



Genetic enhancement of rice for nutritional traits – Present status and future strategies

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ABSTRACT

Rice is an indispensable diet for over 3 billion people in over 100 nations worldwide, but vitamins, essential amino acids, minerals and phytochemicals are lacking in it. A better understanding of the processes that are involved in the synthesis, uptake, metabolism of macronutrients and their transportation is needed to improve the nutritional aspects of rice. Several attempts have been made over the last decade to brush up nutritional and other quality traits, either through traditional breeding or biotechnological approaches. The present study provides a concise overview of the accomplishments and challenges associated with genetic as well as metabolic engineering approaches aimed at improving the quality of rice grains.

Keywords: Rice quality, Biofortification, Nutritional quality, Transgenic rice and malnutrition

INTRODUCTION

Rice (*Oryza sativa*) is a consumable starchy cereal grain of the family Poaceae. In South Asia, China, Korea, Thailand and Japan, rice is extensively grown cereal. It can be broadly categorized into white rice, black rice, red rice and green rice based on the caryopsis colour (Malik *et al.*, 2022). The most popular type of rice among these is white rice. Rice may be made in several forms, including steamed rice, fried rice, rice cakes, noodles, dumplings, parched rice, and sticky rice (Sharma and Singh, 2022). The world's rice crop is mostly consumed by humans, accounting for 95% of its use. Rice serves as the primary food supply for the bulk of the global population, particularly in Asia's east and southeast. It supplies over 25% of the necessary caloric intake for half of the world's inhabitants. However, it provides up to 76% of the daily calories for most of the population of Southeast Asia (Bhardwaj *et al.*, 2020; Fitzgerald *et al.*, 2008; Miura *et al.*, 2011). People typically eat polished (or milled) rice, which has had the embryo and bran layers removed during preparation, leaving only the endosperm. Hence,

the nutritional composition of polished rice is determined by the endosperm, which consists mostly of starch (70-80%), along with a relatively small proportion of proteins (about 7-10%) and lipids (less than 1%) (Yang *et al.*, 2018). Food with higher nutritional value is always favored for the sake of human health. This staple meal contributing at least 20% of the daily protein intake, 3% of the daily fat intake and other vital components. Almost fifty percent of the global population obtains up to 70% of their daily calorie consumption from rice, which serves as an essential grain and reservoir for nutrients. However, this main staple crop for food only contains a small amount of many important micro and macro elements that are crucial for human health such as vitamins, minerals, some phytochemicals, essential amino acids, and fatty acids (Birla *et al.*, 2017). Therefore, the inadequate supply of crucial nutrients poses a big problem for rice consumers. Agricultural techniques, post-harvest processing, type of cultivar as well as tactics for selection for breeding purpose and genetic means are some of the elements that affect the dietary value of rice (Singh and Kumar, 1996; Singh *et al.*, 2018).

Over the past few years, significant effort has been made to improve the nutritional content of rice through traditional breeding, marker-assisted breeding, genetic, or transgenic techniques. To develop the varieties of rice with increased nutrition content over the past ten or so years has largely been a result of mutation breeding as well as genetic engineering.

However, losses which occur after post-harvest are seldom ever taken into concern for nutritional evaluation. After the rice is harvested, it is stored, then it is processed, washing is done for cleaning purpose and then it is cooked. All these procedures can all affect its nutritious content. To mitigate these deficiencies in nutrients, it is possible to increase the levels of minerals such as pro vitamin A, zinc and iron in the endosperm of rice, particularly targeting individuals in rural and urban areas who have limited access to fortified foods and diverse diets. Consequently, it is necessary to boost the dietary significance of rice by firmly established breeding procedures or cutting-edge biotechnology. Plant biologists are now using a diverse range of methods to achieve this objective (Das *et al.*, 2020).

NUTRITIONAL COMPOSITION AND RICE STRUCTURE

For billions of people worldwide, rice is the main source of carbohydrates and is an essential component of a healthy diet. The hull, pericarp or seed covering, starchy endosperm and germ or embryo make up rice seeds. The hull is taken during the milling process, leaving whole grain called brown rice with its bran coat and germ. White rice is produced by removing the outer covering (called bran) from brown rice. The embryo of the grain contains majority of the mineral substances, about one quarter of the protein, roughly all the vitamins, and around three-quarters of the fat. In contrast, the endosperm consists largely of carbohydrates as well as protein. Rice bran contains minerals, phenolic compounds, sterols, vitamins such as niacin, thiamine, tocopherol, tocotrienol, b-carotene, lutein and several health-promoting phytochemicals with antioxidant, anti-inflammatory and anti-hypercholesteric properties (Goffman *et al.*, 2004; Lonsdale, 2006). Despite several benefits, brown rice lacks popularity among customers due to its limited shelf life and incoherent sensory feature (Fitzgerald *et al.*, 2009). The disparities in nutrient levels between husked and milled rice are shown in Table 1, while

Table 1. Nutrient composition per cup of brown or white rice (Tian *et al.*, 2004)

Product	Brown rice	White rice
Protein (g)	5.54	4.43
Fat (g)	1.96	0.39
Carbohydrate (g)	51.7	53.2
Fiber (g)	3.32	0.56
Calories (Kcal)	248	242
Iron (mg)	1.1	2.8
Phosphorus (mg)	208	68.8
Magnesium (mg)	78.8	24.2
Zinc (mg)	1.4	0.8
Copper (mg)	0.2	0.1
Manganese (mg)	2.0	0.7
Thiamine	0.4	0.3
Vitamin B-6 (mg)	0.3	0.1
Niacin	5.2	3.4
Folate (mcg)	18.2	108

Table 2 provides the recommended micronutrient intake for a healthy individual.

