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Influencing Factors of Grain Nutritional Quality and its Genetic Improvement Strategy in Rice

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Abstract

Rice is one of the most important food crops in the world, and about half of the world's population uses it as the main food source. China's annual rice output accounts for about 34% of the world's annual rice output, ranking first in the world, thus China is known as the "Rice Kingdom". Rice has high nutritional value, providing the human body with 35% of the total energy intake and about 28% of the required protein. Therefore, the nutritional value of rice is directly related to human nutrition and health. In this paper, the three aspects of rice nutrition and human health, factors affecting rice nutritional quality and genetic improvement of nutritional quality of rice are reviewed. The new challenges of rice nutrition quality were analyzed, and the prospect of improving rice nutritional quality was prospected. The results

provide theoretical basis for genetic improvement of rice nutrition quality and cultivation of new high-quality rice varieties in the future.

Keywords: Nutritional quality; Rice; Protein; Starch; Influence factors; Genetic improvement.
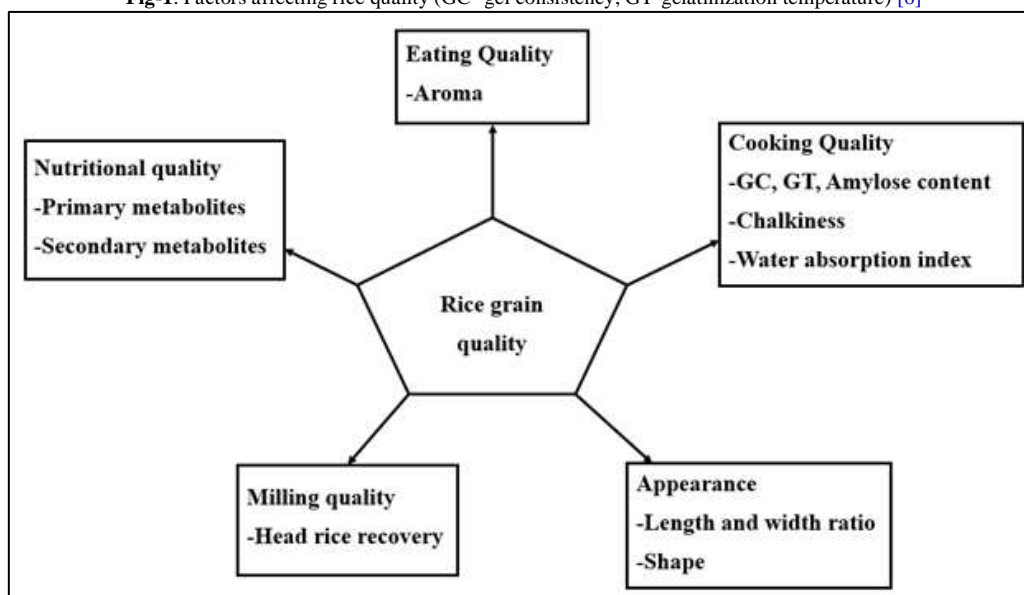
1. Introduction

Rice is the staple food for more than 3 billion people in more than 100 countries in the world, which meets more than 25% of the world's population [1]. Different geographical regions or nationalities have different preferences for rice quality [2]. For example, consumers in Southeast Asia prefer slender grains, while consumers in North Asia prefer stout, short grains, while people in India and Pakistan prefer longer grains. Within the same country, there will be also difference, consumers in southern China prefer fine rice, while consumers in northern China prefer short round rice [3]. Although there are regional differences, rice still occupies a very important position.

Rice is composed of husk (lemma and palea), bran layer (pericarp, aleurone layer, subleucine layer), embryo and endosperm. The most common layer of rice bran is brown rice, so when the husk is removed, it is called "brown rice". The outer layer of natural brown bran removes most of the fiber, vitamins, minerals and amino acids in the polishing process to form "white rice", which is also the general consumption form of rice. In most cases, *indica* rice has a long and thin shape, while *japonica* rice has a short and round shape, and in many cases, short or slender grains are preferred by consumers [4]. However, ironically, rice lacks a lot of biologically available substances, such as vitamins, minerals, essential amino acids, fatty acids and phytochemicals, which can prevent chronic diseases such as type II diabetes, heart disease, cancer and obesity. With the improvement of people's living standards, the access to food has shifted from grain to meat, milk, eggs, vegetables and fruits. The demand for food is no longer simply "enough", but more and more "delicious" and "healthy".

