

Biofortified Crops – Boon for Nutritional Security

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Micronutrient deficiencies of iron, zinc, and vitamin-A are such serious global health issues, that it affects one out of every three people worldwide. The intensity of this “hidden hunger” compels us to acknowledge global nutritional security issues. Plant based food are the most popular and trending choices for people of all rungs of the social ladder. Biofortification is a sustainable and promising process of improving nutrition in plant based food through different agronomic approaches, conventional plant breeding and genetic engineering. Biofortified crops have been developed with high iron, high zinc, vitamin-A, with other nutritional quality enhancements and these crops have already proved to be a ‘boon for nutritional security’. This review highlights some selected Biofortified crops with special reference to rice (*Oryza sativa*), as 50% of the global population relies on it.

Key Words: Hidden hunger, Biofortification, Nutritional Security, Rice, Wheat, Maize, Cassava, Legumes

Introduction

Human population is ever increasing and is currently more than 7 billion (Fig. 1A). According to a United Nations new report, we will be 9.8 billion by 2050 and 11.2 billion by 2100 (www.un.org/en/desa, accessed on July 18, 2022). An increase in the global food production (Fig. 1B) is the need of the hour to ensure food security. Food may satisfy hunger but only nutritionally enriched food can satiate “hidden hunger”.

Malnutrition has affected more than 2 billion people around the world, mostly in countries of South Asia, Africa and Latin America (FAO, 2015). It stealthily compromises the immune system, declines growth of the mind and body and increases chances of mortality. Daily, more than 24,000 people die globally owing to malnutrition (Fiaz *et al.*, 2019). Iron deficiency anaemia (IDA), zinc deficiency, and vitamin-A deficiency (VAD) are common among the malnourished population. Children of sub-Saharan Africa, South and Southeast Asia are suffering from VAD and its associated risks more than other countries in the world (Wu *et al.*, 2021). Such deficiency makes these children vulnerable to VAD associated infectious, diarrheal diseases, blindness, sensory losses, and premature death (Schmitz *et al.*, 2012).

Adequate supply of nutrient enriched diversified food, and food supplements may not be sustainably available to the affected malnourished population. One of the sustainable approaches to combat malnutrition in all the affected parts of the world is through nutrition enriched food crops (Welch, 2002). Cereal crops contribute the most in the daily energy intake in malnourished populations. People from Asia and Africa depend on such staple crop meals to meet 60–80% of their per day energy requirement (Fig. 1C). Rice, wheat (*Triticum aestivum*) and maize (*Zea mays*) are the most popularly produced cereal crops worldwide (Fig. 1D). It is understandable that nutritional improvement of these crops could have a massive positive health impact on the world population.

Crops that have been developed for the purpose of fighting against malnutrition are called biofortified crops. These can be developed by means of conventional breeding and genetic engineering. As many as 35 countries have accepted and released such biofortified crops (conventionally bred) for cultivation and were consumed by a population of 40 million (Fig. 1E) (Mishra *et al.*, 2022).

Recently, a high provitamin-A rice variety (Golden Rice) developed through genetic engineering has been released in the Philippines (Majumder *et al.*, 2022).

