



Genomic-Assisted Breeding Tools for Grain and Nutritional Quality Improvement in Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

More than half of the world's population consumes rice as a staple food in their diet. Rice grain quality is receiving attention from both consumers and producers as living standards in rice-consuming regions has improved and this emerged as a critical rice breeding target. Grain quality traits have a strong influence on the market value of rice and the adoption of new varieties by farmers. Consumer preferences for specific nutritional, physical, eating, and cooking characteristics are at the root of these. Uniform grain width, length, colour (translucence or whiteness), and chalkiness are all desirable physical properties. Cooking and eating (organoleptic) characteristics such as; cooking time (gelatinization temperature and viscosity), the ability of rice to remain soft after cooking (gel consistency), and textural properties of cooked rice (amylose content and aroma) are also very important. Plant breeders are always working hard to meet these demands by developing new rice varieties. The genomics era provides many opportunities to

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assist breeders in addressing these challenges. While not complete, our understanding of the genetic basis underlying many grain quality traits is expanding. There have been reports of quantitative trait loci (QTL) controlling grain appearance, chalkiness, protein content, and grain length. Genomic-assisted Breeding techniques such as marker-assisted selection, QTL mapping, genome editing, genome wide association studies, and genetic engineering can provide further a better understanding of the genetic basis and thus help to improve rice grain nutritional quality traits.

Keywords: Genetic engineering; genome editing; genomic-assisted breeding; marker-assisted selection; nutritional quality; QTL mapping; rice.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a most common staple food, supplying more than half of the world's calorific demands [1] and in most Asian countries the day would be incomplete without rice. However for most individuals in Southeast Asia, it contributes up to 76% of their daily calories [2,3]. Public demand of rice is varies starting from producer, seller to the end user (consumer) as per the preference (Fig. 1). The need for additional food resulted due to fast population expansion and parallel decline in cultivable land. So we have to develop new varieties with great yielding capability, better possessing quality and more resilience for abiotic and biotic stresses should became top priority. Quality is defined as "the sum of traits and attributes of a product or service which bears its ability to satisfy expressed or implied needs". Features are observable aspects of a product that can be linked to quality attributes. The characteristics and qualities of rice or products of rice matching the requirement of end-users are referred to as rice grain quality. Grain quality encompasses a wide range of characteristics, from biochemical to physical, including efficacy of milling, appearance and form of grain, ease while cooking, nutrition and edibility [2]. The ultimate yield and frequency of broken kernel from rice after milling are determined by milling quality, which is a source of concern for both customers and farmers. To assess the efficiency and quality of the milling process, three primary metrics are used: milled rice restoration, head rice restoration, and brown rice restoration. One of the most important characteristics that influence market acceptability of rice grain is its appearance. The texture of the grain after milling is determined by its shape (long or round), size, translucency, and chalkiness. The physical characteristics of rice kernels are described by terms of grain length, width, and thickness, while shape of grain is defined as its length to width ratio [4]. The cooking easeness, stiffness and

stickiness nature of rice after being cooked, are all determined through its cooking and eating qualities. The amylose content of rice, temperature of gelatinization, consistency of the gel, and its pasting viscosity are all easily measured physicochemical parameters that influence rice eating and cooking quality [5].

Rice dietary constituents include: (1) macronutrients, which are present in grams/100 g of rice, such as proteins, lipids(oils), carbohydrates and fibre; (2) micronutrients, which are present in milligrams/100 g of rice, such as vitamins, secondary metabolites and minerals; (3) anti-nutrients, which limit nutrient bioavailability like phytate; and (4) allergens, such as toxins and intolerance. However, numerous accessible minerals, vitamins, critical fatty and amino acids, and several phytochemicals which are known for preventing severe illnesses like diabetes-II, heart disease, obesity and cancer are frequently found to be insufficient. As a result, the first two components will be strengthened, while the latter two will be minimized or eliminated in order to strengthen nutritional qualities of the product [6]. Following starch, protein is found to be the 2nd most prevalent component in rice (milled). Lysine, according to human needs, is the essential amino acid constituent found in rice. Rice's nutritional value is determined by two essential parameters: lysine and protein content. Brown rice is higher in vitamin content, phenolics, minerals and fibres in comparison to milled rice for human health [7]. To satisfy the daily dietary intake, significant improvements in vitamins (E, A and folate), essential amino acids (lysine), minerals (Fe) and flavonoids have been accomplished in rice edible component, *i.e.*, endosperm (bio-fortification).

New generation of breeding (molecular) has evolved as a result of developments in marker technology and molecular biology, dramatically

speeding up the pace of plant breeding. Marker to trait association study in larger scale like; Genome-wide association studies (GWAS) has been facilitated through phenotyping platforms and high-throughput genotyping technologies, allowing researchers to carefully deconstruct genetic structure of plant traits. Agronomically, significant QTL mapping in large scale, mining of elite alleles/haplotypes, gene cloning and characterization, genomic selection and natural variation exploitation have all opened a path for genomics assisted breeding (GAB). GAB could become a potential tool for accelerating the breeding cycle for crop development as more valuable datasets of genomes become available. GAB allows genomic techniques to be merged with phenotyping (high-throughput) to aid crossing practices by using genetic markers to make phenotype prediction from genotype easier. Breeders can start from a huge numbers of

solely genotypic based described offspring and further to utilize only a subset from the original population for more complex evaluation of phenotypic characteristics owing to GAB [8]. Because of its high precision, direct improvement, quick cycle of breeding, and high choosing efficiency, GAB become particularly beneficial in complex trait's improvement. The ultimate objective of GAB is to uncover the ideal allele (or haplotype) combinations, specific genomic locations and excellent gene networks to aid crop's development [9]. Several efforts have been done in the previous decade to optimize nutritional and other quality attributes, either by traditional breeding using high throughput technologies: marker assisted selection (MAS) and breeding, or through use of transgenic technology. GAB techniques are reviewed in this study by demonstrating their efficacy in improving rice grain and nutritional quality.

