



Revolutionizing Rice Grain Quality: A Holistic Review Integrating Conventional and Molecular Approaches


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ABSTRACT

This review mainly focuses on the rice grain quality based on which rice varieties are distinguished into several quality classes like, physical quality, eating and cooking quality and nutritional quality. The rice breeding programme needs to focus on the development of nutrient-dense rice for value addition, helping in reducing malnutrition and Vitamin-A deficiency (VAD). There is enough scope to enhance the nutritional and cooking quality in small and medium grain rice germplasm by bringing them into the breeding programme through Fe and Zn and Vitamin-A biofortification, aided with molecular techniques. Many studies have been conducted to find out the quantitative trait locus (QTL) for enhancing the quality of grain. The QTL *qBRR5* and *qBRR3* are responsible for brown rice grain width and length found on chromosome 5 and on chromosome number 3, respectively and for head rice on chromosome number 1, 5, and 6. The QTLs present on chromosomes 3, 5, and 6 are responsible for head rice recovery, chalky appearance of rice grain as well as grain size and shape. The gene *Wx*, located on chromosome 6 linked with the QTL *qAC6* regulate amylose content and gel consistency. The QTL *SSIIa* on chromosome 6, control both the amylopectin and gelatinization temperature. The QTLs for Fe and Zn content in rice grain are located on chromosome 2, 3, 8, 11 and 12. Development of “Golden rice” and innovations with *Arabidopsis* genes can significantly enhance β -carotene levels and address Vitamin-A deficiency, while RNAi techniques improve storage quality.

KEYWORDS: Biofortification, golden rice, hidden hunger, QTL, rice

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than half of the world's population, and as it is extensively cultivated across the world, it is known as "Global Grain" (Prasad et al., 2019; Fukagawa & Lewis, 2019; Harisha et al., 2022). Asia's contribution to rice production and consumption is more than 90% in the world (Bandumula, 2018; Sharma and Khanna, 2020; Venmuhil et al., 2020). Indeed, Asians get 60% of the calories from rice (Khush, 1997; Singh et al., 2023). China holds 1st rank in rice production, contributing over 28%, followed by India, accounting for about 22%, and Indonesia with 10%, of the world's total rice production (Anonymous, 2017). Nowadays, rice is extensively cultivated, produced, and consumed throughout the world. So, the demand for rice is gradually increasing day by day. With this increasing requirement for rice, rice grain quality has become a key factor for marketing and consumers are giving preference to rice grain based on rice grain quality attributes (Rao et al., 2014). The qualities of rice are determined by its texture during cooking, flavor, and nutritional value. The primary parameter is physical appearance of rice kernel quality which directly influences consumers' willingness to purchase (Custodio et al., 2019).

Grain quality is important for poor people and where rice is taken as the major staple food. Grain quality in rice is very difficult to define as the preference of grain quality varies with demography, country, and continent, as preference of cooking quality varies from country to country (Azeez and Shafi, 1966; Sultana et al., 2022). Grain quality fundamentally comprises grain appearance, milling quality, eating, cooking, quality, and nutritional quality as shown in Figure 1 (Unnevehr et al., 1992; Yu et al., 2008; Chen et al., 2012; Bao, 2014; Gong et al., 2023; Naik et al., 2023). Brown rice and head rice are of primary interest to the plant breeders as they are significant factors in determining and maintaining rice grain quality standards in different nations (Bao, 2019; Zhou et al., 2020). For the most part, Southeast Asians prefer long grains with soft gel consistency and intermediate amylose content, whereas South Asians prefer long grains with high amylose content and a hard gel consistency (Khush and Juliano, 1985; Butardo et al., 2019). Moreover, the preference for quality rice varies based on the preparations for which rice would be used. However, consumers in different locations have different preferences for rice eating and cooking quality but generally superior-graded rice which are usually unbroken, clean, aromatic, supple grains that are elastic but non-sticky, delicious, and can retain their texture when it becomes cold after cooking (Fitzgerald et al., 2009; Demont et al., 2017; Zhou et al., 2020). Malnutrition is a significant global issue,