Rice quality is a complex character reflecting the opinions of producers, processors, sellers and consumers on rice production, processing, marketing and consumption. Rice quality consists of cooking and eating quality, appearance quality, milling quality and nutritional quality. These attributes may evolve over time to meet the different needs of consumers and various industries (Fig. 1) [5]. As an important food crop, rice has become a reference for functional genomic research of monocotyledons [5]. Improving rice quality is an important goal of rice breeders. In the past few decades, the cultivation of semi dwarf varieties and hybrids has made great contributions to the realization of high-yield requirements, but it has reduced the grain quality, which has caused a lot of researchers' in-depth research. Therefore, in rice breeding, we should not only pay attention to high yield, but also pay attention to high quality [5]. In order to improve the nutritional quality of rice, it is necessary to better understand the regulation of synthesis, absorption, transportation and metabolism of macro (starch, seed storage protein and lipid) and micronutrients (vitamins, minerals and phytochemicals). With the publication of rice quality genome sequence, great progress has been made in the identification, isolation, identification of new genes and their regulation on rice nutritional quality.

Fig-1. Factors affecting rice quality (GC- gel consistency, GT-gelatinization temperature) [6]

The nutritional quality of rice is determined by its practical characteristics, including starch content, protein content, essential amino acid content, lipid content and trace elements; Protein content is an important index of rice nutritional quality, which is close to some animal proteins and is rich in essential amino acids for human body. Therefore, it is one of the important topics in rice quality breeding to improve protein content and cultivate high nutrition rice varieties. In the past decade, both traditional high-throughput breeding techniques, such as marker assisted selection and breeding, or transgenic methods have been used to improve the nutritional quality and other quality traits of rice. The levels of vitamins (A, E and folate), minerals (iron), essential amino acids (lysine) and flavonoids in the edible part of rice, namely endosperm (bioaugmentation), were significantly increased to meet the

daily dietary needs. However, the bioaugmentation of rice remains to be studied. Despite many efforts, the commercialization of bio fortified rice has not yet been realized [5]. At present, only in the experimental stage of rice nutrition quality of new progress, but also can be regarded as a success, for the sake of human health, all kinds of risks cannot be ignored, strive to cultivate high nutritional value and usable bio fortified rice to improve people's quality of life.

2. Grain nutritional Quality and Human Health

Rice is an important source of protein. It contains 8 kinds of essential amino acids, and its content is balanced. It plays an important role in the health of hair and skin, the improvement of vision, the nutrition of heart and lung, the improvement of nervous system and brain function. Its nutrients include gamma aminobutyric acid, resistant starch, lysine, iron, zinc, β - carotene, folic acid, anthocyanins, various carotenoids and flavonoids. As a staple food, its nutritional quality has a great impact on the health of consumers, especially in the poor areas where fruits, fish, meat and other food can not afford, and only rely on rice. Therefore, strengthening the nutrition of rice is a matter of great importance to human health.

According to the color of caryopsis, rice can be divided into white rice, black rice, red rice and green rice. Among them, white rice is the most common variety. In addition to steaming rice, it can also be used to make rice cakes, noodles, dumplings and glutinous rice flour [7]. Brown rice of black rice varieties is usually used as rice soup, and glutinous rice with very low amylose content (< 2%) is more suitable for rice soup. Some special health promoting metabolites such as PAS and anthocyanins (provided by colored rice) are only present in the pericarp of brown rice. Therefore, there are also glutinous rice varieties obtained by cultivating colored rice to improve the taste of rice soup and promote consumption [7]. At present, the successful golden rice shows that we have gone further and further on the road of strengthening the cultivation of colored rice, and have occupied a place.

It is known that 70% of human food comes from the endosperm of gramineous crops. The endosperm of these crops is composed of aleurone layer and starch endosperm. In this kind of crop rice, people usually eat polished rice. The germ layer and bran layer are removed by processing, leaving only endosperm. Therefore, the nutritional value of milled rice is determined by rice endosperm, which is mainly composed of starch (70-80%), and the proportion of protein and lipid is very small, about 7-10% and less than 1% respectively [8]. Besides the low level of lipids, minerals, vitamins and other micronutrients in milled rice, rice bran is also rich in polyunsaturated fatty acids, crude fiber and anthocyanins. Brown rice is relatively more nutritious, containing more lipids, minerals, vitamins, dietary fiber, micronutrients and bioactive compounds, especially in the outer layer of brown rice, which is rich in fiber, vitamins, minerals and amino acids. Therefore, all rice consuming countries should advocate increasing brown rice intake. Intervention and epidemiological studies have shown that eating more brown rice can reduce the risk of chronic diseases such as cardiovascular disease, obesity, type II diabetes and some cancers [9], which is conducive to people's health.

