Basmati-1 | Sona × Basmati-370 | Medium tall, spikelet awned, grains: Long slender | http://drdpat.bih.nic.in/ |
| 5 | Prabhavati (PBN-1) | Mutant of Ambemohar local variety | Dwarf, grains: Coarse, tolerant to iron chlorosis | http://drdpat.bih.nic.in/ |
| 6 | SYE-ER-1 (IET-9296) | Sona × SYE-44-3 | Grains: Short slender, moderately resistant to blast | http://drdpat.bih.nic.in/ |
| 7 | Indrayani (IET-12897) | Amb-157 × IR-8 | Semi dwarf, grains: Long slender, moderately susceptible to leaf scald, resistant to blast | http://drdpat.bih.nic.in/ |
| 8 | Mahi Sugandha | BK-79 × Basmati-370 | Semi-dwarf, grains: Long slender, strongly scented | http://drdpat.bih.nic.in/ |
| 9 | Karjat-3 (IET-12481) | IR-36 × Karjat 35-3 | Grains: Short bold | http://drdpat.bih.nic.in/ |
| 10 | Taraori Basmati | Pure line selection from local Basmati | Tall, grains: Long slender | http://drdpat.bih.nic.in/ |
| 11 | Ranbir Basmati (IET-11348) | Pure line selection from Basmati-370-90-95 | Grains: Long slender | http://drdpat.bih.ni5n/ |
| 12 | Pusa Basmati-1 | Pusa150 × Karnal local | Extra elongation upon cooking, fine texture, grains: Long slender | Siddiq et al. (2009) |
| 13 | Basmati 385 | TN1 × Basmati370 | Grains: Long slender | Nagaraju et al. (2002) |
| 14 | Pusa 1121 | Sister line of Pusa Basmati-1 | Grains: Extra-long slender, high cooked kernel elongation ratio | Singh et al. (2018) |
| 15 | Pusa RH 10 | Pusa 6A × PRR 78 | Superfine grain, awnless | Siddiq et al. (2009) |

(continued)

**Table 3.2** (continued)

| Sr. no | Name of variety | Parentages | Salient features | Reference/ source |
|---|---|---|---|---|
| 16 | Vasumathi | PR 109 × Pak. Basmati | Semi-dwarf high yielding | Shobha Rani and Singh (2003) |
| 17 | Bhogavati | Selection from Basmati composite | High yielding, resistant to leaf and neck blast | Kumbhar and Sarawate (2010) |
| 18 | Pusa Basmati 6 | Pusa Basmati 1 × PB 1121 | Cooked rice uniform in shape, strong aroma | Siddiq et al. (2012) |
| 19 | Jazzman | Arkansas variety Ahrent × Chinese aromatic rice line 96a-8 | Soft-cooking, grains: Long slender, no seed dormancy, high yielding, moderately early-maturing, strong aroma | Sha et al. (2011) |
| 20 | Jasmine 85 | IR262 × Khao Dawk Mali 105 | Disease resistant, soft texture | Marchetti et al. (1998) |

two-fold increase in 2AP content (Kaikavoosi et al. 2015). Niu et al. (2008) reported that RNAi-based downregulation of OsBADH2 expression influences aroma accumulation in rice. Transcription activator-like effector nucleases (TALENs) method was used by Shan, Zhang et al. (2015) to target and disrupt the OsBADH2 gene for development of aromatic rice, their result revealed significant increased in the 2AP content in transgenic line, this method provides rice breeders with a new approach to breed fragrant rice. A single customized sgRNA (single guide RNA) was used to bring target mutations in three rice genes, OsBADH2, OsMPK2, and Os02g2382 used by Shan et al. (2013) and their result revealed that CRISPR/Cas9 system has higher mutation frequency as compared to TALENs. All these studies will give us important information about the development of biofortified aromatic rice varieties.

## 3.5 Advantages of Biofortification

Biofortification can be a supportable approach for culminating nutritional security along with other strategies like dietary supplementation, diversification, and commercial fortification. The ideal advantages of biofortification are:

1. From an economic viewpoint, it is a one-time investment to develop biofortified seeds, thus cost-effective, and it is beneficial for controlling micronutrient deficiencies, which ultimately improves human health.
2. The most important advantage of biofortification is it does not compromise with yield; therefore, it is economically sustainable to farmers.

3. It capitalizes on the regular daily consumption of large and consistent amounts of staple food across populations regardless of gender, age, and economic status.
4. Constant monitoring is not needed for biofortified crops as it is highly sustainable.

## 3.6 Challenges

For biofortification to be successful and acceptable by consumers, many broad questions must be addressed.

Can biofortification increase the micronutrient density in food to a target level that makes a significant and measurable impact on nutritional status?

Will farmers accept to grow the biofortified varieties of staple crops, and will consumers eat/buy them in sufficient quantities? To answer these questions, research must be carried out.