impacting the health and development of many people. Iron deficiency, also known as iron deficiency anaemia (IDA), zinc deficiency, and vitamin-A deficiency (VAD) are the most common nutritional deficiencies that have detrimental effects (Majumder et al., 2019). "Hidden hunger" highlights that even with adequate food intake, individuals may still lack essential nutrients, leading to undernutrition problems (Ofori et al., 2022; Zulfiqar et al., 2024). Therefore, the nutritional quality of the grain is equally important like its production and cooking quality and the primary objective is to produce rice genotypes with improved grain nutritional and cooking quality (Asante, 2017; Parikh et al., 2023). The aromatic rice varieties are already famous to the consumers market due to their superior quality attributes like superfine slender grains, fine cooking quality, pleasant aroma, and lengthwise kernel elongation during cooking (Bhattacharjee et al., 2002; Rajendran et al., 2021) and are sold in the market at a premium price. Moreover, molecular marker may be used to identify the quantitative trait loci and the development of the varieties with quality and nutritional value.

The review articles published hitherto focused either on agronomic practices and its impact of rice quality (Gong et al., 2023), specific biotechnological methods for quality improvement (Butardo et al., 2019) or exploring specific genetic pathways (Panda et al., 2020). The present review encompasses multifaceted aspects of rice grain quality (Figure 1). Additionally, emphasis has been given on molecular basis of rice grain quality involving the quantitative trait loci (QTLs) associated with different quality parameters including iron and zinc content in kernel and their corresponding positions in chromosomes.

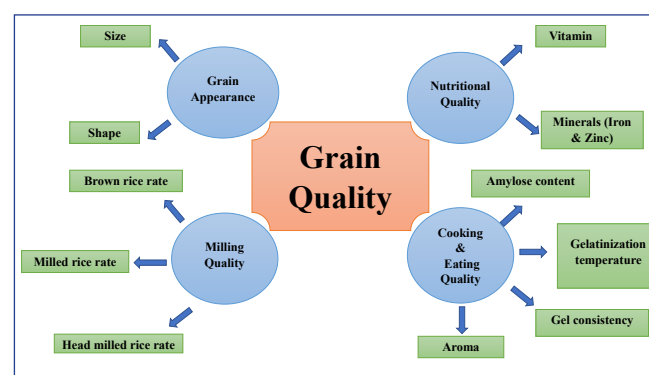


Figure 1: The major determining factors of rice grain quality

2. GRAIN APPEARANCE

According to market requirements and consumer preferences, rice grains are distinguished and grouped into four types of classes (extra long, long, medium, and short) based on their size as shown in Table 1 and four types of classes (slender, medium, bold, round) on the basis

of their shape as shown in Table 2 (Cruz and Khush, 2000). Kernel size and shape are considered as the most common, important quality factors in different phases like processing, drying, handling equipment, breeding and grading. So, grain size and shape or dimensions are the prime quality characters for developing new varieties (Owens, 2001).

Table 1: Size classification

Scale	Size	Length (mm)
1	Extra long	>7.50
3	Long	6.61–7.50
5	Medium	5.51–6.60
7	Short	Less than or equal to 5.50

Source: Aromatic rice: Cruz and Khush (2000)

Table 2: Shape classification

Scale	Size shape	L/B ratio (mm)
1	Slender	Over 3
3	Medium	2.1–3.0
5	Bold	1.1–2.0
9	Round	1.0 or less

Source: Aromatic rice: Cruz and Khush (2000)

3. MILLING QUALITY

Consumers have built up their preference of rice grain quality based on rice grain appearance, size, shape, and its post-cooking behavioral properties like, taste, tenderness, stickiness, aroma of cooked rice etc. Milled rice grain outlook also influences the preference of consumers. Milling quality is estimated from brown rice rate (BRR), milled rice rate (MRR) and head milled rice rate (HMRR). Brown rice is produced by removing the lemma and palea from rice grain by hulling and can be utilized for eating and cooking purposes. Milled rice is produced by the milling of brown rice and removing all the bran, consisting of aleurone, pericarp and germ or embryo. Head rice is milled rice with a greater length or equal to 3/4 of the average length of the kernel. Among these three milling quality parameters, head milled rice is the most important and has a great market price. So, from the plant breeding point of view, the breeder should consider the character of rice grain size and shape as the prime selection criteria for developing new varieties (Adair et al., 1966).