Table 1 clearly shows that, aside from carbohydrates, rice includes very little phosphorous, iron, and protein. The outer brown coating of the grain contains the bulk of the nutrients and minerals. The outer layer of rice, known as bran, is abundant in nutrients such as proteins, fibre, gamma oryzanol, tocotrienols, vitamins and minerals, (Tian *et al.*, 2004; Iqbal *et al.*, 2005; Schramm *et al.*, 2007). Due to these factors, brown rice is the most nutritious of all rice varieties. Rice is composed of complex carbohydrates that are metabolized into glucose, which is primarily used as a source of energy and cognitive fuel. Brown rice has a prolonged starch digestibility, resulting in the retention of some starch molecules that remain unaltered into sugar and pass

Table 2. Essential micronutrients for optimal human health (Sun *et al.*, 2010)

Micronutrient types	Infants	Adults	Pregnancy
Thiamin (mg/day)	0.2	1.2	1.4
Riboflavin (mg/day)	0.3	1.3	1.4
Niacin	2	16	18
Vit B6 (mg/day)	0.1	1.3	1.9
Folate (µg/day)	80	400	600
Vit B12 (µg/day)	0.4	2.4	2.6
Vit C (mg/day)	25	45	55
Vit A (µg /day)	375	500	800
Vit D (µg/day)	5	10	5
Calcium (mg/day)	300	1000	1200
Iodine (µg/kg/day)	15	2	3.5
Iron (mg/day)	0.55	0.6	100
Zinc (mg/day)	0.84	3.37	5

through the large intestine without being broken down (Dolson, 2009). As a result, those with Type II diabetes should prefer brown rice over white. Eating brown or whole-grain rice has some benefits which includes reduce the chance of diabetes (Sun *et al.*, 2010). Despite all these advantages, consumers do not favor brown rice as much as white rice due to its brief shelf life and variable sensory qualities (Fitzgerald *et al.*, 2008). Brown rice's short shelf life and declining nutritional content during storage are caused by lipid peroxidation via the lipoxygenases (LOXs), LOX1, LOX2, and LOX3 (Shirasawa *et al.*, 2008; Kaewnaee *et al.*, 2011).

About 90% of white milled rice is comprised of carbohydrate including both amylose and amylopectin, 5-7% is protein, and 0.5-1% is lipids. When it comes to micronutrients, many individuals who eat rice as their main food have severe deficiencies in Fe, Ca, Zn, iodine and vitamin A (Bhullar and Gruissem, 2013, Rani *et al.*, 2024). According to Ahuja *et al.*, (2008), iron and zinc, which are required for the creation of hemoglobin and various enzymatic processes are abundant in red and black rice. Additionally, red rice contains vital nutrients like copper, manganese and potassium that the body needs for healthy nerve, brain, and metabolic processes. Natural brown rice contains minerals that support the hormonal system, control blood pressure and aid in the healing of scars. The issues of chronic diseases and malnutrition have been linked to the possible health benefits of eating whole rice grains (Dipti *et al.*, 2012). In addition, Genotypes with enlarged embryos and smaller endosperms have more phytochemicals than those with typical embryo/endosperm ratios. Therefore, altering embryo size is crucial for the nutrient makeup of rice grains. Chemical mutagenesis was employed to create mutated rice giant embryo (*ge*) rice from wild rice (Chen *et al.*, 2015). Using such

mutants and a map-based methodology, Nagasawa *et al.*, (2013) were able to clone the gene regulating the giant embryo (GE) trait.

NEED FOR IMPROVEMENT IN THE NUTRITIONAL PROFILE OF RICE

Rice consumption is associated with a notable problem of micronutrient deficiencies in individuals. Rice eaters often increase their rice consumption in response to a calorie need, without proportionally increasing their consumption of other nutritionally dense meals. Plenty of individuals choose not to consume a diet abundant in whole grains, veggies, protein and fruits.

Different levels of nutrients are available in the rice's outer shell and inner endosperm. Rice has a low lysine content despite being a magnificent source of the vitamin B group, thiamine, riboflavin, niacin, aspartic, and glutamic acids. Because of climate change, the nutrient value of rice and other cereal crops has dropped (Smith and Myers, 2019). In rice-producing countries, elevation in CO₂ levels results in reduction in rice protein, B vitamins and micronutrients (Table 3), which may have a detrimental influence on prenatal and child health. Nevertheless, the nutritional evaluation seldom accounts for the losses incurred as a result of post-harvest procedures. After the rice is collected, how it is stored, processed, washed, and cooked all these activities have an impact on fluctuating nutrition in grains. Rice post-harvest nutrient losses can have a significant adverse influence on food safety. The post-harvest loss of a certain commodity refers to the measurable reduction in both quality and quantity (Malik and Chaudhary, 2002). After harvesting, paddy rice undergoes dehydration and is then milled to remove the unwanted hull. Brown rice, also known as hulled rice, contains 90%

Table 3. Environmental and other factors affecting nutrient quality in rice

Factors	Affect	References
CO ₂ in the atmosphere	<ul style="list-style-type: none"> • 17% reduction vitamin B₂, B₁ • 13% reduction vitamin B₅ • and 30% reduction vitamin B₉ 	Smith and Myers (2019)
Solar rays, soil nitrogen, fertilizer application	<ul style="list-style-type: none"> • Change in protein, zinc and iron content 	Malik and Chaudhary (2002)
Cooking process of rice in India	<ul style="list-style-type: none"> • 50% reduction in calcium and phosphorus, 10% decrease in protein, and 75% decline in iron 	
Cooking with excess water	<ul style="list-style-type: none"> • Reduction over 25% of riboflavin, thiamine and niacin 	
Removal of the bran layer	<ul style="list-style-type: none"> • Minerals and vitamins 	

endosperm, 2-3% embryo, and 6-7% fiber (Chen *et al.*, 1998). These nutrient deficiencies can be reduced by raising the amount of pro vitamin A carotenoid, zinc and iron in rice endosperm. Therefore, it is crucial to magnify the nutritional sum of rice using established breeding techniques or cutting-edge biotechnological approaches. Multiple strategies have been used by plant biologists to achieve this objective.

However, the question of whether the increase in micronutrients, amino acids or protein in rice endosperm alone can significantly satisfy the nutritional needs of people who rely on rice is still up for debate. The crop's nutritional value may also rely on how well the plant grows and is strongly influenced by the environment. Salinity, drought, and high or low temperatures are additional factors that limit growth and development of rice. Therefore, while using rice varieties that are abundant in micronutrients or essential amino acids, the superior features are preferred to be paired with additional qualities such as abiotic stress resistance.