The consumption of brown rice is very important for comprehensive dietary nutrition and greatly improves the nutritional value of this staple food. However, after rice polishing, almost all major nutrients (except protein) were significantly reduced, including fat, dietary fiber, vitamin B, iron, calcium and bioactive compounds such as gamma aminobutyric acid (GABA). Although a small number of health conscious people advocate brown rice, consumers' acceptance of brown rice is hindered by its relatively poor texture. Rice is not only a staple food, but also a source of essential nutrients or bioactive compounds through appropriate genetic modification.

Because of the great difference in nutritional components of different rice components, the improvement of rice structure is expected to significantly change the nutritional quality of rice. With the rapid development of biotechnology, several bio enhanced rice germplasm resources have been developed, such as "golden rice" (GR) rich in β-carotene, folic acid fortified rice, purple endosperm rice rich in anthocyanins and astaxanthin enriched rice [8]. In general, each bioaugmentation solves only one individual's deficiency. However, there are also some examples of multi nutrient bioaugmentation in rice. For example, Singh et al. Reported that bio fortified rice, which is rich in iron, zinc and beta carotene, is used to solve the problem of micronutrient deficiency in similar populations around the world. Another representative example of multiple bioaugmentation in rice is the study of giant embryo black rice derived from the cross between black rice and giant embryo mutant rice. In addition, by using mutagenesis, MAS and other conventional technologies, we have also successfully obtained bio fortified rice resources that can be used as functional food, such as giant embryo rice of hypertension patients, high Rs of diabetic patients, iron and zinc rich rice of poor people, etc. one by one successful cases have closely linked the nutritional quality of rice with human health.

Rice nutrition is also affected by the external environment. Rice is the most important food in the world, which provides more calories than any other food. With the rise of carbon dioxide in the global atmosphere, the environmental conditions under which we grow rice are also changing. Rice grown at this level of carbon dioxide will reduce the content of nutrients such as vitamin B, thiamine and folic acid, which are essential for the normal function of the nervous and cardiovascular system by 17-30% [10], Therefore, we must protect the environment to avoid the loss of unnecessary rice nutrition, in order to be more conducive to human health.

3. Analysis of the Factors Affecting the Grain Nutritional Quality in Rice

The nutritional quality of rice is closely related to human life and is regulated by a variety of influencing factors, which can be roughly divided into two types: one is the agricultural intermediary effect, including pesticides and fertilizers, soil type and soil quality, as well as light, water and carbon dioxide in the atmosphere; the other is post

harvest processing, including cooking, milling and storage. The nutrient content of rice is also controlled by abiotic stress factors such as salinity, drought and extreme temperature. Studies by the International Rice Research Institute (IRRI) have shown that there may be significant differences in nutrient content and composition among different varieties. Experiments in controlled environments have shown that sunlight, soil nitrogen, plant maturity and fertilizer application change protein, iron and zinc content.

The development cycle of rice includes vegetative period and reproductive period. The change of oil-water tension in reproductive period has the most significant effect on the content of trace elements in rice grain. Different chemical fertilizers directly affect the nitrogen content of rice to a great extent, which makes the detected trace elements in rice different. There is a significant positive correlation between the nitrogen content of rice and the amount of nitrogen fertilizer used. The lower the nitrogen content of rice, the lower the protein content of rice. The use of different fertilizers can also lead to differences in soil types and soil quality. Because rice roots absorb nutrients from soil for their own growth and development, irrigation methods are also particularly important. The dry cultivation technique of rice covered with plastic film has the characteristics of high efficiency, water saving, fertilizer saving, high yield and high quality. Intermittent Flood irrigation (also known as AWD wet dry alternate method) not only represents a good environment for rice production, but also helps to improve the quality and safety of grain. Irrigation and management of rice are also related to the concentration of arsenic, lead and cadmium in rice seeds. Soil aeration can reduce the content of arsenic and keep the content of lead and cadmium at an acceptable level. The increase of water stress in vegetative period not only increased the contents of Cd and Cu, but also decreased the absorption of Mn and Co.