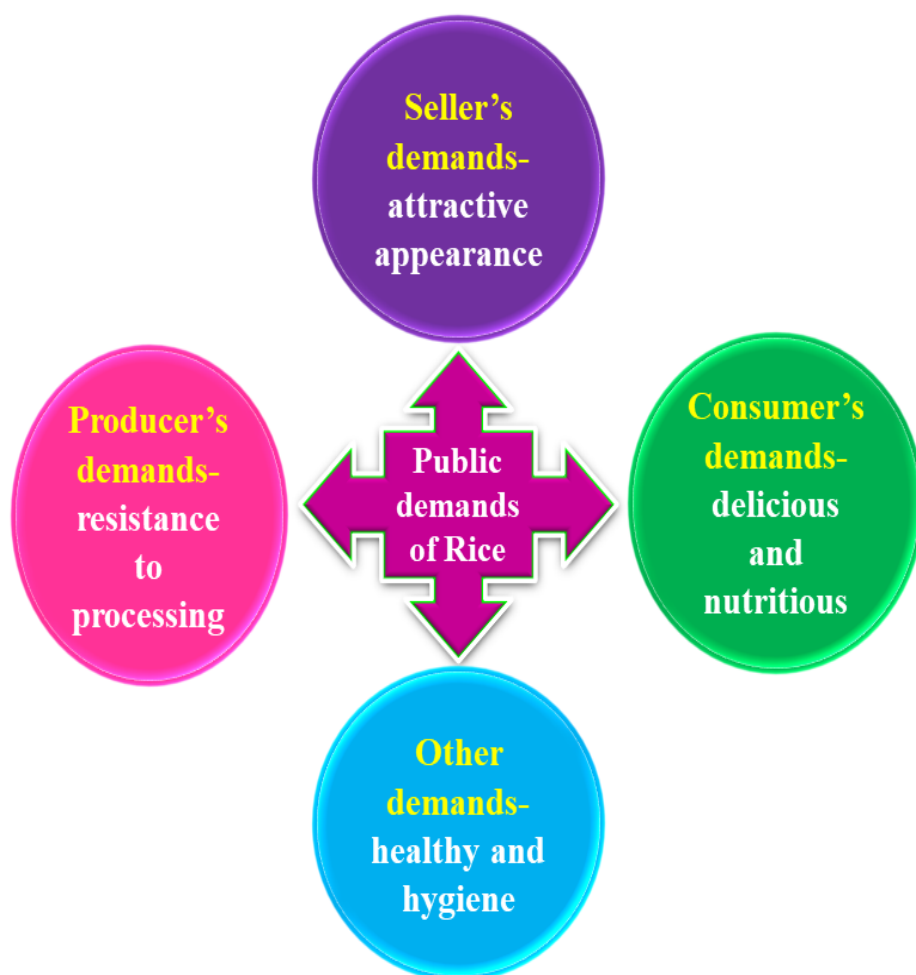


Fig. 1. Public demands of rice based on producer, seller and consumer's preference

2. TARGETED QUALITY TRAITS IN RICE

Quality traits are distinguishable features in the rice grain, which are being assessed physiologically, biochemically by the breeders for better improvement of rice variety. Good quality rice is very much important to consumers, end-user, and processors. To meet the large populations, rice grain quality has become a major concern for rice breeders [10]. This assessment helps to increase the grain yield as well as the grain quality (GQ) traits in rice [11, 12]. Certain factors like environmental factors, crop management, and their interaction affect the rice quality traits [13]. The most important targeted rice grain quality traits are appearance, fragrance, milling quality, cooking and eating quality, nutritional quality (Fig. 2) [14, 15].

2.1 Grain Appearance Quality: Color, Shape and Size

This quality denotes the grain shapes like the length, width, and their ratio, and grain chalkiness [16]. Chalkiness could be classified into white belly, white back, and white core according to its location in the endosperm. The higher chalkiness rice grains are not mostly acceptable by the market [14].

2.2 Milling Quality: Head Rice Recovery

The milling quality of rice denotes the retaining ability of rice kernels after dehusking and polishing without breakage. The final recovery of head rice is a key factor in milling quality. Three main parameters like head rice recovery (HRR),

the percentage of head rice to rough rice), milled rice recovery (MRR, the percentage of milled rice to rough rice), and brown rice recovery (BRR, the percentage of brown rice to rough rice) are used [17].

2.3 Cooking and Eating Quality: Amylose and Gel Consistency

It depends on the gelatinization temperature, amylose content, and gel consistency of the rice grain [18]. 20-25% amylose content rice is mostly accepted and in gelatinization temperature trait case it should be low [19]. Gel consistency assay is used to determine the softness or texture of rice when cooked [20].

2.4 Nutritional Quality: Carbohydrate, Protein, Vitamin and Minerals

The main nutrition of rice is protein. Besides that, it also contains fats, various vitamins, like thiamin and niacin, and minerals, like zinc and phosphorus. Based on the human requirements lysin is the first limiting essential amino acid required to determine the nutritional value of rice [14].

2.5 Fragrance: Pleasant Aroma

Fragrant rice is widely consumed all over the world and taken as the most important quality parameters [11, 21]. 2-acetyl-1-pyrroline (2AP) is mainly responsible for aroma [22]. Besides that, other volatile compounds belonging to the class of aldehydes, ketones, alcohols, phenols, etc., have also been required [23].



Fig. 2. Four facets of grain quality traits in rice

Table 1. Important genes/QTLs related to quality traits in rice

Sl. No.	Target traits	Genes/QTLs	Positioned on the chromosome no.	Function	Reference
1.	Grain appearance	<i>GW7/GL7/SLG7, GLW, SGDP7, GS9, Smg11, OsMADS1, OsSNB, qGRL1.1, GW5, GW2, GS5, GS2, GS3, GL3.1</i>	2, 3, 4, 5, 8, 9, 12	Regulates grain shape	[14, 16]
		<i>OsPPDK 3, SSIIa, GIF 1, ms-h, FLO-2, OsRab5a, Chalk5, PFP 1, qPGWC-7, qPGWC-8</i>	2, 3, 4, 5, 8, 9, 12	Regulate grain chalkiness	[14, 16]
2.	Milling quality	<i>qBRR1.1, qBRR1.2, qBRR2.1, qBRR2.2, qBRR3, qBRR4, qBRR7, qBRR9, qBRR11</i>	1, 2, 3, 4, 7, 9, 11	Controls brown rice recovery (BRR) trait	[17]
		<i>qMRR1, qMRR2, qMRR3, qMRR7, qMRR9, qMRR10, qMRR11, qMRR11.1, qMRR11.2</i>	1, 2, 3, 7, 9, 10, 11	Controls milled rice recovery (MRR) trait	[17]
		<i>qHRR3, qHRR4, qHRR5, qHRR6, qHRR8, qHRR9, qHRR12</i>	3, 4, 5, 6, 8, 9, 12	Controls head rice recovery (HRR) trait	[17]
3.	Cooking and eating quality	<i>Wx</i>	6	Help in amylose synthesis and maintain the gel consistency	[18, 19]
		<i>SSIIa</i>	6	Controls gelatinization temperature	[18, 19]
4.	Nutritional quality	<i>qGPC-1, qGPC-10, qCPC5, Ra, Rc, Rd</i>	1, 5, 7, 10	Control the protein, fat and phenolic content	[14, 24]
5.	Rice fragrance	<i>OsBADH2, P5CS, TPI, GAPDH</i>	3, 4, 5, 8	Responsible for rice aroma	[21, 23]