Getting consumers to accept biofortified crops will be a challenge, but with the help of good seed systems, development of products and markets, and demand creation, this can be solved (Nestel et al. 2006). A successful biofortification strategy requires the adaptation of fortified crops by farmers and consumers.

The success of biofortified crops also relies on good market networks and channels for the distribution of agricultural information. In developing countries, lack of agricultural infrastructure leads to a significant challenge for the adaptation of biofortified crops.

Though the several crops were developed as a proof of concept for biofortification, precise studies of their nutritional impact are needed so that the demand for biofortified food will increase.

Adequate information programs are needed to create public awareness and will play an essential role in ensuring acceptance by the public and farmers.

Strong policy interventions are needed to create interlinking between biofortified producers and various national programs like Rashtriya Krishi Vikas Yojana. High profit would make farmers interested to grow these improved cultivars. Several government-sponsored programs such as the National Food Security Mission and integrated Child Development Program and integration of biofortified grains in mid-day meal scheme would provide an impetus for its popularization.

## 3.7 Organizations

The HarvestPlus program is an international organization that aims to develop micronutrient-rich staple foods to address human micronutrient malnutrition (Singh et al. 2017). The program has targeted grain Zn levels of brown and polished rice. A combination of agronomic and genetic strategies is required to raise grain Zn concentration as many rice fields have low availability of Zn. The transgenic approaches can be advanced through germplasm screening of traditional varieties, old landraces, and wild species to create novel genetic tools to enhance the Zn level in rice grain (Nakandalage et al. 2016). Other organizations viz. DBT, ICMR, and

ICAR, along with international organizations like HarvestPlus, IRRI are now giving their research efforts to biofortification for product development validation and testing. With a proper idea, planning, implementation, and execution, biofortified crops will help to reduce micronutrients malnutrition with a minimum investment in research in India and have a measurable impact on the lives and health of millions of people in the country. In 2004, DBT initiated the India biofortification program for wheat, maize, and rice biofortified with Zn, Fe, and provitamin A. In XI plan, DBT funded biofortification of pigeon pea and groundnut for enhancing vitamin A and sorghum for high Zn and Fe content.

## 3.8 Future Prospects

To increase micronutrient concentrations in staple/edible crops, future research should focus on the identification of the mechanisms involved in micronutrient uptake, mineral-homeostasis in plant cells, integration of agronomic and transgenic approaches to develop a novel strategy to increase mineral transport to tissues. There is a need to refine the planning and monitoring of biofortification programs, considering the biofortification technologies and stakeholders/NGOs that fund biofortification programs. Although the HarvestPlus program is performing a great job, it is required to set priorities and indicators to evaluate the performance of biofortification programs. Regarding the production and consumption of biofortified crops, there is a need to develop marketing and communication strategies considering all issues. Quality assurance and food safety are vital; possible risks of excessive intake must be addressed. Considering environmental changes, possible toxicities, and allergies related to enhanced micronutrient intake still require explosive work. National policies and international standards on food content information and health claims must be highlighted. The transgenic approach requires a convenient regulatory framework for its adaptation. To reach as many as world’s population by 2030, with biofortified crops, policymakers must give high priority to the role of agriculture to refine health. National governments and other institutions must ensure that biofortification is included or based on the nutrition agenda. For further strengthening, research coordination between the agriculture and nutrition specialists requires to decide the target level of micronutrients and proteins, their storage, processing, cooking, and potential levels of consumption by the consumer. Food processors and other factors, along with the value chain, must incorporate biofortified crops in their products. Only through a collaborative effort that reaches across the value chain, biofortification will become successful in upcoming years to overcome the problem of micronutrient malnutrition and hidden hunger.

## 3.9 Conclusion

Recent research reports and developments conclude that an increase in the concentration of micronutrients can be retained in the edible parts after processing, and nutrients are available after consumption by humans. Biofortification is now a proven technology to fight against micronutrient malnutrition and hidden hunger, especially in developing countries where most people rely on staple food crops, which are inherently low in micronutrient concentrations. Enhanced use of fertilizers with required micronutrients, conventional breeding, and genetic engineering are used to develop biofortified crops, and it is being introduced in many countries as a strategy to improve human health. No single type of intervention can, by itself, solve the problem of micronutrient malnutrition. Therefore, biofortification is one of the several evidence-based interventions which can complement the existing interventions with its own implications in improving the overall quality of the diet achieving nutritional security. In recent years biofortification has been shown as a feasible, cost-effective, and promising approach of delivering micronutrients to populations who may have limited access to diverse diets and other inventions and efforts are going on to improve global nutrition by its use further. Although biofortification has many advantages, to ensure sustained impact, it requires continued investment and interest by governments for monitoring the delivery, and in addition, investments by donors for both existing and new programs can further improve impact and footprint. Large scale biofortification should be integrated into nutrition-sensitive and nutrition-specific efforts to control and prevent micronutrient malnutrition.

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