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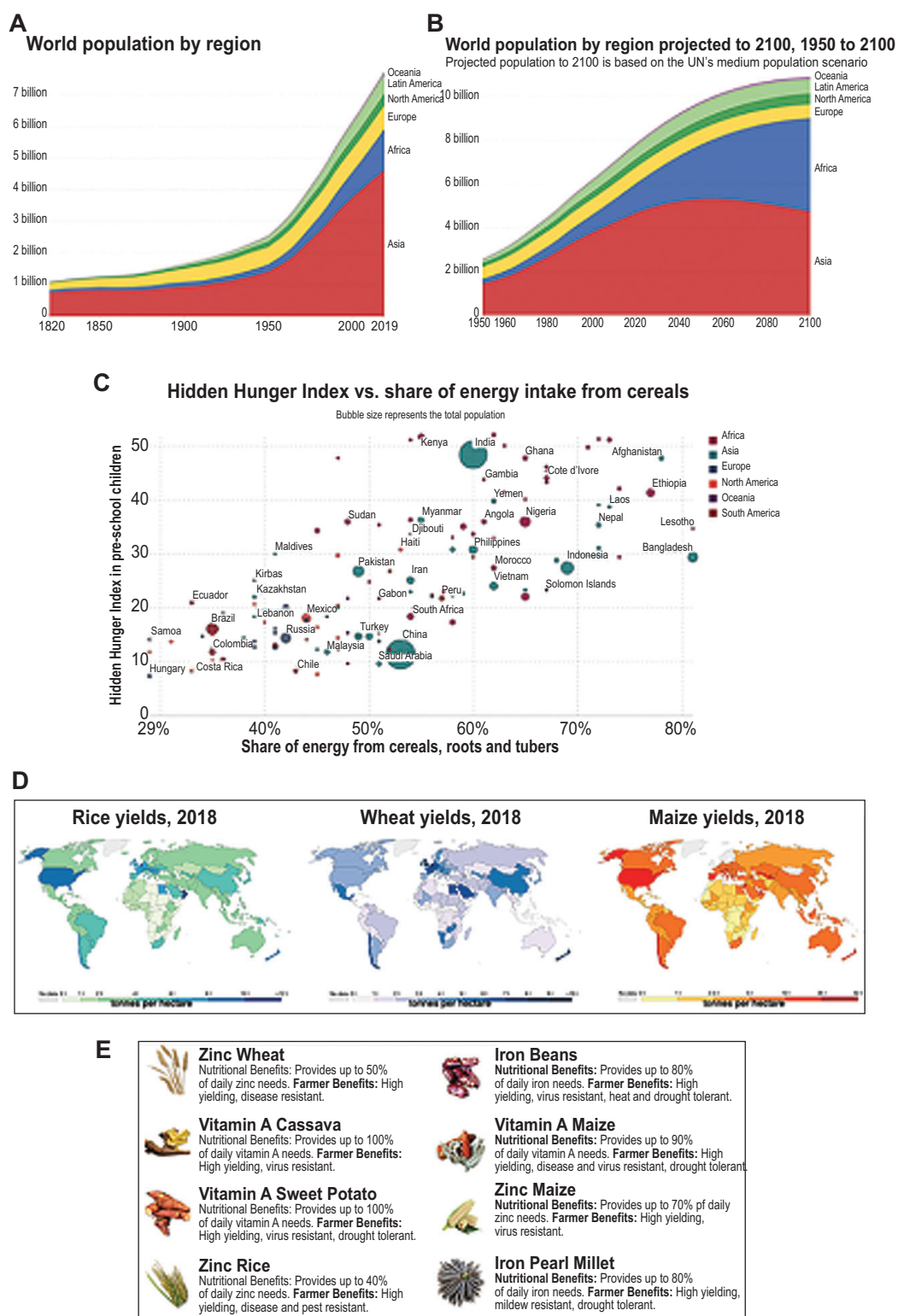


Fig. 1. The world population and cereal nutrition. Current world population by region (A). World population by region projected upto 2100 (B). Share of energy intake from cereals (C). Worldwide yield (tonnes/hectare) of three major cereal crops- rice, wheat, and maize according to 2018 data (D). Released biofortified crops created by conventional breeding approaches (E). This infographic is created based on the information from <https://www.harvestplus.org/> and <https://ourworldindata.org/micronutrient-deficiency>, accessed on July 20, 2022.

Biofortified Rice

Rice is undisputedly the leading cereal crop as a major part of the global population rely on it for upto 70% of their daily calories (Mishra *et al.*, 2018). Modern techniques of molecular breeding were essential to the development of many biofortified rice varieties. The provitamin-A enriched Golden Rice, is one such variety which was recently approved in the Philippines and hopefully it will be also approved soon in Bangladesh (Wu *et al.*, 2021). Different approaches have been successfully applied to developed high iron and high zinc biofortified rice. Recently, the CRISPR/Cas9 genome editing tool was used to develop high oleic rice (Abe *et al.*, 2018).

Golden Rice

Invention of golden rice was a much celebrated application of metabolic engineering. Bacterial and plant genes were introduced into rice to produce β -carotene, a pro-vitamin A precursor. Synthesis of β -carotene in rice endosperm, specifically in non-photosynthetic tissue like endosperm (edible part), requires overexpression of phytoene synthase (PSY) from daffodil (*Narcissus pseudonarcissus*) and bacteria (*Erwinia uredovora*) phytoene desaturase (CRT-I) (Datta and Datta, 2020). Presence of β -carotene in the endosperm adds the golden (yellow orange) colour (Fig. 2).