4. EATING AND COOKING QUALITY

The eating and cooking quality (ECQ) of rice are the most important desired properties, where rice is consumed as a staple food because rice is taken as a whole, milled or cooked. The eating and cooking quality of rice

depends on amylose content, gelatinization temperature, and gel consistency, which are the important factors for determining starch properties. Amylose content is the most essential character as it defines the rice cooking and processing behaviour. The rice which comprises low

Table 3: Standard analytical methods for estimating Amylose content (Juliano, 1971)

Grain type	Range of amylose (%)	Type of cooked rice	Rice water ratio
Waxy	0–8	Moist, sticky, glossy	1:1.3
Non-waxy			
Low amylose content	8–20	Sticky, soft	1:1.7
Intermediate amylose content	20–25	Dry, flaky, soft	1:1.9
High amylose content	25–32	Cook dry, flaky, hard	1:2.1

amylose content is commonly found to be sticky and moist, contrariwise, the rice with high amylose content is usually found to be more in expansion, non-sticky, and have flakiness as shown in Table 3 (Juliano, 1979).

Gelatinization temperature of rice grain had been identified as one of the most important determining factors of cooking and eating quality (Bao et al., 2004). Rice grains that have low gelatinization temperature (55–69°C) show complete disintegration, whereas, rice grains having intermediate gelatinization temperature (70–74°C) disintegrate partially and the grains that have high gelatinization temperature (>74°C) has no effect in alkali solution (Table 4) (Little et al., 1958).

Table 4: The relationship between gelatinization temperature and alkali spreading value

Clarification	Rating	GT
1–2	Low	High >74°C
3–4	Low intermediate	High, intermediate (71–74°C)
5–6	Intermediate	Intermediate (70°C–74°C)
7	High	Low <70°C

The cohesiveness, tenderness, colour, and glossiness of cooked rice grains extensively depend on gel consistency. It estimates the tendency of cooked rice grain to be hardened when it cools down (Table 5). Commonly, rice having medium amylose content, intermediate gelatinization temperature, and soft to medium gel consistency, get preference from the consumers (Khush et al., 1979).

Table 5: Measure of gel consistency (Cagampang et al., 1973)

Gel length (mm)	Description
27–35	Hard
36–40	Medium hard
41–60	Medium
61–100	Soft

High-quality rice like basmati, which has a strong aroma and extra grain elongation than ordinary rice, is considered as a highly desirable characteristic and has more demand in different countries (Little et al., 1958; Juliano, 1971; Cagampang et al., 1973; Mahindru, 1995). India is known to be one of the chief exporters of basmati rice in the world (Husaini et al., 2009). The worldwide demand for aromatic rice is increasing noticeably day by day and consumers are willing to pay more price for the fragrance (Louis et al., 2005).