Rice cultivation requires light, water, temperature and air. The increase of carbon dioxide in the air reduced the contents of protein, micronutrients and vitamin B in rice. Compared with rice grown under conventional CO_2 concentration, in rice with increased CO_2 concentration, vitamin B1 decreased by 17%, vitamin B2 decreased by 17%, vitamin B5 decreased by 13%, and vitamin B9 decreased by 30%. According to reports, zinc decreased by an average of 5%, iron decreased by 8%, and protein decreased by 10%. In terms of temperature control, the chalkiness of rice cultivated at high temperature are higher, and genes related to starch biosynthesis, grain filling and starch granule structure will hinder chalkiness [11, 12]. Granular chalk determines the quality and price of grain [13], resulting in poor grinding, cooking, edible and nutritional quality [14]. Based on these observations, genes that controlling grain chalkiness, including *qPGWC-8* [15] and *qPGWC-7* [16], have been finely mapped. However, the mechanism of grain chalkiness remains a mystery. A new chalky locus *qtlqpgwc-2* was discovered between C63 and C563 [14]. *Chalk5* is also a major chalkiness gene, which increases chalkiness of rice and reduces HRR of rice [17]. Previous studies have also shown that in extremely hot weather, α - amylase is activated during the development of rice seeds and leads to the loss of rice quality by blocking the accumulation of stored starch in endosperm. Therefore, temperature control during cultivation is very important.

In the late harvest period of rice, storage, processing, washing and cooking methods will affect its nutritional value. Indirect nutrition loss in the later stage of rice harvest is a qualitative and quantitative loss in rice, which will directly affect food safety. After rice harvest, 65% white rice, 10% bran and 25% shell can be obtained. The rice was dehydrated and milled to eliminate the inedible shell, so as to obtain shelled rice. Hulled rice or brown rice consists of 2-3% embryo, 90% endosperm and 6-7% bran. Further elimination of bran can produce white rice. In short, as the bran layer is further removed, more minerals and vitamins are lost. For example, washing rice before cooking loses 2-7% protein, 11-26% riboflavin, 22-59% thiamine, 20-60% niacin and 20-41% potassium. Cooking with excess water will lose more than 25% of riboflavin, thiamine and niacin. Indian rice loses 50% calcium and phosphorus, 10% protein and 75% iron during cooking. High temperature fried rice will lose more than 50% thiamine. It is known that increasing the levels of iron, zinc and provitamin in the endosperm of rice can reduce these nutrient deficiencies, especially among the rural and urban poor who do not have access to rich food and a diversified diet. Therefore, it is necessary to improve the nutritional quality of rice through traditional breeding or new biotechnology methods.

4. Genetic Improvement Strategy of Grain Nutritional Quality in Rice

The nutritional quality of rice is mainly determined by starch content, protein content, amino acid content, lipid content and trace element content. The rapid development of genetic breeding provides a new genetic improvement strategy for improving the nutritional quality of rice.

4.1. Genetic Improvement of Grain Starch in Rice

As the most important staple food for human beings, rice has a starch content of up to 90% in its grains, which can provide humans with 40% of the caloric intake and is the number one nutrient in rice. Starch can be divided into amylose and amylopectin because of the different connection of glycosidic bonds. However, they are eventually digested and absorbed by human body in the form of glucose, providing energy for various life activities of human body.

Amylose in the endosperm of rice is synthesized by combining starch granules encoded by *Wx* gene with starch synthetase (GBSSI). Amylose content was positively correlated with hardness, and negatively correlated with viscosity and water absorption. However, the rice with high protein content generally has higher hardness, which shows that the nutritional quality of rice is also related to the amylose content. The operation of enzymes involved in starch biosynthesis pathway is often used to improve rice quality traits. For example, enzyme AGPase is the rate limiting enzyme in this pathway [18]. By down-regulating (antisense or RNAi methods) the expression of enzymes involved in the production of amylose, thereby leading to the production of amylose [6]. The multi-gene method is

adopted to make the grain larger and better appearance [19]. α - amylase is an essential starch hydrolase for seed germination of cereals. It is also expressed in mature stage. Because inactive or heat-labile α-amylase is more suitable for cultivating high-quality rice, it combines error-prone PCR random mutagenesis and single filtration activity test of recombinant enzyme expressed in E. coli, filter paper reactive dyeing method can be used for high-throughput screening of temperature sensitive and inactivated mutation of a- amylase in rice, so as to improve rice quality. Professor Liu Qiaoquan's research team edited the key cis acting elements on rice *Wx* gene promoter to regulate gene expression through CRISPR / cas9 technology, and created a number of new *Wx* alleles that can fine tune amylose content, providing new germplasm and new methods for high-quality rice breeding.

4.2. Genetic Improvement of Grain Protein in Rice

Rice contains a large amount of storage protein, which is the second major nutrient in rice after starch. The protein content of different varieties of rice varies between 5% and 16%. According to the solubility and different extraction methods of storage proteins in rice seeds, they can be divided into four categories: water soluble albumin; dilute hydrochloric acid globulin; alcohol water mixture soluble gliadin and dilute acid or alkali soluble glutelin. Glutelin is the most easily digestible protein among the four proteins, and it is also the most abundant protein in rice seeds. Recent studies have shown that the value of rice protein is mainly reflected in its hypoallergenicity, no pigment interference, soft and nonirritating taste and high nutritional value, and can play a role in determining the gelatinization and texture characteristics of rice [14]. Therefore, making full use of and improving the use value of rice protein is also very important to improve the nutritional quality of rice.