Introduction of Daffodil *PSY* gene into japonica rice variety (Taipei-309), a carotenoid synthesis pathway intermediate phytoene was induced in rice endosperm Burkhardt *et al.* (1997). This proved the principle that provitamin-A can be synthesised in a non-photosynthetic, carotenoid-lacking plant tissue (endosperm). This report is considered as the first golden rice (golden rice-1) development. The same research group also transformed Daffodil *PSY* and lycopene β -cyclase (*LCY*) with bacterial (*E. uredovora*) *CRT-I* into Taipei-309 giving results of 1.6 $\mu\text{g/g}$ total carotenoids (Ye *et al.*, 2000). Combination of these genes gave similar amounts of total carotenoids (1.05 $\mu\text{g/g}$) in other rice cultivars of IR 64, BR 29, Nang Hong Cho Dao and Mot Bui as reported by Datta *et al.* (2003). Till date, 37.0 $\mu\text{g/g}$ of total carotenoids have been reported (golden rice-2) using maize *ZmPSY* and *E. uredovora CRT-I* genes in japonica rice cultivar-Asanohikari (Paine *et al.*, 2005).

In the last two decades, continuous improvement has been observed in golden rice research (Fig. 3). Initially, golden rice was developed using different japonica rice varieties but about 90% of the rice consumers preferred indica rice worldwide (Khush 2001). Therefore, Golden indica rice development was emphasised by multiple research groups with different promoters and gene combinations for better accumulation of carotenoids

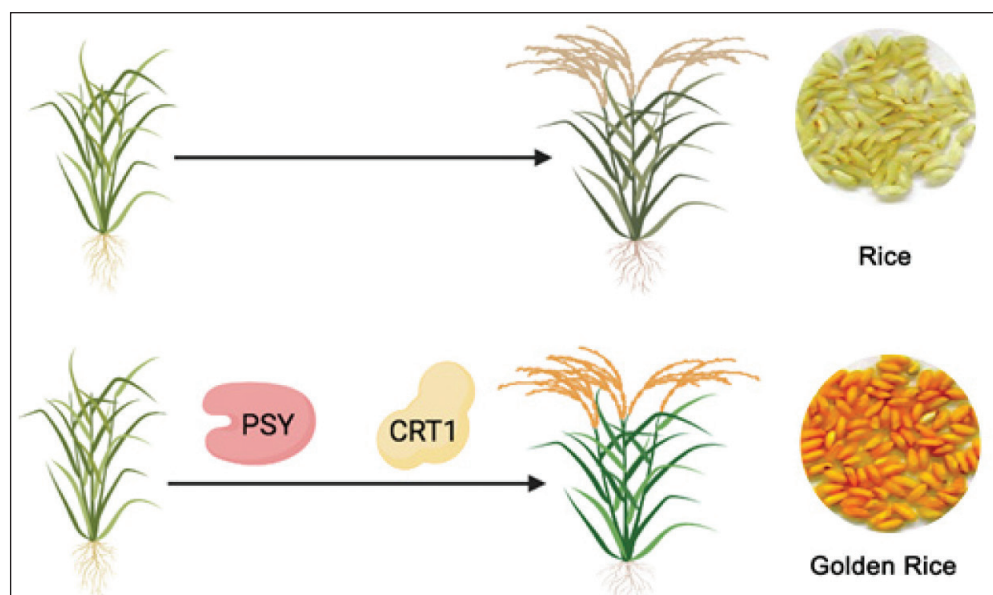


Fig. 2. Metabolic engineering in rice and β -carotene expression in rice grains. Conventional rice is unable to produce β -carotene in its endosperm but golden rice harbours *phytoene synthase* (PSY), and *phytoene desaturase* (CRT-I) genes from other sources and can produce β -carotene in the endosperm. Source of the golden rice picture is from Datta et al., 2007.