5. NUTRITIONAL QUALITY

Human micronutrient deficiencies are a relatively serious issue in those areas where rice is consumed as a staple food. Iron and zinc deficiencies are the most commonly found diseases and major global threat to human health, which affects more than half of the world's human population (Anonymous, 2002; White and Broadley, 2009). Due to iron and zinc deficiency, there is a reduction of the crop yield and causes complex problem in human health. The iron and zinc deficiencies are also known as 'hidden hunger' causes of poor growth in children and compromised psychomotor development of the children, minimize immunity, fatigue, irritability, weakness, hair fall, wasting of muscles, sterility, morbidity, and may lead to death in acute cases (Stein, 2010). Almost 1.62 billion people are suffering from iron deficiency anaemia, which resembles 24.8% of the population. Approximately 41% of pregnant women and 27% of pre-school children worldwide are suffering from iron deficiency anaemia (Anonymous, 2008). Anaemia has extensively spread in India, 58.6% of children, 53.2% of non-pregnant women and 50.4% of pregnant women were found to be suffering from anaemia (Anonymous, 2016). Almost 81% of the rural children of West Bengal are suffering from iron deficiency anaemia (Arlappa, 2010). It had been estimated that 800000 deaths worldwide took place due to iron deficiency anaemia. Almost one third world population are zinc deficient. Zinc deficiency is responsible for approximately, 2.1 million deaths among Indian children under the age of 5 every year. These deficiencies can be overcome by increasing the iron and zinc content in rice, specifically among the urban and rural poor people, those having few or no chance to access to supplements such as enriched foods and diversified balanced

diets. The recommended daily allowance (RDA) of iron, for children is 13 mg day⁻¹; for adult, 17 mg day⁻¹; and of zinc 7 mg day⁻¹ for children, 12 mg day⁻¹ for adult is essential but in India mostly people have monotonous diets which rely on cereals that are deficient in micronutrients, iron, and zinc (Anonymous, 2009). But common cultivars comprise about 12 mg kg⁻¹ of Fe and 25 mg kg⁻¹ Zn, whereas in traditional landraces Fe varied from 7.8 to 24.4 mg kg⁻¹ and Zinc varied from 13.5 to 41.6 mg kg⁻¹ (Gregorio et al., 1999). The malnutrition issue is expected to be increased further with the increasing population as the poor people in developing countries like India solely depend on cereal meals i.e. rice and cannot afford to balance their diet like vegetables, milk, meat and fruit supplements (Poletti et al., 2004). A quick meal can satisfy hunger, but the problem of "hidden hunger", can be only solved by nutritionally enriched food. The technique which is known as "biofortification" incorporates the genes for micronutrients and allows the crops to provide micronutrients that are vital to human health. It is also a sustainable and feasible strategy to minimize micronutrient deficiencies for people who only consume rice and have restricted access to diversified food. There are few released varieties with high zinc content like IET 25477 (Zinco Rice), IET 24760 Surabhi (NSL), IET 24555 (DRR Dhan 48), IET 24557 (DRR Dhan 49) and IET 23832 (DRR Dhan 45). IR68144 was developed by use of the traditional breeding method by International Rice Research Institute (IRRI), which contains more than two times higher Fe concentration in seeds than local varieties in the Philippines (Gregorio et al., 2000).

Rice is important energy-providing food and can be an essential source of Vitamin B complex, proteins, minerals and other vitamins. Human gets almost 20 to 27% of their daily energy from rice in the form of carbohydrates (starch) and about 15 to 20% of daily required protein comes from rice and it has high demand because it is easy to digest (Naves, 2007; Bottini, 2008). Rice has very little to no amounts of fat and has zero cholesterol.

6. MOLECULAR BASIS OF GRAIN QUALITY

The comparison of quality parameters among different cultivars and varieties, cultivated in the same environment, explores the significant variation among these cultivars and varieties (Adu-Kwarteng et al., 2003; Cameron and Wang, 2005; Kang et al., 2006; Vidal et al., 2007). So, the regulating factor for the grain quality resides in the rice genome itself in the locus which encodes starch synthetases and storage proteins. Control of these genes on both transcription and post-transcription levels may also lead to important roles. Moreover, different studies on genetic variation in starch synthetases explored important markers that influence different traits. Further, mapping-

based methods gave new methods of finding essential genes engaged in the regulation of rice quality. Nowadays, different studies reveal that many characters are not only regulated by a single gene, but also controlled by quantitative trait loci (QTLs). The rice quality measuring characters are quantitatively inherited, regulated by multiple genes or QTL (Tan et al., 2000) and the environment, where it is growing has a great influence on rice quality (Zhao et al., 2015).