3. UNDERSTANDING THE GENETICS FOR QUALITY IMPROVEMENT IN RICE

The term “quality” is defined as “the sum of the characteristics and characteristics of a product or service that carries its capacity to meet the stated or stated needs”. Though grain quality is complicated, genetic researchers have proved multiple genes and quantitative trait loci (QTL) for grain quality. Some key genes have been cloned, and their functions in specific pathways such starch, protein, lipid, and flavonoids production have been established. Some QTLs have been finely mapped in preparation for map-based cloning and quantitative measurement. To increase breeding and selection efficiencies, known genes or QTLs have been effectively utilized in breeding programmes for marker-assisted selection (MAS). Fragrant material is dominated by a single repetitive gene (formerly believed to be *fgr* on chromosome 8) according to genetic research. Cloning of the position revealed that *fgr* incorporates the enzyme *betaine aldehyde dehydrogenase 2* (*BADH2*) [25]. *OsBADH2* is a full-length gene with 15 exons and 14 introns containing 503 amino acids. The full-length gene *OsBADH2*, which produces 503 amino acids, has 15 exons and 14 introns. 7-bp removal in exon 2 (named *badh2-E2*), 8-bp removal with 3-bp SNP in exon 7 (designated *badh2-E7*), and 803-bp removal between exons 4 and 5 (designated *badh2-E4 / 5*) reported fragrant rice during the sequence between the aromatic and non-aromatic rice varieties [26, 27, 28].

A few key QTLs associated with rice quality have been identified as a result of advanced mapping technology. The discovery of brown rice is influenced by two large QTLs (*qBRR3* and *qBRR5*) which are also connected to chromosomes 5 and 3 [29, 30]. Based on this conventional method is effective one but certain advanced technology has been used just like CRISPR/Cas9 mediated rice grain quality improvement. Conventional breeding methods rely heavily on genetically engineered mutations. The introduction of desirable traits into the selected germplasm requires a series of retreats, followed by human congestion, which takes a lot of time and effort. On the other hand, genetic mutations accelerate the reproduction of plants by arranging the genome in specific areas. The effectiveness of choosing between generations with different genetic factors contributes to the genetic development of any character. It has been assumed that genetic functions are related

to the number of reproductions. The width and length of the grain intertwine QTLs; *qBRR3* and *qBRR5*. Other studies have identified the QTL *qHRR3* key which is linked to head rice intake and grain length. It is found on the third chromosome. There is a strong correlation based on this investigation. The fraction obtained for head rice is equal to grain size or shape. The four QTLs associated with grain size were chemically modified and analyzed for performance, including the *GW2*, *GW5*, *GW8*, and *GS5* [29, 30, 31]. Glutaminous, low, medium, high I, and II AC high all have five common alleles: *Wx*, *Wxt*, *Wxg1*, *Wxg2*, and *Wxg3*. Chalkiness is also an important factor in determining quality and, ultimately, the economic value of rice grains. Appearance, digestion, cooking, and nutrient content, as well as the restoration of head rice, are all damaged by overcrowding. External and internal signs play a major role in chalky [30]. The use of genome targeting to improve the quality of rice grains is a quick, long-term, and cost-effective method. Use of CRISPR/Cas9 takes a few steps. The first step is to identify the key genes. The genes of Q have the potential to have a negative impact on grain quality (any type of crop that contributes to the deterioration of the quality of rice grains when they are produced). Both forward and backward genes can also be used to identify genes that cause genetic variation [32]. Chalkiness is a negative quality factor that leads to a decline in market acceptance. Grain status is considered part of the crop and plays an important role in determining the quality of the grain grain. *Gn1a*, which determines the amount of grain, and *GS3*, which controls grain length, have recently been successfully converted to four types of rice [33].

The ability to conduct MAS is aided by the provision of several molecular markers of rice, as well as the fact that many features are marked with molecular markers. However, MAS is only useful if the target features are present in the rice germplasm, and it does not work if interesting features are not present in the rice germplasm. Transgenic engineering can be useful in this situation, as it can introduce new features of rice by transferring the targeted gene from another gene. The introduction of CRISPR/Cas9 technology provides a simple, affordable, and effective genome planning platform for researchers, compared to the previous two generations of genome planning technology. The forum not only promotes basic research, but also accelerates the conversion of research results into applications. New Cas9 variant proteins and

homologous proteins, such as *Cas9-VQR*, *Cas9-VRER*, *Cpf1-RR*, *Cpf1-RVR*, and *SaCas9*, have been produced and applied in the rice genome as a result of the existence and optimization of the CRISPR/Cas system in rice. Compared with the wild species, T1-free transgene plants had longer grain lengths and a thousand better grain weights. Similarly, the CRISPR/Cas9-mediated multiplex genome gene was used to target three other key genes, *GW2*, *GW5*, and *TGW6*, which are negative grains of grain weight. The findings show that the genome sequencing of these genes improves grain size and grain weight by as much as a thousand ounces [34]. In the same way, the text control features associated with dCas9 can limit or activate typing in an object. CRISPR-Act2.0 (*dCas9-VP64* linked to *MS2-VP64* via gRNA2.0) was developed by Lowder et al. [35] in the genre and could activate several gene transcripts. The system makes typing more effective than the first-generation *dCas9-VP64* program previously reported [36]. The CRISPR/Cas9 system is the best choice for improving the quality of rice grains based on presented facts and well-documented genomics of effective rice grains, as well as the availability of genetic resources and high mutation efficiency.

4. GENOMIC-ASSISTED BREEDING TOOLS FOR QUALITY TRAIT IMPROVEMENT

The nutritional content and quality of grains have become a top priority in today's world, for both producers and consumers. Rice grain provides about 49 nutrients that are generally vital for appropriate development and growth of an individual [37]. Role of minerals among all other micronutrients is substantial in the human metabolism. With the context to other major crops such as legumes, wheat, maize and tubers, rice has considerably lesser proportion of several key inorganic elements like Zn, Ca, and Fe [38]. The causes of anemia and osteoporosis have been discovered to be inadequate amounts of iron and calcium respectively, in developing and industrialized nations [39]. To address these issues, these factors necessitate for an improved rice grain quality and nutritional content. The industrialized world is coping with inadequacy by creating food fortification management systems, however, similar methods may be too expensive for the developing nations. As a result, alternative and economical ways for improving rice nutritional quality have been realigned, to develop high yielding varieties in combination

with nutrient rich cultivars through suitable breeding methods and other genetic principles [40]. Potential GAB Tools such as; Marker Assisted Selection (MAS), Genetic Engineering (GE), and Genome Editing (Fig. 3) have been extensively examined and inspected by researchers in order to considerably improve these quality attributes in rice crops.