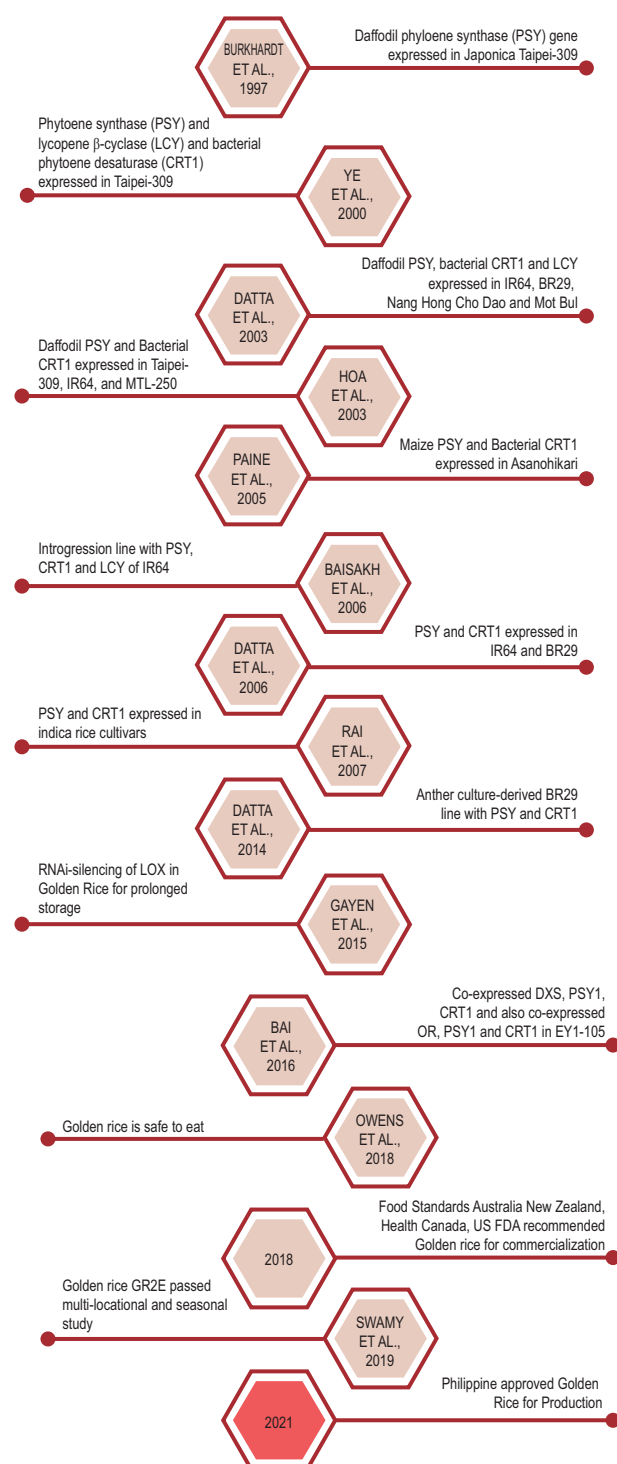


Fig. 3. Timeline (1997-2021) of Golden rice development and its advancement research

in the rice seeds (Datta 1999; Datta *et al.*, 2003, 2006, 2007, 2014; Parkhi *et al.*, 2005; Rai *et al.*, 2007; Rehana *et al.*, 2018; Ghosh *et al.*, 2019).

One limitation of golden rice is deterioration of its carotenoid level during storage (Datta *et al.*, 2021). This is caused by lipoxygenase (LOX) that catalyses lipid peroxidation resulting in the seed quality deterioration. LOX produces hydroperoxy fatty acids which oxidise carotenoids and decolourize seeds (Gayen *et al.*, 2015). Diminishing LOX activity in golden rice seeds can give better stability to carotenoids during storage. Seed specific RNAi-mediated *LOX* gene silencing in golden rice showed less deterioration of carotenoids and stabilised seed quality (Gayen *et al.*, 2014, 2015, 2016).

Recently the three distinguished agencies for international food safety regulation - the Food Standards Australia New Zealand, the Health Canada, and the United States Food and Drug Administration, highly approved ‘Golden Rice’ for commercialization (Datta and Datta, 2020). The Philippines, is the first country in the world for planting and cultivation of golden rice (Wu *et al.*, 2021). Positive perception is gradually developing towards commercialising golden rice with the availability of positive biosafety data on it (Owens *et al.*, 2018).

High Iron Rice

According to the World Health Organization (WHO) estimation, around 800 million children and women are affected by anaemia (WHO, 2015). Developing countries (regions like South-East Asia, Eastern Mediterranean and African) endure IDA more than the higher-income zones (North America, Europe and some parts of Central Asia) (Majumder *et al.*, 2022). Iron biofortified rice could save many lives from these regions. High iron rice has been achieved by multiple strategies some of which are discussed here.