6.1. QTLs for brown rice recovery

In eight studies, a sum of 20 QTLs has been recognized covering all the chromosomes except chromosome number 2. A major QTL *qBRR5* has been found on chromosome 5, at the interval between markers RM42 and C734b liable for rice grain width (Tan et al., 2001) and a QTL *qBRR3* has been found on chromosome number 3, which shares the same genomic region, responsible for grain length (Lou et al., 2009). Among Five QTLs (Li et al., 2004), three were found to be expressed in two years, which implies the presence of QTL-by-environment interaction effects.

6.2. QTLs for milled rice recovery

In seven studies, a sum of 19 QTLs has been recognized covering all the chromosomes except chromosome number 8. It has not been found any strong or reproducible QTLs for the milled rice recovery. Three individual studies showed the presence of QTL for milled rice recovery on chromosome number 5 (Tan et al., 2001; Aluko et al., 2004; Zheng et al., 2007), but these are not in the same region. Among four QTLs, two were found to be expressed in two years, which implies the presence of QTL-by-environment interactions effects. (Li et al., 2004).

6.3. QTLs for head rice recovery

Till yet in 10 studies, a sum of 34 QTLs has been recognized covering all the chromosomes from 1 to 7. Another research identified a major QTL *qHRR3* located on chromosome number 3 is responsible for grain length and head rice recovery (Tan et al., 2001; Jiang et al., 2013), which indicates that there is a genetic relationship between grain shape or size with the percentage of head rice. Other studies recognize that the QTL on chromosome number 3, there might be a principal gene for head rice (Aluko et al., 2004; Dong et al., 2004; Li et al., 2004; Jiang et al., 2005; Lou et al., 2009). On the other hand, three individual studies identified QTLs on chromosome number 1, 5, and 6. Three QTLs were found for head rice, but they had been recognized in specific year, which implies that the head rice is greatly influenced by the environment (Li et al., 2004). Genetic variation of the head rice was due to main-effect QTL than QTL×environment effect found in Cypress/RT0034 RIL population, while the main effect of QTLs have a low genetic variation than QTL × environment effect in the Cypress/LaGrue RIL population (Nelson et al., 2011). It

has been found that QTL for head rice recovery with early-heading QTLs in the hot region, suggesting the presence of an environmental effect (Nelson et al., 2011). Few genetic population were evolved from cultivated and wild rice, but all the milling with yield increasing effects derived from the cultivated plants (Septiningsih et al., 2003; Aluko et al., 2004). The genetic areas linked to head rice recovery and chalky appearance coincided with grain size and shape QTLs present on chromosomes 3, 5, and 6. Cloned genes *GS5* and *GW8* regulate rice cell proliferation positively. The genes linked to grain size, shape, and weight are important regulators of brassinosteroid signal transduction and cellularization, as well as the selective proteolysis pathway (Shomura et al., 2008; Sreenivasulu and Wobus, 2013).

6.4. QTLs for raw and cooked rice grains width and length

In a genome-wide association study (GWAS) utilizing 2.9 million single nucleotide polymorphisms (SNP) and 393,429 indels on 591 rice landraces to find the genetic basis of cooked and raw grain length, breadth, and shape. It was found that a fine-mapped genomic region (GW7.1) is strongly correlated with both raw and cooked grain width. Furthermore, genomic region GW7.2 was discovered for grain dimension. It was also discovered that unique areas on chromosomes 10 and 11 were connected to the raw grain width and cooked grain shape, respectively (Misra et al., 2017). In another study with rice width and weight also showed these were mostly influenced by the QTL *GW5*, which was also thought to control cell division during seed formation by acting in the ubiquitin-proteasome pathway. This work indicates that *GW5* might be a very useful tool for the development of high-yielding crops (Weng et al., 2008). The functional characterization and cloning were also performed and found that the four QTLs were associated with grain size, namely *GW2* (Song et al., 2007), *GW5* (Wan et al., 2008), *GW8* (Wang et al., 2012) and *GS5* (Wan et al., 2008).