4.1 Marker-Assisted Selection (MAS): Made Selection Easy through Molecular Markers

Novel cultivars with enhanced nutritional content provide several benefits and are also an advancement in a step towards reducing malnutrition in developing countries. As per the "International Rice Research Institute (IRRI)", 843 cultivars are established so far, employing both traditional and molecular breeding practices. Genetic markers are the forerunners in today's agricultural production system, having significantly assisted to the generation of newer varieties with increased precision, dependability, and expediency. As a result, it has made substantial contributions to precision breeding of plants and reliance on molecular approaches such as marker-assisted selection (MAS) and quantitative trait loci (QTL) is crucial for increased agricultural yield in the forthcoming scenario. Rice being a model crop, as well as sequencing the genomes of the indica and japonica, allowed the breeders with resources they need for marker aided breeding. The genetic mapping influencing agronomic characteristics, along with the increasing prevalence of SSR markers and rapid DNA isolation approaches has armed rice breeders with a good chance to adopt MAS strategies [41]. Assemblage of genetic markers as well as the availability of rich genetic maps in rice plants, has enabled the use of MAS [42]. Gene identification, mapping of QTLs and the genomic basis of mineral nutrient accumulation in rice grains, provide a foundation for developing ways to upsurge the mineral content of rice [43].

MAS necessitates the use of more closely related markers which results in the increased fine-mapping of the gene at a higher resolution. Genes can be mapped with a resolution of less than 1 cM in populations larger than 400. In most cases, this will result in MAS-ready markers. Markers that are closely associated to the candidate gene could be designed once the gene has been identified. These markers are frequently investigative of the

beneficial allele present within a diverse array of germplasm, since they don't undergo recombination in breeding populations. Identification of functional nucleotide polymorphism result in a functional marker [44], sometimes known as the "perfect marker". Nevertheless, the SSRs are still widely used, they will continue to be useful for MAS, within or next to the gene. New generation markers claim in reducing the expense of MAS and make it appealing to breeders. So, this MAS technique successfully overcomes the drawbacks associated with conventional breeding, such as labor intensiveness, phenotypic dependence for characterization, unessential gain of linked genes, phenotypic development stage and season dependence, time consumption so on.

Almost all rice breeding programmes put a lot of emphasis on both cooking quality (namely flavour and texture) and grain appearance. The starch content of rice is composed of amylose and amylopectin. The proportion of amylose content is a key indicator for cooking quality of

the rice grain. The amount of Wx protein (granule bound starch synthase, GBSS) found in the Wx locus of the 6th chromosome determines the quantity of amylose present [45]. A microsatellite marker with a varied amount of CT repeats that is primarily linked to Wx is related to amylose content and grain quality. Larkin and Park [46] discovered that the exons 6 and 10 of the two SNPs were associated with the changes in the apparent concentration of amylose and viscosity parameters of rice varieties. Another key quality indicator is gelatinization temperature (GT), with moderate GT being a desirable objective for many regulars. Two polymorphic starch synthase II (*SSIIa*) gene, *Alk* situated on chromosome number 6 was discovered by Waters et al. [47] that clarified variances in the GT content. Numerous breeding programmes value fragrance. A significant gene located on the 8th chromosome, betaine aldehyde dehydrogenase 2 (*BAD2*) has been positionally cloned, with fragrance signifying a function loss induced by an eight base pairs deletion [48]. This polymorphism has resulted in the development of an ideal MAS

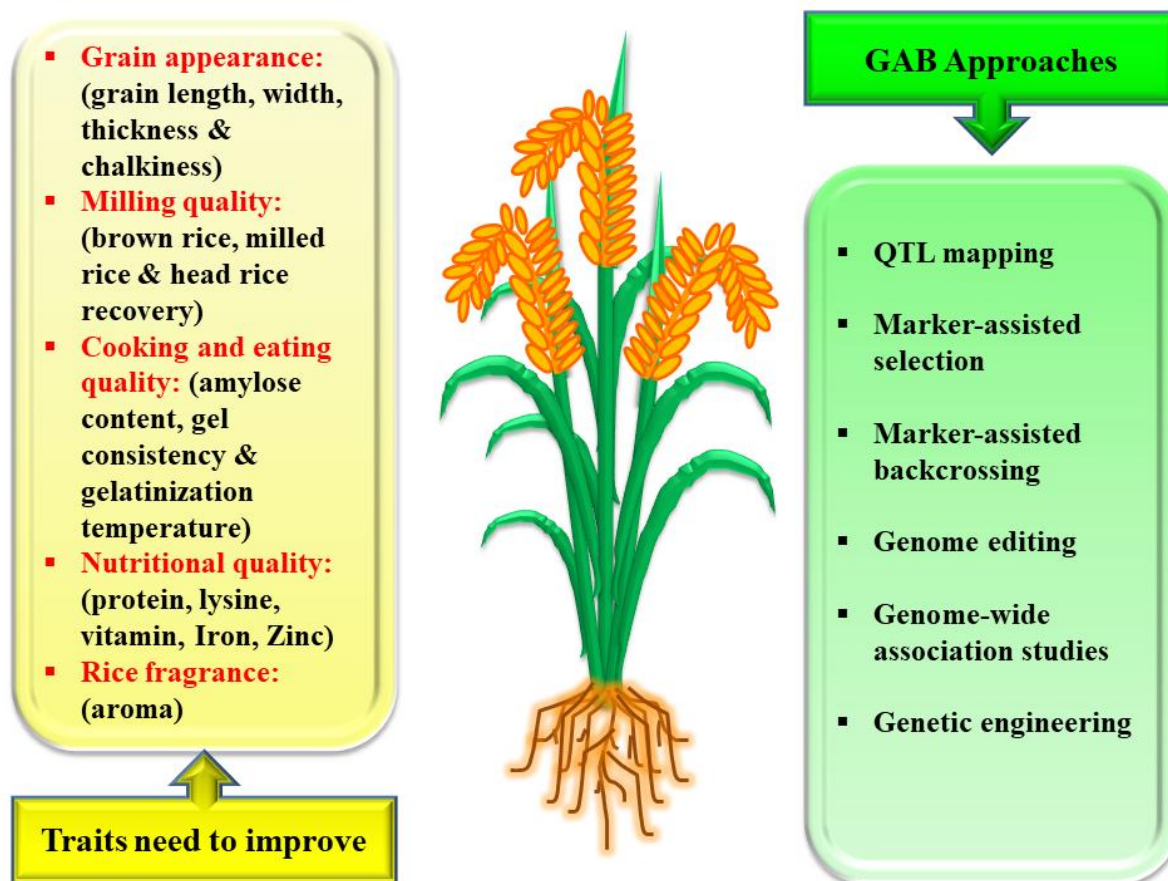


Fig. 3. An illustration for Genomic-assisted breeding (GAB) techniques to improve the quality traits in rice

marker [49]. Grain appearance like shape, size are all essential quality variables, with most consumers preferring certain size and features. Several studies have shown a QTL for length of grain and weight on the 3rd chromosome [50, 51]. A Quantitative Trait Loci on chromosome 8 was discovered in being relevant in the chalkiness and added quality factors [52]. Rice has just a limited nutritional characteristic that have been identified and mapped genetically. Brown rice has been shown to exhibit QTLs for cadmium (Cd) content when grown in soil rich in Cd [53]. This has suggested a way of producing cultivars that are tolerant of high-Cd soils. Mutants with low phytic acid contents have been explored for their potential to enhance animal feed quality and for low phytic acid cultivars to improve Fe absorption in micronutrient deficient populations. A 47-kb area on chromosome 5 was linked to a low phytic acid mutant, *Lpa1* [54]. Due to the availability of whole genome sequencing data, markers related with quality attributes have been discovered. This also aids in the selection and targeting of certain genes by allowing for the use of linked molecular markers to resourcefully characterize rice types as prospective donors or acceptors for improved cross design.