Overexpression of ferritin gene

Upto 4500 iron atoms can form a complex (non-toxic) with the multi-subunit protein ferritin and from this complex the human intestine can absorb iron ions (Andrews *et al.*, 1992). Soybean (*Glycine max*) *ferritin* genes *SoyferH1* and *SoyferH2* were found to be useful for developing iron biofortified rice (Kok *et al.*, 2018). Soybean *ferritin* gene was expressed in rice with the help of globulin and glutelin promoters (Goto *et al.*, 1999; Lucca *et al.*, 2002; Vasconcelos *et al.*, 2003; Qu *et al.*, 2005; Khalekuzzaman *et al.*, 2006; Oliva *et al.*, 2014). Rice *ferritin* gene (*Osfer1*) was transferred into Pusa Sugandhi II rice under the control of a glutelin promoter that accumulated over 2-fold iron in milled

rice grains (Paul *et al.*, 2012). Upto 4.4-fold increment of iron content has been reported in IR64144 rice using the Soybean *ferritin* gene (Vasconcelos *et al.*, 2003) and upon being introduced into the Swarna rice variety, this gene produced 2.54-fold more iron in milled rice than the control (Paul *et al.*, 2014).

Silencing of Phytic Acid in Rice Grains

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate or IP₆ or InsP₆) is commonly found in cereals. It predominantly accumulates in the seeds as insoluble complex salt of mineral ions, known as phytate. It is highly potent in chelating divalent ions like iron (Fe²⁺), zinc (Zn²⁺), magnesium (Mg²⁺), and calcium (Ca²⁺), thereby reducing their bioavailability in the body (Majumder *et al.*, 2019). Down regulation of *myo*-inositol-3-phosphate synthase (MIPS), which catalyses the initial step of phytic acid biosynthesis pathway in rice seeds, successfully generated low phytate seeds. Initially the down regulation process was driven by constitutive CaMV35S promoter (Feng and Yoshida, 2004) and later by seed specific promoters like Glutelin B-1 (Kuwano *et al.* 2006) and Oleosin 18 (Kuwano *et al.*, 2009 and Ali *et al.*, 2013a) to eliminate the undesirable effects on vegetative tissue. Reduction of phytic acid level in rice seeds was also achieved by tissue specific downregulation of *myo*-inositol trisphosphate kinase (ITPK) in Khitish rice cultivar (Karmakar *et al.*, 2020 and Sengupta *et al.*, 2021) and *myo*-inositol pentakisphosphate-2-kinase (IPK1) in Pusa Sugandhi II (Ali *et al.*, 2013b). Recently, Sengupta *et al.* (2021) reported that seed specific downregulation of rice *ITPK-2* gene reduces 37% of phytic acid content in the transgenic seeds.

Degradation of Phytic Acid

Phytase is an enzyme that can degrade phytate (a salt form of accumulated Phytic acid) and release chelated minerals, including phosphate. Unfortunately, the digestive tract of monogastric animals, including humans, possess negligible or no phytase activity (Colombo *et al.*, 2020). Due to that, monogastric animals can break down only about 10% of the phytate during digestion and causes major unavailability of important divalent ions of iron, zinc, magnesium, and calcium. Fortunately, degradation of phytate was achieved in rice by seed specific expression of the phytase gene- *Aphytase*, derived from fungi (*Aspergillus fumigatus*) (Wirth *et al.*, 2009; Boonyaves *et al.*, 2016, 2017) and the phytase gene-*appA*, derived from bacteria (*Escherichia coli*)

(Bhattacharya *et al.*, 2019). Recent study in Khitish indica rice cultivar showed that seed specific expression of the *E. coli appA* gene not only increased twofold iron content but also increased zinc by threefold and inorganic phosphorus (Pi) levels by fourfold in rice grains (Bhattacharya *et al.*, 2019). Such rice biofortification strategies can improve the bioavailability of multiple, nutritionally important, divalent ions at a time.

Improvement of Iron Bioavailability

Studies have shown that absorption of non-haem iron can be increased by cysteine-rich metallothionein (MT) (Taylor *et al.*, 1986; Hsieh *et al.*, 1995). A combination of MT gene and *Aphytase* iron-biofortified Taipei-309 rice was developed that could complete the degradation of phytic acid (Lucca *et al.*, 2001). Thereby genes or combination of genes can be used to ensure better absorption of divalent ions to improve bioavailability.