Rice protein is rich in essential amino acids, especially lysine, which is significantly higher than other cereals. Due to the low content of crude fiber and tannin, and the biological value of protein is related to the concentration of lysine, the truly digestible protein in rice protein is higher than that in other cereals. The limited nutritional value of rice seed protein is mainly due to the deficiency of some amino acids, such as lysine and tryptophan [6]. The protein content of polished rice seeds in the most common rice varieties is about 5-7%, of which gluten accounts for about 70-80% of the total protein of seeds [20]. But the higher the protein content, the worse the taste [21]. Since most people in South Asian countries take rice as their staple food, many attempts have been made to increase the concentration of its nutrients to meet people's nutritional needs.

The research team of academician Wan found a rice protein transport mutant named *gpa4*, in which 57 kDa glutenin precursor was accumulated excessively, forming two abnormal endoplasmic reticulum derived structures, *gpa4* encodes an evolutionarily conserved membrane protein GOT1B. GOT1B interacts with the vesicle component *Sec23c* of COPII to regulate the formation of COPII vesicles, which in turn regulates the endoplasmic reticulum output of gluten [22]. Through positional cloning, Peng et al. cloned the *OsAAP6* gene, which encodes an amino acid transport protein that has a positive regulatory effect on the content of four storage proteins. *OsAAP6* is a positive regulator of grain protein (GPC) and can significantly increase The total amount of amino acids affects the formation of protein bodies through amplification [13]. Chen et al. Identified many known and unknown QTLs through genome-wide association analysis of storage proteins in 527 cultivars. They analyzed the different haplotypes of the cloned protein genes *OsAAT2*, *RA17*, *RM1* and *Rp6* and the effects of the cloned starch metabolism-related genes AGPS2a, ISA2, PUL and Waxy on the content of various storage proteins [23]. It is generally believed that environmental conditions have great influence on PC. Many of the identified QTLs for PC and PI were consistent with previously reported QTLs, which enhanced the recognition of PC genetic components.

4.3. Genetic Improvement of Amino Acids in Rice

Rice is rich in 8 kinds of essential amino acids, and its content is balanced. It has a great effect on the health of hair and skin, the improvement of eyesight, the nutrition of heart and lung, the improvement of nervous system and brain function. The order of the content of various amino acids is basically similar in different varieties. Glutamic acid and leucine are higher, and tryptophan and methionine are lower. The contents of various amino acids increase and decrease correspondingly to different degrees with the increase or decrease of rice protein content. Amino acids are not only the components of soil nitrogen that plants can use directly, but also the most important form of nitrogen redistribution in plants. Rice protein lacks lysine, threonine, tryptophan, and sulfur-containing amino acids, especially lysine as the first limiting amino acid. Besides the low lysine content, the threonine content in rice is very low. It has been shown that the increase of threonine content is closely related to the regulation of lysine content, and many attempts have been made to achieve this feat. Through synthetic biology methods, Two genes were synthesized by combining endogenous rice gene with lysine (k) / threonine (T) motif (TKTKTKK). They are named *TKTKK1* and *TKTKK2*, and their respective proteins contain 73.1% and 83.5% lysine and threonine. Compared with wild rice, the contents of lysine (33.87%), threonine (21.21%), total amino acids (19.43%) and crude protein (20.45%) were significantly increased after these two genes were introduced into rice genome independently.

The key gene *OsLHT1*, which is responsible for the absorption of amino acids in rice roots, was screened by analyzing the association between the whole genome amino acid transporter gene and the uptake rate of 15N labeled aspartic acid by *indica* and *japonica* rice roots. The loss of *OsLHT1* function led to a 40-66% reduction in the absorption of amino acids by roots, and a significant reduction in the distribution and transportation of amino acids synthesized by roots to the shoot, which significantly affected the growth and development [24]. The essential amino acids of rice can be increased by knocking out some storage proteins, and then increasing the contents of other storage proteins by proteome rebalancing. Taiji*, et al.* [25], constructed transgene knockout lines of glutelin, globulin and gliadin, and found that the lysine content of transgenic lines without 13kda gliadin increased by 56% compared with that of wild type. Maize lysine ketoglutarate reductase / saccharin dehydrogenase (LKR / SDH) is a bifunctional