4.2 Genome Editing: Manipulate the Targeted Genes

CRISPR/Cas mediated genome editors provide a greater opportunity to improve rice quality by targeting the desired gene. The actual mechanism for improving rice quality trait by genome editing is to manipulate the target gene by mutation or gene knockout, so that the activity of that gene either suppressed or overexpressed. This results in the change of the protein expression and subsequently affect the quality trait. Several genome editors like adenine base editors (ABE), cytosine base editor (CBE), RNA base editor (RBE), and prime editing strategy were used [55]. ABEs generally target A nucleotide and convert it to G nucleotide. The adenosine deaminase deaminates adenine into inosine and the nCas9 generates a nick at target strand [56]. So ABEs convert an Adenine-Thymine base pair to a Guanine-Cytosine base pair. Like ABE, CBE converts Cytosine-Guanine base pairing to Thymine-Adenine base pair by cytidine deaminase and nCas9 [55]. RBEs changes single nucleotide by using the programmable RNA and deactivated Cas13b protein in the mRNA [55]. But Prime editing system utilizes the modified Cas9 protein and guide RNA which guide the cut site in a single

strand with high accuracy [57]. Base editors, that is CBE3 was targeted to *OsBE1b* and *OsPDS* genes to generate a point mutation cause an increase in rice amylose contents [58]. Like that, CBE 3 also altered the amylose content by targeting *Wx* gene or GBSS1 protein [59]. Beside this, CRISPR/Cas-9 mediated editing also improve the quality traits. For improving eating and cooking quality *OsAAP6* and *OsAAP10* gene was targeted in Yanggeng-158, Nangeng-9108 Wuyungeng-30 cultivars, which helps in increasing the protein content [60]. *OsBADH2* gene expression was also reduced to increase the rice flavour. Knocking out *OsNramp5* gene by CRISPR/Cas9 reduces the cadmium percentage in indica rice [61]. *OsOr*, *SSU-crtI*, *ZmPsy* were targeted to increase in provitamin-A content [62, 63]. Certain genes like *DEP1*, *Gn1a*, *TGW6*, *GW2*, *GS3* and *GS5* were knocked out and *OsSPL16/GW8*, *OsFWL* were expressed for rice shape and appearance [64-67].

4.3 Genetic Engineering (GE): Incorporation of Transgenes

Because of the incapability to manufacture essential amino acids, humans are very keen in enhancing the quantities of certain amino acids in agricultural plants. Plant genetic studies and genetic engineering technologies have greatly aided in the enrichment of major crop plants by increasing their required amino acid and protein content. Biotechnology has outperforms than traditional breeding and it's critical to ensure that any differences in grain size, colour, or texture have no severe adverse agronomic implications for consumers when a nutritionally enhanced rice variety is developed using genetic engineering technology. Previously, conventional breeding, marker aided breeding, and transgenic technologies have all been used to improve rice nutritional quality. The genetic engineering strategy was used to improve the important micronutrients in rice, including zinc (Zn), iron (Fe) and Vitamin-A. The transgenic technique boosted nutritional content by improving nutrient absorption and translocation via modification of several ion transporters. Some anti-nutritional factors like phytic acids, were decreased by gene insertion. Increased Fe, Zn and carotenoid concentration in the rice endosperm helped alleviate nutritional deficits, especially amid rural and urban people who lacked intake of the supplemented foods and diverse diets. Therefore, either traditional breeding or developing biotechnology methods must be used to upsurge the nutritional content of rice. So as to

achieve this objective, plant researchers have to utilize a variety of methods. Tryptophan and lysine in particular, received a lot of attention since their supply in cereal crops is limited. Constitutive expression of bacterial lysine insensitive DHDPS in Arabidopsis plants and transgenic tobacco or genetic mutations in the tobacco DHDPS resulted in the overproduction of lysine in the tissues of the plant [68, 69]. In order to increase cereal lysine content, a new technology has been developed in recent years that involve inserting lysine at alternative codons during translation utilizing a recombinant tRNA lysine. The expression of recombinant tRNA in the transgenic rice might considerably increase the content of lysine in seed [70].

Rice breeding procedures are continuously progressing, with transgenic assisted breeding processes being used to generate nutrient-rich rice types. Goto et al. [71] used transgenic techniques to observe a threefold enrichment of Fe levels in endosperm of rice by inserting the soybean ferritin gene. As a result, some scientists have taken a chance to improve Iron (Fe) elements in the endosperm by overexpressing candidate genes that are involved in the absorption of Iron from the soil and translocation from roots to the shoot and flag leaves to the grains, as well as by enhancing ability of Iron accumulating proteins [72, 73, 74]. Overexpression of NAS (nicotianamine synthase gene) or other relevant transporter genes has been shown to boost Iron and Zinc content in rice in a number of studies. Furthermore, three similar NAS proteins that are *OsNAS1*, *OsNAS2*, and *OsNAS3* were overexpressed to elevate Fe and Zn accumulation in refined rice by twofold [75]. In comparison to other wild-type grains, Lee et al. [76] found that introducing *OsNAS3-D1* increases Cu, Zn and Fe accumulation from 1.72 to 2.90 times in the seedlings of rice. In the contrast to milled rice seeds, various genes were introduced, including *IDS3* genomic regions of barley, NAS gene overexpression, *OsSUT1* promoter drive *OsYSL2*, and ferritin gene under endosperm-specific promoter control [73]. Supplementation using pharmaceutical preparations, enrichment of food, dietary variety, and sickness reduction are the most common predictable ways for addressing deficiency caused due lack of micronutrient [77]. None of them so far are successful in sinking the incidence of anemia, caused due to iron deficiency in developing countries for several reasons. Enhancement of diet staples either by plant breeding or recombinant DNA technology,

would be a more sustainable choice [78,79]. Upsurging seed ferritin, the natural Fe storage, is recommended as a way to enhance the iron levels [79]. The above-mentioned findings showed that a multi-gene targeting method would be more beneficial for improving mineral accumulation and nutritional quality in rice grains.