6.5. QTLs for eating and cooking quality of rice

The eating and cooking quality (ECQ) of rice is strongly linked with parameters amylose concentration (AC), gel consistency (GC), and gelatinization temperature (GT), which directly influences the taste of rice. A genome-wide association research on variables relevant to ECQ was carried out utilizing 1.2 million single nucleotide polymorphisms (SNPs) and the phenotypic information on 173 rice accessions. One QTL was found for gel consistency, five for amylose content and two for gelatinization temperature had been found (Jiang et al., 2024). The chromosomal position of QTL *qGT6* coincided with the alkali digestion gene (Gao et al., 2003). A significant gene *Wx*, located on chromosome 6 corresponded with the QTL *qAC6* responsible for regulation of amylose content (AC) and gel consistency

(GC) (Lapitan et al., 2009; Zhang et al., 2021). Moreover, different AC classes also contain a large number of *Wx* alleles. The five frequent alleles *Wx*, *Wx'*, *Wx^{g1}*, *Wx^{g2}*, and *Wx^{g3}* were found for the five prominent classes of Amylose content i.e., glutinous, low, intermediate, high I, and high II, respectively (Ni et al., 2011; Teng et al., 2012). Furthermore, chalkiness is a crucial factor that influences the quality and, eventually, it decides the commercial worth of rice grains. Chalkiness had a detrimental effect on head rice recovery as well as on physical appearance, milling, cooking, and nutritional properties (Nevame et al., 2018). For instance, rice grown in hot climates has a greater chalkiness level, and genes related to grain filling, starch granule shape, and starch biosynthesis all have negative impact on chalkiness (Yamakawa et al., 2017; Bergman, 2019). These findings have led to the fine-mapping of genes, of *qPGWC-8* (Guo et al., 2011) and *qPGWC-7* (Zhou et al., 2009) regulating grain chalkiness.

A significant QTL *SSIIa* presents on chromosome 6 regulates both the amylopectin and gelatinization temperature (GT) (Lapitan et al., 2009; Umemoto et al., 2002; Wang et al., 2007). But the genes *SBE1*, *BE3*, *AGPLar*, *PUL*, and *ISA* linked to starch biosynthesis and the genes *SSI*, *SSIIa*, *SSIII-2*, and *SSIV-2* were found be linked with starch production (Bao et al., 2002; Tian et al., 2009). All these genes influence the eating and cooking quality (ECQ) of rice.

6.6. QTLs associated with iron and zinc-containing rice

SSR microsatellite markers are comparatively inexpensive and have greater estimations of polymorphism (McCouch et al., 1997), extensively utilized for the study of phylogenetic relations among the genotypes belonging to three different ancestries, such as indica, japonica and tropical japonica. Three SSR marker RM12796 on chromosome 2, RM489 on chromosome 3, RM287 on chromosome 11 significantly showed association with the grain zinc content (Indurkar, 2016). RM535 on chromosome 2 was common to both Fe and Zn concentrations and four markers RM535, RM137, RM152, RM260 situated on chromosomes 2, 8 and 12 were linked solely to Fe concentration. Among these, only one maker RM535 on chromosome 2 explained high phenotypic variance (Anuradha et al., 2012). Three SSR markers RM263, RM152 and RM21 showed 100% polymorphism among the RIL population on chromosome numbers 2, 8 and 11, respectively (Gande, 2014). Among 25 markers RM3322 and RM7488 were associated with both iron and zinc (Kiranmayi, 2014).