lysine degrading enzyme, mainly accumulated in endosperm [26]. Under the control of embryo-specific glb-1 promoter, inhibition of LKR/SDH by RNAi resulted in a 10-fold increase in free lysine content in corn grains compared to the control [27]. The same technique of down regulating LKR/SDH was applied in rice, and the free lysine level was also increased by 10 times compared with wild type [28]. Ying, *et al.* [29] Developed a high free lysine (HFL) line through polymerization feedback insensitive overexpression lines [29]. At the same time, several substitutes (Leu28, Asp112, Cys149, Trp201, Asp204, Gly295, Leu300 and Cys342) which lead to the loss of activity of amino acid residues were also identified, as well as a variety of heat sensitive substitutes Asp83, Asp187 and Glu252 [12]. Therefore, the content of lysine is very important to the protein content of rice. The regulation of lysine level by molecular biology technology can significantly affect the protein content of rice, and effectively improve the nutritional quality of rice.

4.4. Genetic Improvement of Lipids in Rice

Rice is one of the important cereal crops, known as the main food resources of human beings. There is no evidence that transgenic rice with higher oil content can be produced. In this regard, it is mainly controlled by three genes, including *WRI1*, a transcription factor that regulates several genes involved in lipid production, *Ole*, which encodes oil body protective protein, and *DGAT1*, which is the key control factor in the last step. At present, the use of metabolic engineering methods to increase lipid content in different parts of plants (including leaves and stems) can be considered as an innovative platform to obtain more energy. *WRI1* is one of the important transcription factors. It regulates the fatty acid biosynthesis network and the accumulation of TAG by balancing the carbon flux between carbohydrates and lipids. In addition, the oleosin-encoding gene (*Ole*) can protect TAG from degradation by being encapsulated in oil bodies. In the current study, four important TAG assembly and protection genes (*AtDGAT1* and *AtPDAT*, *AtWRI1* and *AtOle*) were overexpressed in rice under a constitutive promoter. Compared with control plants, transgenic seeds showed a significant increase in TAG content (P≤0.05) reached 26%, and the content of oleic acid and palmitic acid increased significantly by 28% (from 32% to 41%) and 27% (from 11% to 14%) respectively. In metabolic engineering lines, the significant increase in total grain and leaf oil content is aimed at increasing the oil content and energy density of seeds and nutrients.

4.5. Genetic Improvement of Trace Elements in Rice

Rice minerals contain iron, zinc, copper, selenium four trace elements, rice also provides phosphorus, potassium and other nutrients to improve blood circulation, maintain water balance and internal harmony. Rice contains vitamin B1, B2, B3, B4, B6, B7, B9 and vitamin E, but does not contain vitamin A, vitamin C and vitamin D. The lack of iron, zinc, selenium and other basic mineral elements can lead to "hidden hunger" of human beings, and more than half of the world's population has varying degrees of mineral malnutrition [30]. In order to improve this phenomenon, genetic improvement of trace elements in rice is particularly important.

Rice contains a variety of nutrients, but may also contain heavy metals (arsenic, cadmium, lead and mercury), pesticide residues and allergens and other toxins. Arsenic and chromium are the main toxic elements found in rice. Arsenic mainly exists in the form of arsenite, and cadmium exists in soil in the form of cadmium sulfide, cadmium carbonate or Cd^{2+}. Their absorption and migration mechanisms overlap with those of iron and zinc. At the same time, arsenic can also reduce selenium content in rice. Ishikawa et al. Proposed a method to simultaneously reduce cadmium and arsenic in Rice by using *OsNRAMP5* gene mutant. After AWD treatment, the milled grains of *OsNRAMP5* mutant plants did not contain cadmium, and the arsenic content decreased by 27% compared with the control [31]. It can be seen that arsenic is easy to be washed off after grinding, which also provides a favorable way to reduce arsenic content.

In the process of rice milling, because most of iron is stored in aleurone layer, the iron content in rice will be significantly reduced during rice milling. Using Soybean Ferritin Gene, *glub-1*, an endosperm specific promoter, was used to increase iron content in rice. Compared with the untransformed seeds (11.2 ± 0.9 mg / g DW), the iron content increased three times (38.1 ± 4.5 mg / g DW). Another way to alleviate iron deficiency is to introduce metal chelators such as nicotinamide and ephedrine [32, 33]. Nicotinamide (NA) is also a metal chelator, which is involved in the transportation of metals in plants. Overexpression of *OsNAS1* in endosperm significantly increased the concentration of Na and Zn, but the concentration of Fe did not. Lee and his colleagues found that iron and zinc contents in rice seeds were significantly increased by overexpression of Na synthase genes *OsNAS2* and *OsNAS3* [32] . Johnson et al. Also obtained *OsNAS2* overexpression lines containing 19 ug/g Fe in polished grains through constitutive overexpression of *OsNAS* gene family. In *OsNAS2* system, the zinc concentration also increased by two times [34]. Therefore, the increase of iron content often has a positive synergistic effect with zinc, which can achieve better nutritional effect by adding iron.