5. CONCLUSION AND FUTURE DIRECTION

Rice grain quality is a complex trait that includes appearance, milling quality, cooking and eating quality, and nutritional quality. Research on the genetic control of quality traits has made significant progress, particularly in the areas of appearance, cooking, and eating quality. Because people are increasingly concerned about the health benefits of the food they eat, the nutritional quality of rice will be a new area for further research. Nutritional quality encompasses a wide range of characteristics, such as protein, amino acids, fat, and phenolics. More genetic research is needed to improve milling and nutritional quality of rice. The advancement of molecular genetics in grain quality has made marker-assisted selection (MAS) more efficient in this direction. However, only MAS for cooking and eating quality and genetic engineering for nutritional quality have made some progress. More molecular breeding practises, such as genome sequencing, genome editing, and genome-wide association studies, are required to improve grain quality. Because only a small number of QTLs have been identified, and none have been finely mapped or cloned, genetic understanding of milling quality is quite limited. Natural variation in protein, amino acid, fat, and fatty acid compositions has been studied in this field, but only a few genes have been identified. Each nutrient in rice grain is formed through a complex pathway involving many genes or enzymes. Recent advances in protein and fatty acid biosynthesis in other crops and Arabidopsis may aid in understanding the rice pathways. To conduct in-depth research on milling quality, rapid and accurate analytical tools are required to measure the trait, as well as finely dissection of QTLs with large effects. Because no milling quality mutants have been reported, mutants induced by T-DNA insertion may provide a good starting point for characterising the genes responsible for milling quality. Two finely mapped QTLs for grain chalkiness await further characterization, and a transcriptome for chalkiness formation during seed development has been described. More progress in this area

appears to be on the horizon. Hotspots for QTLs controlling a variety of grain quality traits have been identified on chromosomes 3, 6, and 8, indicating that there are still many novel genes to be discovered. The genes associated with these QTLs are beginning to be identified and characterised, such as *BETADINE ALDEHYDE DEHYDROGENASE 2 (BADH2)*, which controls the flavour and aroma of Jasmine and Basmati rice and has previously been reviewed by many researchers. The *Waxy* locus, which controls the amylose content and grain texture of japonica and indica rice, and *GRAIN INCOMPLETE FILLING 1 (GIF1)* have been described. The potential to use biotechnology to engineer these traits into high-yielding varieties will become a reality as we gain a better understanding of the genotype underlying varieties with diverse grain quality traits. All appearance-quality traits are typical quantitative traits that are influenced by environment and controlled by multiple genes. Numerous QTL have been identified on all 12 chromosomes in various mapping populations in rice as a result of the advancement of rice functional genomics study and molecular marker technology. Many grain shape genes have been identified, including *GW2*, *GW5*, *GS2*, *GS3*, *GS5*, *GS9*, *GL3.1*, *GL7/GW7/SLG7*, *GLW7*, *SGDP7*, *smg11*, *OsMADS1*, and *OsSNB*. Some genes for chalkiness have been identified, including *OsPPDKB*, *SSIIIa*, *GIF1*, *ms-h*, *FLO2*, *OsRab5a*, *Chalk5*, and *PPF1*. Some QTL, such as *qGRL1.1* for grain shape and *qPGWC-7* and *qPGWC-8* for grain chalkiness, have been fine-mapped. Cooked rice elongation, a quantitative trait, is being closely monitored in the international rice market. Because the inheritance pattern of cooked rice elongation is inconsistent across crosses, elucidating its genetic basis, which has remained ambiguous, has proven difficult. Basmati rice is a desirable variety because it has high elongation without increasing in width when cooked. The discovery and fine-mapping of QTLs can be used for genomic selection of desirable traits in rice varieties. Genes can be cloned to investigate allelic variation in polymorphisms or transformed into high-yielding lines to meet regional grain quality preferences. However, with the ability to sequence the entire genomes of a large number of rice accessions now available, breeders will soon be able to identify the genes underlying grain quality traits and mine for allele variation. This provides a once-in-a-lifetime opportunity to learn more about how allelic variation affects phenotypic variation. The challenge for molecular breeders then becomes determining how to best apply this knowledge.

The major drawbacks of traditional transformation technologies are consumer acceptance and the inability to eliminate the endogenous target gene, so modern tools such as targeted genome editing technologies are playing an increasingly important role.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kusano M, Yang Z, Okazaki Y, Nakabayashi R, Fukushima A, Saito K. Using metabolomic approaches to explore chemical diversity in rice. *Mol. Plant*. 2015;8:58-67.
2. Fitzgerald MA, McCouch SR, Hall RD. Not just a grain of rice:the quest for quality. *Trends Plant Sci*. 2009;14:133-139.
3. Miura K, Ashikari M, Matsuoka M. The role of QTLs in the breeding of high-yielding rice. *Trends Plant Sci*. 2011;16:319-326.
4. Juliano B. Rice chemistry and quality. International Rice Research Institute, Los Baños, Laguna. 2003.
5. Philpot K, Martin M, Butardo V, Willoughby D, Fitzgerald M. Environmental factors that affect the ability of amylose to contribute to retrogradation in gels made from rice flour. *J Agric Food Chem*. 2006;54:5182-5190.
6. Uncu AO, Doganlar S, Frary A. Biotechnology for enhanced nutritional quality in plants. *Crit. Rev. Plant Sci*. 2013;32:321-343.
7. Bao JS. Nutraceuticals properties and health benefits of rice. *Cereals and Pulses. Nutraceutical Properties and Health Benefits*. Yu LL, Tsao R, Shahidi F, (eds). Wiley-Blackwell, John Wiley and Sons, 2012;37-64.
8. Cooper M, Messina CD, Podlich D, Totir LR, Baumgarten A, Hausmann NJ, Wright D, Graham G. Predicting the future of plant breeding: complementing empirical evaluation with genetic prediction. *Crop and Pasture Science*. 2014;65:311–336.
9. Xu Y, Lu Y, Xie C, Gao S, Wan J, Prasanna BM. Wholegenome strategies for marker-assisted plant breeding. *Molecular Breeding*. 2012;29:833-854.
10. Bazrkar-Khatibani L, Fakheri BA, Hosseini-Chaleshtori M, Mahender A, Mahdinejad N, Ali J. Genetic mapping and validation of Quantitative Trait Loci (QTL) for the grain