Chelation-based Strategy

When the iron concentration is low, roots of graminaceous staple crops (including rice) secrete phytosiderophores (PS) like mugenic acid (MA) and avenic acid at a low concentration which chelates iron or zinc from the soil (Romheld and Marschner, 1990; Marschner and Romheld, 1994). Two genes – *nicotianamine synthase* (NAS) (*OsNAS1*, *OsNAS2*, and *OsNAS3*) and *nicotianamine transferase* are responsible for PS synthesis in rice (Huguchi *et al.*, 1999; Nozoye *et al.*, 2011). The rice NAS gene was overexpressed to develop high iron biofortified rice (Lee *et al.*, 2009; Johnson *et al.*, 2011; Lee *et al.*, 2012). In some cases *NAS* genes (*HvNAS1*) from Barley (*Hordeum vulgare*) and Barley's *IDS2* and *IDS3* genes increased iron content in grains of polished rice (Masuda *et al.*, 2008, 2009; Suzuki *et al.*, 2008). This seed specific expression of PS (chelation based) strategy provided double iron concentration in biofortified rice.

Zinc Biofortified Rice

The zinc-regulated, iron-regulated transporter-like proteins (ZIP) family protein in rice facilitate Zn and Fe uptake and homeostasis. Overexpressing the ZIP family genes *OsIRT* and *MxIRT* elevated zinc and iron concentration in rice grain (Lee and An, 2009; Tan *et al.*, 2015). Zinc accumulation in seeds was increased by using a combination of *AtIRT1*, *AtNAS1* with *Pvferritin* gene expression in biofortified rice (Boonyaves *et al.*, 2017). Approaches for rice iron biofortification also gave positive results of zinc accumulation in seeds.

Strategies of expressing the *Osfer2* gene (Paul *et al.*, 2012), and silencing of important genes of the phytic acid pathway- *MIPS* (Ali *et al.*, 2013a), *IPK1* (Ali *et al.*, 2013b), and *ITPK* genes (Karmakar *et al.*, 2020; Sengupta *et al.*, 2021) in rice was accompanied by increased zinc content in the seeds along with iron.

High Oleic Rice

Oleic acid (18:1, monounsaturated) is one of the fatty acids found in rice grains that has many health benefits. It helps maintain healthy blood pressure, prevent heart attack, stroke and other cardiovascular diseases (Lopez-Huertas, 2010). Four fatty acid desaturase 2 (FAD2) genes – *OsFAD2-1*, *OsFAD2-2*, *OsFAD2-3*, and *OsFAD2-4* have been identified in rice. *OsFAD2-1* is directly involved in oleic acid to linoleic acid conversion (Zaplin *et al.*, 2013). Down regulation of the *OsFAD2-1* gene can help maintain a higher amount of oleic acid in rice by preventing conversion to linoleic acid. CRISPR/Cas9 knockout *OsFAD2-1* gene from Nipponbare rice confirmed this hypothesis as the concentration of oleic acid in rice seeds surged by twofold (Abe *et al.*, 2018). Rice bran oil (RBO) made from such CRISPR-ed (knockout *OsFAD2-1*) rice cultivars can have high commercialization potential as RBO is favoured by many Asian households as ‘healthy cooking oil’.

Wheat Biofortification

After rice, wheat is the second most popular and produced cereal crop in the world (Fig. 1D). It is a staple food for around 30% of the world population (Lobell *et al.*, 2011). Agronomic, genetic approaches and available genomic resources for wheat biofortification have been recently reviewed in multiple articles (Ludwig and Slamet-Loedin 2019; Saini *et al.*, 2020; Ali and Borrill, 2020). Research on wheat biofortification is limited compared to rice.

Provitamin-A biofortification was achieved in wheat, by maize *PSY* and bacterial (*E. uredoovora*) *CRT-I* gene introduction into an elite wheat variety EM12 (Cong *et al.*, 2009). In another report provitamin-A content increased 76-fold upon expressing the bacterial *CRT-B* and *CRT-I* genes in Bobwhite wheat (Wang *et al.*, 2014). When the *OsNAS2* gene was made to overexpress under a maize ubiquitin promoter in wheat, the iron content increased by 2.1-fold and zinc content by 3.7-fold in the grains (Singh *et al.*, 2017). Similar results were reported in another study where overexpression of the *OsNAS2* gene in wheat increased iron content by 1.4-

fold (Beasley *et al.*, 2019). Seed specific expression of the soybean *ferritin* gene in wheat resulted in 40 µg/g of iron accumulation in the wheat foliage (Drakakaki *et al.*, 2000) and 44.5 µg/g surged iron in the biofortified wheat endosperm (Borg *et al.*, 2012). A vacuolar-iron transporter- *TaVIT2* gene was expressed under a seed-specific promoter, which resulted in a two-fold increase in iron content than its control (Connorton *et al.*, 2017).