Selenium is also one of the essential micronutrients for human body. It plays an important role in enhancing human immunity, preventing cancer and resisting various diseases. Nearly 70% of China's population live in the soil selenium deficient areas, so it is urgent to supplement selenium scientifically. Rice is the main food crop in the world, so it is very important to research and develop selenium rich and safe rice. The results showed that low concentration selenium treatment (< 5 mg/kg) promoted rice photosynthesis, enhanced rice antioxidant capacity and increased rice yield, but high concentration of selenium would cause toxicity. NO through promoting rice growth and photosynthesis, increasing antioxidant capacity, down regulating the expression of *OsPT2*, *OsSAMS1* and *OsSBP1* genes in root system, down regulating the expression of *OsPT2*, *OsCS* and *OsSBP1* genes in shoot, and reducing the absorption and accumulation of selenium in rice, so as to effectively increase the resistance to selenium stress of rice and reduce the high selenium toxicity [35]. At present, a new rice breeding material with low cadmium

and high zinc, low cadmium and High Selenium rice has been created through molecular marker assisted screening. It can be seen that the addition of selenium has been paid great attention to in the cultivation and breeding of rice, and achieved good results.

In addition, vitamin A deficiency is a worldwide problem. Ye et al. Cultivated Jinmi 1 with carotenoid content of 1.6mg/g by expressing Narcissus plant ene synthase gene, bacterial plant ene desaturase and lycopene β-cyclase in endosperm [36]. Paine et al. transformed rice with maize lycopene synthase gene instead of Narcissus, and cultivated Jinmi 2 with total carotenoid content of 37mg/g. Gayen et al. solved the problem of carotene degradation during storage by regulating the expression of lipoxygenase gene [37, 38]. Vitamin A deficiency has been greatly improved by transgenic technology, and it also provides a new idea for the addition of other low content vitamins.

Similar to trace elements, the contents of anthocyanins and proanthocyanidins in rice are very little. It is known that the anthocyanin synthesis in leaves is significantly different between wild rice and cultivated rice. The vast majority of modern cultivated rice have no anthocyanin accumulation in leaves and other vegetative tissues, while *O.rufipogon*, the ancestor of Asian cultivated rice, generally has anthocyanin accumulation in vegetative tissues, including leaves [39]. Anthocyanins are water-soluble pigments with high antioxidant activity. Anthocyanin synthesis is controlled by three dominant genes: C (chromogen), A (activator) and P (tissue specific regulator). C and A are the basis of anthocyanin pigment formation, while P is the tissue-specific regulator of C and A. Anthocyanins are synthesized by various transcription factors under the catalysis of PAL, CHS, CHI, F3H, DFR and ANS. Procyanidins are a group of polymeric phenolic compounds, which can be obtained by using organic solvents as extractants. They have high degree of polymerization and acylation. They are formed by ANR and LAR catalysis on the basis of anthocyanin synthesis and metabolism. They are considered as natural antioxidants.

Proanthocyanidins and anthocyanins in rice exist in the pericarp of black rice and red rice, respectively. Red skin is controlled by *Rc* on chromosome 7 and *Rd* on chromosome 1. When *Rc* has functions, the peel is brown; when *Rd* has functions, the peel has no color; but when both *Rc* and *Rd* have functions, the peel is red. The black grain of rice is determined by three dominant genes, namely *kala1* (*Rd*), *kala3* and *kala4* (*OsB2*). *Rd* is the anthocyanin synthesis gene, *kala3* is the R2R3Myb transport factor, and *OsB2* is the bHLH transcription factor, corresponding to the determinants of anthocyanin biosynthesis P, C and A. Anthocyanins and procyanidins belong to flavonoid polymers, because of their strong oxidation, they enhance the resistance of plants to biological stress and abiotic stress, and participate in scavenging free radicals in animals, which are beneficial to human health by reducing the risk of some cancers, cardiovascular diseases, diabetes and other chronic diseases [40]. The existence of anthocyanins and procyanidins is very small. In order to play a real role, it is necessary to determine their genes and transfer them into rice to obtain new germplasm with stable inheritance.