- appearance and quality traits in rice (*Oryza sativa* L.) by using Recombinant Inbred Line (RIL) population. *Int J Genomics*. 2019;1-13.
11. Sreenivasulu N, Butardo VM, Misra G, Cuevas RP, Anacleto R, Kishor PBK, et al. Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *Journal of Experimental Botany*. 2015;66(7):1737-1748.
 12. Sun J, Yang L, Wang J, Liu H, Zheng H, Xie D, Zhang M, Feng M, Jia Y, Zhao H, Zou D, et al. Identification of a cold-tolerant locus in rice (*Oryza sativa* L.) using bulked segregant analysis with a next-generation sequencing strategy. *Rice*. 2018;11(1):1-12.
 13. Sharma N, Khanna R, et al. Rice grain quality: Current developments and future prospects. recent advances in Grain Crops Research. 2020;1-17.
 14. Bao J. Genes and QTLs for Rice Grain Quality Improvement. *Intech*. 2014;1-41. DOI:<http://dx.doi.org/10.5772/56621>
 15. Verma DK, Srivastav PP. Introduction to rice aroma, flavor, and fragrance. in science and technology of aroma, flavor, and fragrance in rice (Issue March); 2020. DOI:<https://doi.org/10.1201/b22468-10>.
 16. Qiu X, Yang J, Zhang F, Niu Y, Zhao X, Shen C, Chen K, Teng S, Xu J. et al. Genetic dissection of rice appearance quality and cooked rice elongation by genome-wide association study. *Crop Journal*. 2021;9(6):1470-1480.
 17. Bao J. Rice milling quality. In *Rice: Chemistry and Technology*. 2018, AACCI. Published by Elsevier Inc. in cooperation with AACCI International.
 18. Zhang YF, Tan JX, Li SB, Yu YZ, Xing CG, Xu Q, The three important traits for cooking and eating of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. *Theoretical and Applied Genetics*. 1999;642-648.
 19. Sattari A, Mahdinezhad N, Fakheri B, Noroozi M. et al. Improvement of the eating and cooking qualities of rice:a review. *Int J of Farming and Allied Sci*. 2015;4(2):153-160.
 20. Tran NA, Daygon VD, Resurreccion AP, Cuevas RP, Corpuz HM, Fitzgerald MA, et al. A single nucleotide polymorphism in the Waxy gene explains a significant component of gel consistency. *Theoretical and Applied Genetics*. 2011;123(4):519-525.
 21. Ashokkumar S, Jaganathan D, Ramanathan V, Rahman H, Palaniswamy R, Kambale R, Muthurajan R, et al. Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing. *PLoS ONE*. 2020;15(8):1-18.
 22. Buttery RG, Ling LC, Turnbaugh JG, Juliano BO. Cooked Rice Aroma and 2-Acetyl- 1-pyrroline. *J. Agri. Food Chem*. 1983;31(4):823-826.
 23. Hinge VR, Patil HB, Nadaf AB. Aroma volatile analyses and 2AP characterization at various developmental stages in Basmati and Non-Basmati scented rice (*Oryza sativa* L.) cultivars. *Rice*. 2016;9(1):1-22.
 24. Yang Y, Guo M, Sun S, Zou Y, Yin S, Liu Y, Tang S, Gu M, Yang Z, Yan C. Natural variation of OsGluA2 is involved in grain protein content regulation in rice. *Nature Communications*. 2019;10:1949.
 25. Bradbury LMT, Fitzgerald TL, Henry RJ, Jin QS, Waters DLE. The gene for fragrance in rice. *Plant Biotechnol*. 2005a; 3:363-370.
 26. Kovach MJ, Calingacion MN, Fitzgerald MA, McCouch SR. The origin and evolution of fragrance in rice (*Oryza sativa* L.). *Proc. Natl Acad.Sci. USA* 106. 2009;14444-14449.
 27. Shao GN, Tang A, Tang SQ, Luo J, Jiao GA, Wu JL, Hu PS, et al. A new deletion mutation of fragrant gene and the development of three molecular markers for fragrance in rice. *Plant Breed*. 2016;130:172-176.
 28. Shi W, Yang Y, Chen S, Xu M. et al., Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. *Mol. Breed*. 2008;22:185-192.
 29. Lou J, Chen L, Yue G, Lou Q, Mei H, Xiong L and Luo L. QTL mapping of grain quality traits in rice. *J. Cereal Sci*. 2009;50:145-151.
 30. Tan Y, Sun M, Xing Y, Hua J, Sun X, Zhang Q, Corke H. et al., Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived,from an elite rice hybrid. *Theor. Appl. Genet*. 2001;103:1037-1045.
 31. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat. Biotechnology*. 2013;31:233.

32. Lau WC, Rafii MY, Ismail MR, Puteh A, Latif MA, Ramli A. Review of functional markers for improving cooking, eating, and the nutritional qualities of rice. *Front. Plant Sci.* 2015;6:832.
33. Shen L, Li J, Fu Y, Wang J, Hua Y, Jiao X, Yan C, Wang K. et al., Orientation improvement of grain length and grain number in rice by using CRISPR/Cas9 system. *Chin. J. Rice Sci.* 2017;31:223-231.
34. Xu R, Yang Y, Qin R, Li H, Qiu C, Li L, Wei P, Yang J. Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *J. Genet. Genom.* 2016;43:529-532.
35. Lowder LG, Zhou JP, Zhang YX, Malzahn A, Zhong ZH, Hsieh TF, Voytas DF, Zhang Y, Qi YP. Robust transcriptional activation in plants using multiplexed CRISPR-Act2.0 and mTALE-Act systems. *Mol Plant.* 2018;11(2):245-256.
36. Lowder LG, Zhang DW, Baltes NJ, Paul JW, Tang X, Zheng XL, Voytas DF, Hsieh TF, Zhang Y, Qi YP. A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. *Plant Physiol.* 2015;169(2):971-985.
37. Welch RM and Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp. Bot.* 2004;55:353-364.
38. Adeyeye EI, Arogundade LA, Akintayo ET et al. Calcium, zinc and phytate interrelationship in some foods of major consumption in Nigeria. *Food Chem.* 2000;71(4):435-441.
39. Welch R, Graham RD. A new paradigm for world agriculture:meeting human needs productive, sustainable, nutritious. *Field Crops.* 1999;60:1-10.
40. Gearing ME. Good as gold: Can golden rice and other biofortified crops prevent malnutrition? *Science in the News.* 2015.
41. Xu X, Kawasaki S, Fujimura T, Wang CT. A protocol for high-throughput extraction of DNA from rice leaves. *Plant Mol Biol Rep.* 2005;23:291-295.
42. Choudhary K, Choudhary OP, Shekhawat NS. Marker assisted selection:a novel approach for crop improvement. *Am Eurasian J Agric.* 2008;1:26-30.
43. Zhang MW, Guo BJ, Peng ZM. Genetic effects on Fe, Zn, Mn and P content in indica black pericarp rice and their genetic correlations with grain characteristics. *Euphytica.* 2004;135:315-323.
44. Andersen JR, Lubberstedt T. Functional markers in plants. *Trends Plant Sci.* 2003;8:554-560.
45. Wang ZY, Zheng FQ, Shen GZ, Gao JP, Snustad DP, Li MG, Zhang JL, Hong MM. The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. *Plant J.* 1995;7:613-622.
46. Larkin PD, Park WD. Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (*Oryza sativa* L.). *Mol Breed.* 2003;12:335-339.
47. Waters DLE, Henry RJ, Reinke RF, Fitzgerald MA. Gelatinization temperature of rice explained by polymorphisms in starch synthase. *Plant Biotechnol.* 4:115-122.
48. Bradbury LMT, Fitzgerald TL, Henry RJ, Jin QS, Waters DLE. The gene for fragrance in rice. *Plant Biotechnol.* 2005a;3:363-370.
49. Bradbury LMT, Henry RJ, Jin QS, Reinke RF, Waters DLE. A perfect marker for fragrance genotyping in rice. *Mol Breed.* 2005b;16:279-283.
50. Li JM, Thomson W, McCouch SR. Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. *Genetics.* 2004;168:2187-2195.
51. Ge XJ, Xing YZ, Xu CG, He YQ. QTL analysis of cooked rice grain elongation, volume expansion, and water absorption using a recombinant inbred population. *Plant Breed.* 2005;124:121-126.
52. Wan XY, Wan JM, Weng JF, Jiang L, Bi JC, Wang CM, Zhai HQ. Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. *Theor Appl Genet.* 2005;110:1334-1346.
53. Ishikawa S, Ae N, Yano M. Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (*Oryza sativa*). *New Phytol.* 2005;168:345-350.
54. Andaya CB, Tai TH. Fine mapping of the rice low phytic acid (Lpa1) locus. *Theor Appl Genet.* 2005;111:489-495.
55. Molla KA, Karmakar S, Islam MT. Wide horizons of CRISPR-cas-derived technologies for basic biology, agriculture, and medicine. In *CRISPR-Cas Methods*, Springer Protocols Handbooks. 2020.
56. Molla K, Yang Y, et al. CRISPR/Cas-mediated base editing: Technical