REGULATION AT AMINO ACID AND PROTEIN LEVEL

Humans have a strong interest in boosting the concentrations of certain crucial amino acids in agricultural plants due to their inability to make these compounds. It was made possible by crop genetic research and genetic engineering techniques to enrich the key crop plants by upgrading the amount of essential amino acids and protein in them. Tryptophan and lysine received a lot of focus because cereal crops can only produce so much of them. The limited availability of superior genetic resources for plant breeding has hindered the effectiveness of genetic breeding techniques in some crop species.

Furthermore, because lysine and tryptophan do not have a seed-specific role, genetic traits for elevated levels of these amino acids are typically unified with aberrant plant growth. On the other side, genetic engineering manipulation worked well because it could enable the expression of particular characters of interest in specific seeds. The ability to incorporate the trait into various genotypes and plant species is another advantage of genetic engineering. However, to progress metabolic pathways via genetic engineering, it is important to possess a thorough understanding of how regulatory network pathways interact to control plant production and growth.

MANIPULATION OF PROTEIN CONTENT

Rice grain protein concentration (GPC) is a significant indicator of cereals' nutritional value. Its content depends upon amino acid contents and having polygenic inheritance. Amount of protein in polished grain is 5-17%. Both conventional method and genetic engineering are used for manipulating amino acids of rice. While much success has been achieved by the method called genetic engineering while little by conventional breeding methods. Reason for this little success is lack of plenty of genetic resources in hand for genetic breeding. Rice GPC also vary in *Indica* and *Japonica* species, *Indica* (4.9 to 19.3%) having more GPC as compared to that of *Japonica* (5.9 to 16.5%) species. In comparison to other cereal grains, rice has a comparatively low protein content of about 7%.

The *AmA1* is a seed albumin (a kind of protein found in animal sources) concentrated from the pseudo-cereal *Amaranthus hypochondriacus*. In 2017, Xu *et al.* performed an experiment where they used an *Agrobacterium*-mediated co-transformation system and a twin T-DNA binary vector to introduce *AmA1* into rice. The integration of *AmA1* into the rice genome was confirmed using a southern blot analysis. Real-Time PCR was used to evaluate *AmA1*'s transcription and western analysis was used to confirm that it was expressed. Utilizing a quick amino acid analyzer and the Kjeldahl technique, the contents of amino acids and proteins were found. The total protein content in the seeds of five homozygous lines rose by 1.06 to 12.87% when compared to the wild-type control. Furthermore, the content of several EAAs, such as lysine, threonine and valine substantially increased in the best expressing line. Peng *et al.* (2014) conducted an experiment and demonstrate how the manufacturing and assembling of albumins, glutelins, globulins, prolamins and starch are regulated by the quantitative trait locus (QTL) qPC1 in rice, which regulates GPC. OsAAP6, a putative amino acid transporter that is encoded by the gene qPC1, acts as a supportive regulator of GPC with greater OsAAP6 bearing being correlated with elevated GPC. OsAAP6 affects the distribution of various amino acids and greatly enhances the uptake of different amino acids by the roots. GPC diversity appears to be primarily correlated with two frequent variants in the OsAAP6 5' untranslated regions' potential cis-regulatory elements in *indica* cultivars. Their finding marks an initial step in elucidating the regulatory

mechanism that regulates the natural divergence of GPC in rice.

MANIPULATION OF LYSINE CONTENT

Plants require lysine as an essential amino acid; however, its synthesis is very limited in rice and several important crops. Consequently, a significant number of rice researchers were committed to comprehending the control and administration of lysine metabolism and its utilization in order to enhance the quantity of unbound lysine in the seeds. Several researchers are also keen on employing proteins with a high lysine content. Lysine is generated via the aspartate family path, as well as via another pathway that synthesizes two other significant amino acids, threonine and methionine (Frankard *et al.*, 1992; Shaul and Galili, 1992).

A new approach to enhance the lysine content in cereals involves the incorporation of lysine at different codons during translation, using a modified tRNA-lysine produced by genetic recombination (Wu and Chen, 2003). Recombinant production of this tRNA in transgenic rice could greatly increase the amount of lysine in rice seeds (Wu and Chen, 2003; Yang *et al.*, 2016). In HFL rice, increased serotonin biosynthesis was discovered by Yang *et al.* (2018) using a transcriptomic and metabolomic method. Serotonin is a neurotransmitter that is correlated with endosperm colour. Furthermore, the increased expression of *TDC3*, a pivotal gene involved in serotonin production, provided evidence for this.

Elevated lysine levels in plant tissues may lead to decrease in seed production and atypical vegetative and reproductive development. Long *et al.* (2012) conducted an experiment to create genetically modified rice to upgrade Lysine levels. *Dihydrodipicolinate synthase* (DHPS) and lysine feedback-insensitive *aspartate kinase* (AK) from bacteria were expressed to improve Lys biosynthesis (Table 4). Rice lysine ketoglutaric acid reductase/saccharopinedehydrogenase (LKR/SDH) was inhibited by RNA interference to decrease

catabolism and the combined expression of Free Lys increased by up to a factor of 12 in the leaves and a factor of 60 in the grains of these transgenic plants. Two histone proteins, named as *RLRH1* and *RLRH2*, which are endogenous rice lysine-rich were developed by Wong *et al.*, (2015) in an experiment to be overexpressed in rice seeds to accomplish lysine biofortification. An allergic order-based homology screening on their protein sequences was successful. The levels of these proteins in grains were increased to a modest level by employing an altered rice glutelin 1 promoter with reduced expression intensity to prevent the development of physiological discrepancies such as unfolded protein response.