Maize Biofortification

Provitamin-A (β-carotene) biofortification in maize through transgenic approaches was successfully implemented. The bacterial *CRT-B* and *CRT-I* genes, expressed in maize, surged up 34-fold (9.8 µg/g) more β-carotene in the endosperm than control seeds (Aluru *et al.*, 2008). In another study, corn *PSY-1* and the bacterial (*E. uredoovora*) *CRT-I* genes were introduced in M37W maize variety under wheat glutenin promoter and barley D-hordein promoter, respectively that accumulated 60 µg/g β-carotene in biofortified maize (Naqvi *et al.*, 2009). Updates on maize biofortification for provitamin-A, zinc, vitamin-E, high protein through conventional and molecular breeding, and country wise commercial availability of such biofortified maize varieties have been recently reviewed by Prasanna *et al.* (2020).

Cassava (*Manihot esculenta*) Biofortification

More than 800 million people in the world consume cassava and the sub-Saharan African population gets 50% of the daily calories from it (Howeler *et al.*, 2013). Biofortification for iron, zinc, provitamin-A have been achieved in cassava. For iron biofortified cassava development algal iron assimilatory protein FEA1 has been introduced into cassava and resulted in a threefold increase in iron content (Ihemere *et al.*, 2012). When the *Arabidopsis* vacuolar iron transporter *VITI* gene was transferred into cassava it gave four-times increase in iron accumulation in the root (Narayanan *et al.*, 2015). Combined expression of three genes – *AtIRT1* (iron transporter), *FER1* (ferritin) and *VITI* resulted in 18-times higher iron accumulation and 10-times higher zinc accumulation in the biofortified cassava (Narayanan *et al.*, 2019). For zinc biofortification, *Arabidopsis* zinc transporter gene *AtZIP1* and *AtMTP1* were transferred into cassava (Gaitán-Solís *et al.*, 2015). Carotenoid accumulation upto 6.67 µg/g in cassava has been achieved by expressing the bacterial *CRT-B* gene (Welsch *et al.*, 2010).

Legumes Biofortification

Legumes are an excellent source of nutrition, having high protein, minerals, dietary fibre, and complex carbohydrates. The amount of certain minerals, like zinc and calcium, is low in legumes (Wang *et al.*, 2003). Plant growth-promoting bacteria (PGPB) play a significant role in the biofortification of legumes (Roriz *et al.*, 2020). Iron content of mung beans (*Vigna radiata*) has been increased by 3.4-fold in association with the strain of *Pantoea dispersa* (Patel *et al.*, 2018). The lysine content of pigeon pea (*Cajanus cajan*) was increased significantly in the transgenic line (Thu *et al.*, 2007). Genome-editing technology has been used successfully in several legume crops for biofortification (Bhowmik *et al.*, 2021). Fatty acid and isoflavone content of soybean was increased by CRISPR-Cas9 mediated genome editing technology (Zhang *et al.*, 2020). Amino acid and proteins have been increased by traditional breeding in mungbean (Abbas *et al.*, 2019).

Conclusion

Nutritional security can be achieved sustainably through biofortified crops. The global catastrophe of losing human lives to VAD, IDA, and zinc deficiency and related health problems can be extensively reduced by including provitamin-A, high iron, and zinc biofortified cereals in the diet. Not only do malnourished people need biofortified crops, nutritional enriched foods are important for everyone. Nutrition is crucial for better immunity and to fight the ongoing COVID-19 pandemic that made it evident to the world the urgent necessity for nutritionally enriched, sustainable biofortified foods. World governments and policymakers must find a way to accelerate biofortified crops adoption specifically in those countries where hidden hunger is engulfing the vulnerable masses. The approval of Golden rice cultivation in the Philippines has inspired the world to shun taboos related to GMOs and accept the simplicity of scientific evidence for the noble cause of human wellbeing.

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