7. DEVELOPMENT AND EVOLUTION OF GOLDEN RICE

Consumption of dietary carotenoids offers various health benefits, such as reducing the risk of cancer and eye

problems. Carotenoids like lycopene, lutein, zeaxanthin, and β -carotene aid in cell differentiation, glycoprotein synthesis, and bone growth (Johnson, 2002; Ghosh et al., 2019; Majumder et al., 2019). β -carotene possesses additional advantages because it can be converted into vitamin A, which is absent in brown and polished rice. Vitamin A deficiency (VAD), prevalent in Southeast Asia and Sub-Saharan Africa, affects half of the global child population, causing night blindness and increasing susceptibility to infections (Wirth et al., 2017). β -carotenoid-biofortified rice makes the rice distinctive golden-orange colour, known as golden rice (GR), which helps to alleviate VAD by providing Vitamin-A (Tan et al., 2005; Dutta et al., 2014).

Provitamin A is found in minuscule amounts in rice grains, predominantly in the outer cell layers of the seeds, which are removed during polishing, leaving polished and brown rice devoid of it. Preformed vitamin A is exclusively found in animal products, while β -carotene is primarily present in animal products and the dark green, yellow, and orange parts of plants (Dawe et al., 2002; Zhao et al., 2020). The rice endosperm lacks the entire β -carotene biosynthesis pathway. The endosperm of wild-type rice is only capable of synthesizing geranylgeranyl diphosphate (GGPP), which serves as a precursor for carotenoids. Phytoene synthase (PSY) is essential for the production of β -carotene in the rice grain endosperm, converting geranylgeranyl diphosphate (GGPP) into intermediate phytoene. The β -carotene synthesis from phytoene in plants involves four additional enzymes: phytoene desaturase (PDS), ζ -carotene desaturase (ZDS), carotene *cis-trans*-isomerase (CRTISO), and lycopene β -cyclase (LCYB).

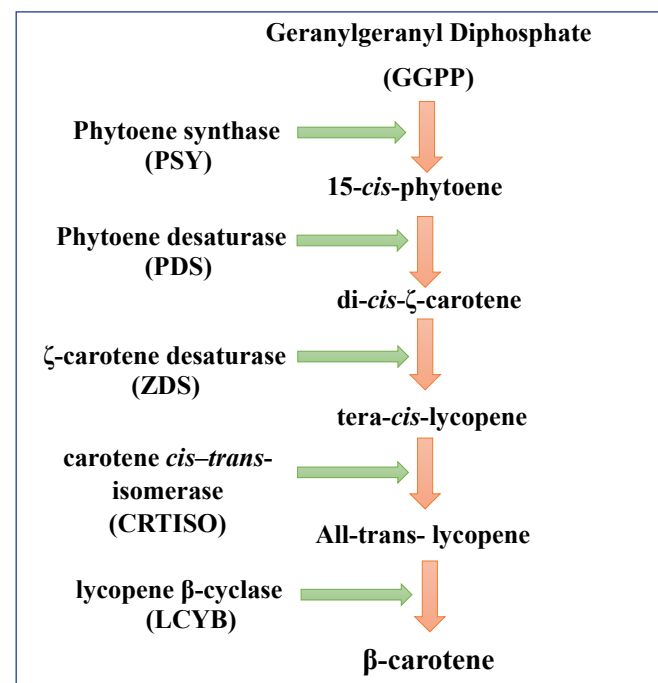


Figure 2: β - carotene biosynthesis pathway in rice

and lycopene β -cyclase (LCYB) (Figure 2).