MANIPULATION OF IRON AND ZINC CONTENT

Iron and zinc have an important appearance in electron transfer in both respiration and photosynthesis. The human organism requires the minerals zinc (Zn) and iron (Fe), which are both essential. The most common micronutrient deficiencies globally are those of Fe and Zn. Polished rice of common varieties holds approximately 2 µg/g Fe. Programs for food or supplement fortifying with iron and zinc haven't always been an alternate method called Fe biofortification includes developing cultivars that efficiently mobilize, absorb, and transport Fe to the edible parts of the plant (Bhardwaj *et al.*, 2022). For a significant period, attempts to enhance additional nutritional characteristics of rice grains via selective breeding were halted as a result of protein-related setbacks. The research indicates that an increase in the concentration of iron and other metals in grains does not consistently lead to a decrease in agronomic production. As an illustration, the rice genotype IR68144, which was developed for high yield with good disease resistance, was found to have high amounts of both Fe (21µg/g in brown rice) as well as Zn (Gregorio *et al.*, 2000). The discovery of QTLs for rice grain Fe concentration in wild species may be useful for improving rice grain nutritional

Table 4. Enrichment of protein and amino acid content of rice using the transgenic approach

Element type	Gene details	Sources	References
Lysine	<i>LRP</i> , <i>RLRH1</i> , and <i>RLRH2</i>	Rice, Bacteria, <i>Psophocarpus tetragonolobus</i>	Yang <i>et al.</i> , (2016)
Total protein and essential amino acids	Seed albumin (<i>AmA1</i>)	<i>Amaranthus hypochondriacus</i>	Xu <i>et al.</i> , (2017)

superiority through marker-assisted selection. The genetic variation that results in variable mineral element quantity in rice grains has been investigated. However, QTL analyses are currently very restricted. A strategy like improving iron uptake from soil was also applied from improving Fe content of rice. The first obstacle to Fe accumulation in edible plant sections is primary rhizosphere uptake. Trans-membrane proteins are always required to enable Fe entry into the plant because Fe (and other cations) cannot diffuse through lipidic membranes. Despite the abundance of Fe in soils and sediments, its availability to plants is often limited due to the propensity of Fe³⁺ to form insoluble Fe oxides (Lemanceau *et al.*, 2009). Dixit *et al.*, (2019) conducted an experiment to identify genomic region(s)/QTL(s) for grain Fe (iron) and Zn (zinc) concentration and developed BC₂F₅ population, which was created by crossing RP-Bio226 and Sampada for the identifications of yield and yield-related traits beside QTL(s). The mapping group was genotyped using 108 SSR markers. The average mineral content ingrain in the mapping population diverse from 10.5-17.5 ppm for Fe and 11.3-22.1 ppm for Zn over the two seasons. A cross-season comparison showed four consistent QTLs for Fe (qFe1.1, qFe1.2, qFe6.1, and qFe6.2) and two QTLs for Zn content (qZn1.1 and qZn6.2). By using marker assisted selection (MAS), the discovered QTLs may be employed to develop varieties that exhibit enhanced yield and higher concentrations of Fe and Zn. Boonyave *et al.* (2017) created transgenic lines of rice that expressed the gene cassettes for *AtIRT1* and *PvFER* and compared the resulting lines to those transgenic lines. Additionally, they evaluated the effectiveness of the *MsENOD12B* and native *AtIRT1* promoters to stimulate the expression of *AtIRT1* in rice employing each type of gene cassettes. It was concluded that the native *AtIRT1* promoter was a superior choice for driving the expression of *AtIRT1* in biofortification strategy. The single insertion transgenic lines revealed a substantial rise in iron concentration in both polished and unpolished grains. However, the combined expression of *AtIRT1*, *AtNAS1*, and *PvFER* proved to be an even more effective strategy in achieving the highest iron levels, reaching up to 10.46 µg/g dry weight.

A rice cultivar that was biofortified with zinc and created through conventional breeding in Bangladesh was made available in 2013 by CGIAR-Harvest Plus. Approximately 1.5 million farming families in India are now cultivating eight distinct

varieties of zinc-fortified rice. IET 23832 (DRR Dhan 45) is a biofortified semi-dwarf, middle duration (125 days), non-lodging plant type that has a zinc concentration of 22.6–4.00 ppm in polished grain. It was developed by the Indian Institute of Rice Research, Hyderabad (retrieved from: <https://icar.org.in/node/6293>, on 14 April 2019). Additionally, using HarvestPlus material with required amylose content (20.7%), guaranteeing moral cooking quality and moderate resistance to rice blast disease (*Magnaporthe grisea*), sheath rot disease (*Sarocladiummoryzae*) and rice tungro virus infection, the IET 23832 was developed through conventional breeding. Ali *et al.*, (2013) found that by using RNAi to silence the MIPS gene in the phytic acid biosynthesis pathway, the quantity of calcium, zinc, magnesium, and iron in milled rice grain increased.

MANIPULATION OF B-CAROTENE CONTENT

Vitamin A insufficiency is extensive in nations where rice is a principal food and fulfils most dietary needs for human health because rice endosperm lacks beta-carotene. A vital micronutrient in an everyday diet is vitamin A, also known as beta-carotene. Dietary carotenoids have a sum of positive health effects, counting a lower chance of cancer and eye disease. Numerous carotenoids, including β-carotene, lycopene, zeaxanthin, and lutein have been investigated for their role in human health, glycoprotein synthesis, cell differentiation, antioxidant properties and bone growth and development. Carotene may have additional advantages because it can turn into vitamin-A. Since dark rice is unable to produce vitamin A. Carotenoid-biofortified rice is often referred to as “golden rice” (GR) due to the grain’s color. Rice’s seed-specific activation of carotenoid synthesis and accumulation of β-carotene are both accountable for the grains’ acquisition of a yellowish orange hue. The proposal of golden rice took root at an International Agriculture forum held in the Philippines during 1984. Ye *et al.*, (2000) deployed endosperm-specific glutelin promoter to introduce the daffodil *PSY* gene and the bacterial phytoene desaturase (*CRTI*) gene from *Erwinia uredovora* into the japonica rice cultivar *Taipei-309* through Agrobacterium-mediated transformation. These gene combination yielded a total carotenoid content of 1.6 µg/g in the endosperm of rice grains. In order to enable pro vitamin A