- considerations and practical applications freely available for limited time. *Cell*. 2019; 8-9.
57. Tabassum J, Ahmad S, Hussain B, Mawia AM, Zeb A, Ju L, et al. Applications and potential of genome-editing systems in rice improvement: Current and future perspectives. *Agronomy*. 2021;1-24.
 58. Li J, Sun Y, Du J, Zhao Y, Xia L. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. *Mol Plant*. 2017; 10:526-529.
 59. Xu Y, Lin Q, Li X, Wang F, Chen Z, Wang J, Li W, Fan F, Tao Y, Jiang Y, Wei X, Zhang R, Zhu Q, Bu Q, Yang J, Gao C, Crops F, Academy J, Sciences A, Academy C. Fine-tuning the amylose content of rice by precise base editing of the. *Plant Biotechnol J*. 2021;19(1): 11-13.
 60. Wang S, Yang Y, Guo M, Zhong C, Yan C, Sun S. Targeted mutagenesis of amino acid transporter genes for rice quality improvement using the CRISPR/Cas9 system *Shiyu. The Crop Journal*. 2020;8(3):1-15.
 61. Tang L, Mao B, Li Y, Lv Q, Zhang L, Chen C, He H. et al. Knockout of *OsNramp5* using the CRISPR / Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific Reports*. 2017;1-12.
 62. Endo A, Saika H, Takemura M, Misawa N, Toki S. A novel approach to carotenoid accumulation in rice callus by mimicking the cauliflower Orange mutation via genome editing. *Rice*. 2019;1-5.
 63. Dong OX, Yu S, Jain R, Zhang N, Duong PQ, Butler C, Li Y, Lipzen A, Martin JA, Barry KW, Schmutz J, Tian L, Ronald PC. Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9. *Nature Communications*. 2020;1-10.
 64. Li M, Li X, Zhou Z, Wu P, Fang M, Pan X, Henry R. Reassessment of the Four *GS3*, and *IPA1* in Rice Using a CRISPR/Cas9 System. *Frontiers in Plant Science*. 2016;7:1-13.
 65. Gao Q, Li G, Sun H, Xu M, Wang H, Ji J, Wang D. Targeted Mutagenesis of the Rice *FW2.2*-Like Gene Family Using the CRISPR/Cas9 System Reveals *OsFWL4* as a Regulator of Tiller Number and Plant Yield in Rice. *Int J of Mol Sci*. 2020;6-9.
 66. Zeng Y, Wen J, Zhao W, Wang Q, Huang, W. Rational Improvement of Rice Yield and Cold Tolerance by Editing the Three Genes *OsPIN5b*, *GS3*, and *OsMYB30* with the CRISPR – Cas9 System, *Frontiers in Plant Science*. 2020;10:1-13.
 67. Usman B, Nawaz G, Zhao N, Liao S, Qin B, Liu F, Liu Y, et al. Programmed Editing of Rice (*Oryza sativa* L.) *OsSPL16* Gene Using CRISPR / Cas9 Improves Grain Yield by Modulating the Expression of Pyruvate Enzymes and Cell Cycle Proteins. *Int J Mol Sci*. 2021; 22(1): 249.
 68. Frankard V, Ghislain M, Jacobs M. Two feedback-insensitive enzymes of the aspartate pathway in *Nicotiana glauca*. *Plant Physiol*. 1992;99:1285-1293.
 69. Shaul O, Galili G. et al. Increased lysine synthesis in transgenic tobacco plants expressing a bacterial dihydrodipicolinate synthase in their chloroplasts. *Plant Journal*. 1992;2:203-209.
 70. Wu XR, Chen ZH. Enrichment of cereal protein lysine content by altered tRNAlys coding during protein synthesis. *Plant Biotechnol*. 2003;1:187-194.
 71. Goto F, Yoshihara T, Shigemoto N. et al., Iron fortification of the rice seed by the soybean ferritin gene. *Nat Biotechnol*. 1999;17:282-286.
 72. Bashir K, Takahashi R, Nakanishi H et al. The road to micronutrient biofortification of rice: progress and prospects. *Front Plant Sci*. 2013;4:15.
 73. Masuda H, Kobayashi T, Ishimaru Y, et al. Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Front Plant Sci*. 2013;4:132.
 74. Slamet-Loedin IH, Johnson-Beebout SE, Impa S. et al. Enriching rice with Zn and Fe while minimizing Cd risk. *Front Plant Sci*. 2015;6:121.
 75. Johnson AA, Kyriacou B, Callahan DL, et al. Constitutive over expression of the *OsNAS* gene family reveals single gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One*. 2011;6(9):24476.
 76. Lee S, Jeon US, Lee SJ. et al. Iron fortification of rice seeds through activation of the nicotianamine synthase gene. *Proc Natl Acad Sci USA*. 2009;106:22014–22019.
 77. Maberly GF, Trowbridge FL, Yip R, Sullivan KM, West CE. Programs against micronutrient malnutrition: ending hidden

- hunger. *Annu Rev Public Health.* 1994;15:277-301.
78. Bouis H. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutr Rev.* 1996;54:131-137.
79. Theil EC, Burton JW, Beard JL. et al. A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *Eur. J. Clin. Nutr.* 1997;51: 28-31.

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