An endosperm-specific promoter integrate the *Psy* gene from daffodils (*Narcissus pseudonarcissus*) and the bacterial phytoene desaturase (*CrtI*) from *Pantoea ananatis* (previously known, *Erwinia uredovora*) into rice to substitute the roles of four plant enzymes, such as PDS, ZISO, ZDS, and CRTISO (Ye et al., 2000). This transgenic rice produced up to 1.6 $\mu\text{g g}^{-1}$ of carotenoids in the endosperm. This unique golden or orange hue is attributed to the inclusion of bacterial *CrtI* and daffodil *Psy* genes, which led to it being called “Golden Rice” (GR). Golden Rice (GR) was engineered to produce carotenoids in rice endosperm. The scientists of Syngenta developed improved second-generation golden rice (GR2) to enhance carotenoid levels up to 37 $\mu\text{g g}^{-1}$ out of which β -carotene was 31 $\mu\text{g g}^{-1}$, approximately 23 times higher than GR. In GR2, substituting the daffodil *Psy* gene with a maize-derived ortholog gene significantly increased β -carotene synthesis in the endosperm while the *Pantoea CrtI* gene remained unaltered. This suggests that the type of *Psy* gene limits the β -carotene synthesis rate in GR (Paine et al., 2005). Subsequently, there were multiple attempts to develop a new and improved version of GR (Bai et al., 2016; Ha et al., 2010; Jeong et al., 2017; Tian et al., 2019; Zhao et al., 2020), either by raising the availability of isoprenoid precursors upstream or by stimulating metabolic sinks downstream. The *Arabidopsis thaliana* genes *AtDXS* and *AtOR*, along with the maize *Psy* gene (*ZmPsy1*) and the *Pantoea CrtI* gene (*PaCrtI*) were incorporated into rice (Bai et al., 2016). While *AtDXS* signals the enzyme 1-deoxy-D-xylulose 5-phosphate synthase, which is involved in the supply of isoprenoid precursors upstream of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, *AtOR* signals the ORANGE gene, which promotes the development of a metabolic sink by transforming colourless plastids into chromoplasts. In the endosperms of rice plants expressing *ZmPSY1+PaCRTI+AtDXS* or *ZmPSY1+PaCRTI+AtOR* proteins, the accumulated β -carotene levels (7.5–16.6 $\mu\text{g g}^{-1}$ and 5.9–10.5 $\mu\text{g g}^{-1}$, respectively) were significantly higher than in the control plants that only expressed *ZmPSY1+PaCRTI* (1.2–2.2 $\mu\text{g g}^{-1}$) (Bai et al., 2016).

In addition to the plastidial MEP route, plants can also produce isoprenoids through the cytoplasmic mevalonate (MVA) pathway, in which 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) worked as the main enzyme and recognized as the rate-limiting enzyme. An improved MVA pathway coupled with *ZmPSY1+PaCRTI* expression is a potential strategy for the development of GR (Tian et al., 2019). According to them, insertion of the *tHMG1* gene, a truncated HMGR from *Saccharomyces cerevisiae*, along with *ZmPSY1+PaCRTI* into rice led to higher β -carotene and total carotenoid accumulation

compared to rice expressing *ZmPSY1+PaCRTI* (Tian et al., 2019). This suggests that enhancing the upstream flow from the MVA or MEP pathways may increase β -carotene synthesis.

It might be difficult to preserve the nutritional quality of GR in the long term due to the presence of the Lipoxygenase (LOX) enzyme in seeds and facilitates the insertion of molecular oxygen into polyunsaturated fatty acids (PUFAs). This reaction generates conjugated hydroperoxide compounds that oxidize carotenoids, thereby diminishing the seeds’ nutritional value (Carrera et al., 2007). The degradation of seed quality is attributed to the *r9-LOX1* gene, one of the 14 LOX protein-encoding gene types present in the rice genome (Gayen et al., 2014). The storage stability and viability of GR seeds were enhanced by RNAi-mediated down-regulation of this *r9-LOX1* gene in GR, which is regulated by the Oleosin-18 promoter (Gayen et al., 2015). This technique may be beneficial for the long-term preservation of rice seeds.

7. CONCLUSION

Market research is essential for developing cultivars that align with consumer preferences and demands. The technique “biofortification” of rice can mitigate the micronutrient and Vitamin-A deficiency in human. The GR effectively combats VAD by producing β -carotene, with advanced versions like GR2. The QTL mapping analysis should be done for grain appearance, brown rice, milled rice, head rice, eating and cooking quality and nutritional quality to identify good candidate QTLs/genes for the development of the varieties.

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