biosynthesis in the endosperm of several *indica* rice cultivars (Dutta *et al.*, 2003) adapted to various ecosystems of different nations, a combination of transgenes was introduced by means of the biolistic method of transformation. The confirmation of stable integration and expression of transgenes were verified through biochemical and molecular analyses. The polished rice grain's golden colour revealed a carotenoid buildup in the endosperm. By using HPLC and spectrophotometric analysis, the colour intensity and the predicted carotenoid content were correlated. Phytoene desaturase (*CRTI*) and Lycopene beta-cyclase (*LCY*) bonded to the peptide transit sequence of the pea-Rubisco small subunit, were regulated by intrinsic cauliflower mosaic virus promoter, while phytoene synthase (*PSY*) was driven by seed-specific rice glutelin promoter (Gt-1 P) (CaMV35S P). Through screening, transgenic plants were produced with either the CaMV35S P-driven hygromycin phosphotransferase (*hph*) gene or the CMP-driven *pmi* (phosphomannose isomerase) gene. One of the prerequisites is development of stable homozygous lines for the selection and assessment of transgenic lines as well as for usage in crossbreeding programs to introduce the transgenes into different backgrounds. It typically takes a minimum of three generations to select homozygous lines when using a transgene as a marker, based on the copy quantity and position of the introduced genes. The production of doubled haploids using another culture method shortens the breeding cycle and quickly corrects homozygosity. Dutta *et al.*, (2014) conducted anther culture of main transgenic plants in order to produce stable, doubled haploid rice that produces β -carotene as soon as feasible. It can be difficult to maintain the nutritional properties of GR in a proper manner. *Lipoxygenase* (LOX), an enzyme found in seeds, catalyzes the incorporation of molecular oxygen into polyunsaturated fatty acids (PUFAs), producing conjugated hydroperoxide products that oxidize carotenoids and diminish nutritional quality of seeds. The r9-LOX1 gene, one of 14 different LOX protein-coding variants found in rice genome, oversees deteriorating seed quality (Carrera *et al.*, 2007). RNAi-mediated down-regulation of r9-LOX1 gene in Golden Rice, regulated by the Oleosin-18 promoter resulted in enhanced storage stability and survival seedlings. In the future, this technique may serve as a supportive technique for the long-term safeguarding of rice seeds (Gayen *et al.*, 2015; Singh *et al.*, 2017). To enhance the concentration of zinc, iron and

carotene in the grain's endosperm, researchers created rice lines that express genes for a single genetic locus for Arabidopsis NICOTIANAMINE SYNTHASE 1 (*AtNAS1*), bacterial CAROTENE DESATURASE (*CRTI*), bean FERRITIN (*PvFERRITIN*) and PHYTOENE SYNTHASE (*ZmPSY*) from maize. The findings provide a proof-of-concept for the simultaneous addressing of multiple micronutrient deficiencies through the multiple -nutrient enhancement of rice kernels from a specific genetic locus.

MANIPULATION OF VITAMIN C

Due to its antioxidant, anti-atherogenic, immunity-boosting and anticarcinogenic qualities, ascorbic acid is a crucial water-soluble vitamin. It carries out a variety of tasks, including the biosynthesis of neurotransmitters, muscular carnitine, catecholamines, and collagen. Since the gluconolactone oxidase enzyme is absent in humans, they are incapable of generating this vital vitamin. Although various ascorbic acid biosynthesis pathways have been described in plants, little is known about this pathway in monocot. A commonly used high yielding rice cultivar was adapted to various abiotic stresses using a biotechnological method of raising foliar ascorbate levels by Holler *et al.*, (2015). The ascorbate biosynthesis gene GDP-L-galactose phosphorylase (*AcGGP*) which was extracted from kiwifruit was engineered to express in the cultivar IR64 (*Oryza sativa L. ssp. indica*). Transgene expression increased ascorbate levels in the leaves by up to >2.5 fold, but no morphological characteristics were altered (grain weight, sterility rate, seed yield, and biomass).

CHALLENGES FOR QUALITY/ NUTRITIONAL ENHANCEMENT

The production of functional proteins and enzymes that affect the metabolic pathways of macro- and micronutrients biosynthesis without interfering with other endogenous metabolism, plant growth, and development is necessary for the usage of genetic/metabolic engineering in improving nutritive excellence. Quality improvement is now feasible, thanks to quick advancements in biochemistry, molecular biology, genetic engineering, omics platforms and analytical tools. Many workers have made successful attempts to incorporate micronutrient genes using transgenic approach (Table 5).

Table 5. Enhancement of micronutrients in rice by transgenic approaches

Element type	Gene(s)	Sources	References
Zinc (Zn) and Iron (Fe)	Zinc and iron content elevation via <i>OsGluB1</i> ferritin, <i>IDS3</i> , <i>NAS</i> , <i>IRT1</i> , <i>OsVIT pro-SoyferH1</i> gene	Rice, Barley, Maize and Soybean	Suzuki et al. (2008); Masuda et al. (2009); Paul et al. (2012); Trijatmiko et al. (2016)
β -carotene	<i>Lycopene β cyclase</i> , phytoene synthase and <i>crtI</i>	<i>Narcissus pseudonarcissus</i> (daffodil), <i>Erwinia uredovora</i>	Ye et al. (2000) ; Tang et al. (2009)
Zinc (Zn), Iron (Fe), and beta carotene	<i>AtNAS1</i> , <i>ZmPSY</i> , <i>CRTI</i> , <i>PvFERRITIN</i>	Arabidopsis, Maize Bean, Bacteria and Bean	Singh et al. (2017)

Though lot of approaches are available, still engineering numerous genes to target several steps of a biosynthetic pathway or distinct pathways to incorporate multiple traits is difficult even nowadays. Thus, transformation technology necessitates the creation of a high-capacity binary vector, creation of a diverse set of effective monocot promoters and the transfer of multiple genes as a single genetic locus. The artificial chromosome (mini chromosome) method is superior to current techniques of randomized integration of one or limited genes transferred at the same period via *Agrobacterium* or biolistic-mediated genetic transformation. However, the question of whether an increase in micronutrients, amino acids or protein in rice endosperm alone can significantly satisfy the nutritional needs of those who depend on rice is still up for debate. The crop's nutritional quality may also be based on the plant's ability to grow properly and is strongly influenced by the environment. Other factors that limit rice development, growth and production include drought, salinity and temperature extremes. Climate change has a negative impact on rice output and grain quality by increasing temperature, CO₂ levels, drought, salinity and flooding. Several authors have investigated the relationship between changing climatic circumstances and decreased yield and poor grain quality. Adverse environmental conditions raise chalkiness and lower fertility have an influence on grain quality (head rice and milling yield), with an apparent decrease in grain weight and grain filling. Therefore, it is suggested to combine the superior features with other qualities like abiotic stress tolerance in order to adopt the essential amino acid and micronutrient rich rice types.

Acceptance of nutritionally enhanced rice or other foods has long been a source of debate due to biosafety issues. Even though numerous risk

assessment studies have been conducted to assuage consumers' concerns, no adverse effects on health or the environment have been noticed thus far. To fully investigate the biosafety of GM products using these high throughput technologies, case-by-case research is required.

CONCLUSION

For a huge figure of poor populations who solely hinge on rice as a primary diet, particularly in developing nations, the poor nutritional superiority of rice and the lack of diversified diets have emerged as a significant problem. Micronutrient deficiencies caused by an unbalanced diet have a detrimental influence on people's diet and well-being. Over 200 million children are undernourished, and about 5 million of them pass away each year from a lack of macro- and micronutrients. Thus, it is imperative to fortify the most significant staple products like wheat and rice. Plants with increased nutritional content can be developed quickly using genetic engineering. It functions by upregulating the gene(s) of the necessary nutrient's biosynthetic pathway or by downregulating the gene(s) of the competing or catabolic pathway. However, the transgenic method has its own drawbacks, just like other technologies. The greatest obstacle is overcoming societal anxiety regarding the acceptance and biosafety of genetically modified plants.

It has already been determined that rice contains a variety of nutrients, including vitamins, minerals, and some secondary compounds. However, in the foreseeable future, it is necessary to include supplementary vitamins and minerals such as thiamine, magnesium, and calcium. Furthermore, one of the biggest obstacles to rice improvement is the dearth of comprehensive information regarding the relevant biosynthetic and regulatory pathways of

the essential nutrients along with the relevant genes and transcription factors. Research is needed to evaluate and consider different rice development strategies that consider regional and/or national preferences, multidisciplinary complementarity for better understanding, along with public-private partnership schemes to systematically distribute the gained benefits at a larger scale.

To prevent malnutrition in the future, the nutritional worth of rice must be improved in emerging nations. In the modern day's rice nutritionists and researchers are collaborating to boost the nutritional worth of rice by utilizing biotechnology or breeding techniques to produce new and improved kinds to serve dietary needs for rice-dependent people. Innovations in biotechnology have come to light as a potential solution that could tackle the prevailing issue of hunger by enhancing the nutritional content of rice. The development of the necessary genotypes will be greatly aided by nutritional value-related genes and QTLs.

REFERENCES

- Ahuja, U., Ahuja, S. C., Thakrar, R. and Singh, R. K. (2008). Rice—a nutraceutical. *Asian Agri-History*, 12(2), 93-108.
- Ali, N., Paul, S., Gayen, D., Sarkar, S. N., Datta, S. K. and Datta, K. (2013). RNAi mediated down regulation of myo-inositol-3-phosphate synthase to generate low phytate rice. *Rice*, 6(1), 12. <https://doi.org/10.1186/1939-8433-6-12>
- Bhardwaj, A. K., Chejara, S., Malik, K., Kumar, R., Kumar, A. and Yadav, R. K. (2022). Agronomic biofortification of food crops: An emerging opportunity for global food and nutritional security. *Frontiers in Plant Science*, 13, 1055278.
- Bhardwaj, A. K., Rajwar, D., Basak, N., Bhardwaj, N., Chaudhari, S. K., Bhaskar, S. and Sharma, P. C. (2020). Nitrogen mineralization and availability at critical stages of rice (*Oryza sativa*) crop, and its relation to soil biological activity and crop productivity under major nutrient management systems. *Journal of Soil Science and Plant Nutrition*, 20(3), 1238-1248. <https://doi.org/10.1007/s42729-020-00208-y>
- Bhullar, N. K. and Gruissem, W. (2013). Nutritional enhancement of rice for human health: the contribution of biotechnology. *Biotechnology advances*, 31(1), 50-57. <https://doi.org/10.1016/j.biotechadv.2012.02.001>
- Birla, D. S., Malik, K., Sainger, M., Chaudhary, D., Jaiwal, R. and Jaiwal, P. K. (2017). Progress and challenges in improving the nutritional quality of rice (*Oryza sativa* L.). *Critical Reviews in Food Science and Nutrition*, 57(11), 2455-2481. <https://doi.org/10.1080/10408398.2015.1084992>
- Boonyaves, K., Wu, T. Y., Gruissem, W. and Bhullar, N. K. (2017). Enhanced grain iron levels in rice expressing an iron-regulated metal transporter, nicotianamine synthase, and ferritin gene cassette. *Frontiers in Plant Science*, 8, 130. <https://doi.org/10.3389/fpls.2017.00130>
- Carrera, A., Echenique, V., Zhang, W., Helguera, M., Manthey, F., Schrage, A. and Dubcovsky, J. (2007). A deletion at the Lpx-B1 locus is associated with low lipoxygenase activity and improved pasta color in durum wheat (*Triticum turgidum* ssp. durum). *Journal of Cereal Science*, 45(1), 67-77. <https://doi.org/10.1016/j.jcs.2006.07.001>
- Chen Y, Liu L, Shen Y, Liu S, Huang J, Long Q, Wu W, Yang C, Chen H, Guo X, Cheng Z, Jiang L, Wan J (2015) Loss of function of the cytochrome P450 gene CYP78B5 causes giant embryos in rice. *Plant Molecular Biology Reporter* 33: 69-83. <http://dx.doi.org/10.1007%2F978-1-105-014-0731-3>
- Chen, H., Siebenmorgen, T. J. and Griffin, K. (1998). Quality characteristics of long grain rice milled in two commercial systems. *Cereal chemistry*, 75(4), 560-565. <https://doi.org/10.1094/CCHEM.1998.75.4.560>
- Das, P., Adak, S. and Lahiri Majumder, A. (2020). Genetic manipulation for improved nutritional quality in rice. *Frontiers in Genetics*, 11, 776. <http://dx.doi.org/10.3389/fgene.2020.00776>
- Datta, K., Baisakh, N., Oliva, N., Torrizo, L., Abrigo, E., Tan, J., Rai, M., Rehana, S., Al Babili, S., Beyer, P. and Potrykus, I. (2003). Bioengineered 'golden' indica rice cultivars with β -carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnology Journal*, 1(2), 81-90. <https://doi.org/10.1046/j.1467-7652.2003.00015.x>
- Datta, K., Sahoo, G., Krishnan, S., Ganguly, M. and Datta, S. K. (2014). Genetic stability developed for β -carotene synthesis in BR29 rice line using dihaploid homozygosity. *PLoS One*, 9(6), e100212. <https://doi.org/10.1371/journal.pone.0100212>
- Dipti, S. S., Bergman, C., Indrasari, S. D., Herath, T., Hall, R., Lee, H., Habibi, F., Bassinello, P.Z., Graterol, E., Ferraz, J.P. and Fitzgerald, M. (2012). The potential of rice to offer solutions for malnutrition and chronic diseases. *Rice*, 5(1), 16. <https://doi.org/10.1186/1939-8433-5-16>
- Dixit, S., Singh, U. M., Abbai, R., Ram, T., Singh, V. K., Paul, A. and Kumar, A. (2019). Identification of genomic region (s) responsible for high iron and

- zinc content in rice. *Scientific Reports*, 9(1), 8136. <https://10.1038/s41598-019-43888-y>
- Dolson, L. (2009) What You Need to Know About Complex Carbohydrates. <http://lowcarbdiets.about.com/od/nutrition/a/starch.htm> (March 24, 2020).
- Fitzgerald MA, McCouch SR, Hall RD (2009) Not just a grain of rice: the quest for quality. *Trends Plant Science*.14: 133-139. <https://doi.org/10.1016/j.tplants.2008.12.004>
- Fitzgerald, M. A., Sackville Hamilton, N. R., Calingacion, M. N., Verhoeven, H. A. and Butardo, V. M. (2008). Is there a second fragrance gene in rice?. *Plant Biotechnology Journal*, 6(4), 416-423. <https://doi.org/10.1111/j.1467-7652.2008.00327.x>
- Frankard, V., Ghislain, M. and Jacobs, M. (1992). Two feedback-insensitive enzymes of the aspartate pathway in *Nicotiana sylvestris*. *Plant Physiology*, 99(4), 1285-1293. <https://doi.org/10.1104/pp.99.4.1285>
- Gayen, D., Ali, N., Sarkar, S. N., Datta, S. K. and Datta, K. (2015). Down-regulation of lipoxygenase gene reduces degradation of carotenoids of golden rice during storage. *Planta*, 242(1), 353-363. <http://10.1007/s00425-015-2314-4>
- Goffman, F. D. and Bergman, C. J. (2004). Rice kernel phenolic content and its relationship with antiradical efficiency. *Journal of the Science of Food and Agriculture*, 84(10), 1235-1240. <https://doi.org/10.1002/jsfa.1780>
- Gregorio, G., Senadhira, D., Htut, H. and Graham, R.D. (2000) Breeding for trace mineral density in rice. *Food and Nutrition Bulletin* 21: 382-386. <https://doi.org/10.1177/156482650002100407>
- Höller, S., Ueda, Y., Wu, L., Wang, Y., Hajirezaei, M. R., Ghaffari, M. R., Wiren, N.V. and Frei, M. (2015). Ascorbate biosynthesis and its involvement in stress tolerance and plant development in rice (*Oryza sativa* L.). *Plant molecular biology*, 88(6), 545-560. <http://dx.doi.org/10.1007/s11103-015-0341-y>
- Iqbal, S., Bhanger, M. I. and Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food chemistry*, 93(2), 265-272. <https://doi.org/10.1016/j.foodchem.2004.09.024>
- Kaewnaee, P., Vichitphan, S., Klanrit, P., Siri, B. and Vichitphan, K. (2011). Effect of accelerated aging process on seed quality and biochemical changes in sweet pepper (*Capsicum annuum* Linn.) Seeds. <https://doi.org/10.3923/biotech.2011.175.182>
- Lemanceau, P., Bauer, P., Kraemer, S., & Briat, J. F. (2009). Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. *Plant and Soil* 321: 513-535. <https://dx.doi.org/10.1007/s11104-009-0039-5>
- Long, X., Liu, Q., Chan, M., Wang, Q. and Sun, S. S. (2013). Metabolic engineering and profiling of rice with increased lysine. *Plant Biotechnology Journal*, 11(4), 490-501. <https://doi.org/10.1111/pbi.12037>
- Lonsdale, D. (2006). A review of the biochemistry, metabolism and clinical benefits of thiamin (e) and its derivatives. *Evidence Based Complementary and Alternative Medicine*, 3(1), 49-59.. <https://doi.org/10.1093/ecam/nek009>
- Malik, K., Rani, M., Sharma, P. and Bhardwaj, A. K. (2022). Rice straw compost: A sustainable residue recycling solution for soil health and productivity enhancement in the rice–wheat cropping system. *Journal of Natural Resource Conservation and Management*, 3(2), 143–151.
- Malik, S., & Chaudhary, P. (2002). Non-conventional tools in the improvement of aromatic rices. *Specialty rices of the world: breeding, production and marketing*, 207-222.
- Masuda, H., Usuda, K., Kobayashi, T., Ishimaru, Y., Kakei, Y., Takahashi, M. and Nishizawa, N. K. (2009). Overexpression of the barley nicotianamine synthase gene HvNAS1 increases iron and zinc concentrations in rice grains. *Rice*, 2(4), 155-166.. <https://doi.org/10.1007/s12284-009-9031-1>
- Miura, K., Ashikari, M. and Matsuoka, M. (2011). The role of QTLs in the breeding of high-yielding rice. *Trends in plant science*, 16(6), 319-326. <https://doi.org/10.1016/j.tplants.2011.02.009>
- Nagasawa, N., Hibara, K. I., Heppard, E. P., Vander Velden, K. A., Luck, S., Beatty, M., Nagato, Y., and Sakai, H. (2013). Giant embryo encodes CYP78A13, required for proper size balance between embryo and endosperm in rice. *The Plant Journal*, 75(4), 592-605. <https://doi.org/10.1111/tpj.12223>
- Paul, S., Ali, N., Gayen, D., Datta, S. K. and Datta, K. (2012). Molecular breeding of Osfer2 gene to increase iron nutrition in rice grain. *GM crops & food*, 3(4), 310-316. <https://doi.org/10.4161/gmcr.22104>
- Peng, B., Kong, H., Li, Y., Wang, L., Zhong, M., Sun, L., Gao, G., Zhang, Q., Luo, L., Wang, G., Xie, W., Chen, J, Yao, W., Peng, Y., Lei, L., Lian, X., Xiao, J., Xu, C., Li, X. and He, Y. (2014) OsAAP6 functions as an important regulator of grain protein content and nutritional quality in rice. *Nature Communications* 5: 4847. <https://doi.org/10.1038/ncomms5847>

- Rani, S., Bhat, M. A., Sharma, M. K. and Prakash, R. (2024). Growth, yield and zinc uptake by wheat in response to zinc fertilization under different saline environments. *Journal of Natural Resource Conservation and Management*, 5(2), 111–123.
- Schramm, R., Abadie, A., Hua, N., Xu, Z. and Lima, M. (2007). Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. *Journal of Biological Engineering*, 1(1), 9 <https://doi.org/10.1186/1754-1611-1-9>
- Sharma, S. and Singh, D. (2022). Zinc for enhancing crop growth of rice: A brief review. *Journal of Natural Resource Conservation and Management*, 3(2), 125–129.
- Shaul, O. and Galili, G. (1992). Increased lysine synthesis in tobacco plants that express high levels of bacterial dihydrodipicolinate synthase in their chloroplasts. *The Plant Journal*, 2(2), 203-209. <https://doi.org/10.1111/j.1365-313X.1992.00203.x>
- Shirasawa, K., Takeuchi, Y., Ebitani, T. and Suzuki, Y. (2008). Identification of gene for rice (*Oryza sativa*) seed lipoygenase-3 involved in the generation of stale flavor and development of SNP markers for lipoygenase-3 deficiency. *Breeding science*, 58(2), 169-176. <https://doi.org/10.1270/jsbbs.58.169>
- Singh, A. K. and Kumar, A. (1996). Responses of promising rice genotypes to nitrogen levels in irrigated lowlands. 46–47.
- Singh, S. P., Gruissem, W. and Bhullar, N. K. (2017). Single genetic locus improvement of iron, zinc and β -carotene content in rice grains. *Scientific Reports*, 7(1), 6883. <https://doi.org/10.1038/s41598-017-07198-5>
- Singh, S. P., Kumar, A., Satyendra, S., Kumar, M., Nahakpam, S., Sinha, S. and Singh, P. K. (2018). Identification of drought tolerant rice (*Oryza sativa* L.) genotypes using drought tolerance indices under normal and water stress condition. *International Journal of Current Microbiology and Applied Sciences*, 7, 4757–4766.
- Smith, M.R. and Myers, S.S. (2019) Global health implications of nutrient changes in rice under high atmospheric carbon dioxide. *Geohealth*3, 3(7), 190-200. <https://doi.org/10.1029/2019GH000188>
- Sun, Q., Spiegelman, D., Van Dam, R. M., Holmes, M. D., Malik, V. S., Willett, W. C. and Hu, F. B. (2010). White rice, brown rice, and risk of type 2 diabetes in US men and women. *Archives of internal medicine*, 170(11), 961-969. <https://doi.org/10.1001/archinternmed.2010.109>
- Suzuki, M., Morikawa, K. C., Nakanishi, H., Takahashi, M., Saigusa, M., Mori, S. and Nishizawa, N. K. (2008). Transgenic rice lines that include barley genes have increased tolerance to low iron availability in a calcareous paddy soil. *Soil Science and Plant Nutrition*, 54(1), 77-85. <https://doi.org/10.1111/j.1747-0765.2007.00205.x>
- Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA (2009) Golden Rice is an effective source of vitamin A. *American Journal of Clinical Nutrition*. 89, 1776–1783. <https://doi.org/10.3945/ajcn.2008.27119>
- Tian, S., Nakamura, K. and Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of agricultural and food chemistry*, 52(15), 4808-4813. <https://doi.org/10.1021/jf049446f>
- Trijatmiko, K. R., Dueñas, C., Tsakirpaloglou, N., Torrizo, L., Arines, F. M., Adeva, C. and Slamet-Loedin, I. H. (2016). Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Scientific reports*, 6(1), 19792. <https://doi.org/10.1038/srep19792>
- Wong, H. W., Liu, Q. and Sun, S. S. M. (2015). Biofortification of rice with lysine using endogenous histones. *Plant molecular biology*, 87(3), 235-248. <https://doi.org/10.1007/s11103-014-0272-z>
- Wu, X. R., Chen, Z. H. and Folk, W. R. (2003). Enrichment of cereal protein lysine content by altered tRNAlys coding during protein synthesis. *Plant biotechnology journal*, 1(3), 187-194. <https://doi.org/10.1046/j.1467-7652.2003.00017.x>
- Xu, M., Zhao, S., Zhang, Y., Yin, H., Peng, X., Cheng, Z., Yang, Z. and Zheng, J. (2017). Production of marker-free transgenic rice (*Oryza sativa* L.) with improved nutritive quality expressing AmA1. *Iranian Journal of Biotechnology*, 15(2), 102. <https://doi.org/10.15171%2Fijb.1527>
- Yang, Q. Q., Zhang, C. Q., Chan, M. L., Zhao, D. S., Chen, J. Z., Wang, Q. and Liu, Q. Q. (2016). Biofortification of rice with the essential amino acid lysine: molecular characterization, nutritional evaluation, and field performance. *Journal of experimental botany*, 67(14), 4285-4296. <https://doi.org/10.1093/jxb/erw209>
- Yang, Q. Q., Zhao, D. S., Zhang, C. Q., Wu, H. Y., Li, Q. F., Gu, M. H., Sun, S.S.M. and Liu, Q. Q. (2018). A connection between lysine and serotonin metabolism in rice endosperm. *Plant Physiology*, 176(3), 1965-1980. <https://doi.org/10.1104/pp.17.01283>
- Ye, X., Al-Babili, S., Klott, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000). Engineering the provitamin A (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, 287(5451), 303-305. <https://doi.org/10.1126/science.287.5451.303>