Genome-wide association study (GWAS) technology was used to identify the determinants of rice leaf anthocyanin synthesis, *OsC1* and *OsRb*, and found that the genome segment where these two genes are located has been significantly artificially selected. In addition, *OsC1* and *OsRb* were found to be leaf tissue-specific regulators, which did not affect the synthesis and accumulation of anthocyanins in the seed coat of black rice varieties [39]. Anthocyanins in rice are only present in the pericarp, not in the endosperm, so they are lost in highly polished rice. Zhu et al. Constructed a construct containing eight anthocyanin related genes (two regulatory genes from maize and six structural genes from Coleus), driven by endosperm specific promoter, as well as a selection marker and a gene for marker excision and transformation of rice. In this study, we developed a new generation of efficient multi gene vector system TGS II (transgene stacking II) by using Cre / loxP recombination system and the newly created irreversible mutant loxP site. Through the use of this system, eight key genes related to anthocyanin synthesis were successfully transferred into rice, realizing the specific synthesis of anthocyanins in rice endosperm, creating the first new rice germplasm "Amethyst rice" rich in anthocyanins and antioxidant activity [41]. Therefore, colored rice is favored by consumers.
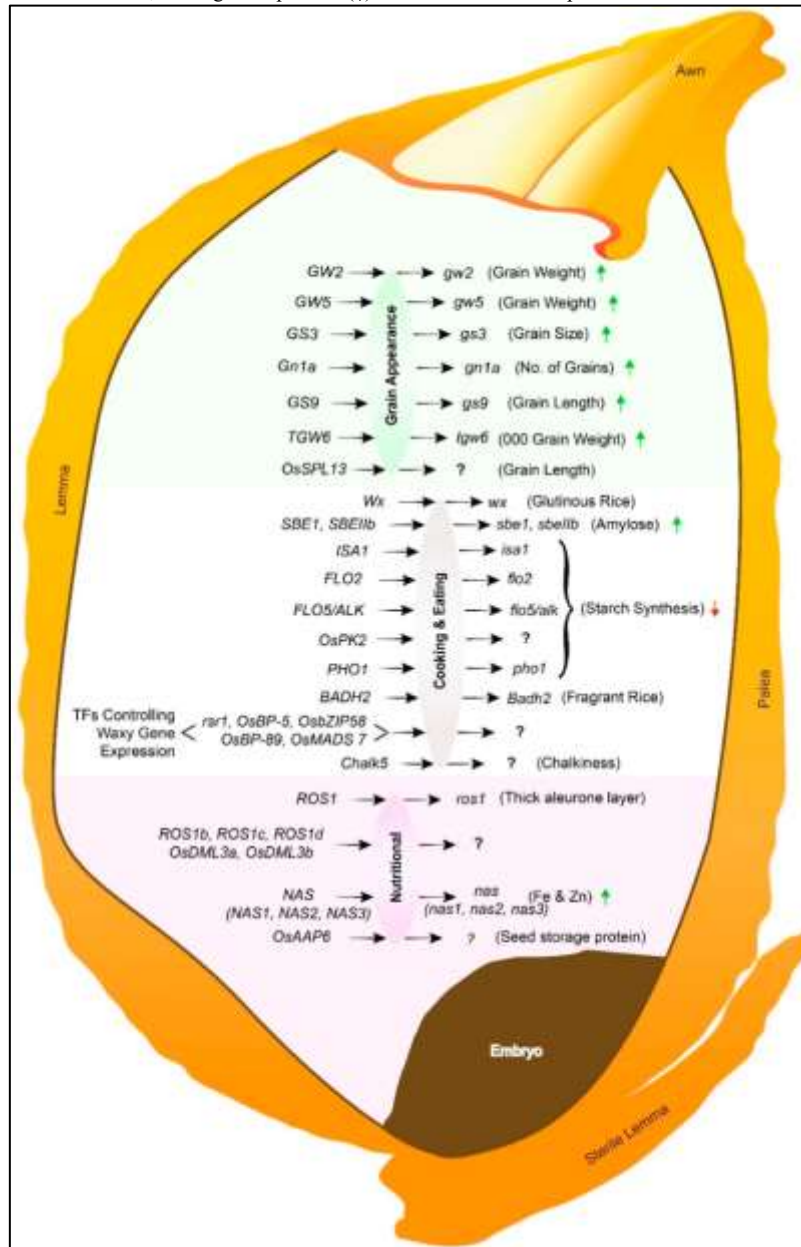
4.6. New Measures of Genetic improvement and Cultivation

The emerging genomic technology can provide a new way for rice breeding through the identification of various alleles, and greatly promote the nutritional improvement of rice. Through conventional breeding or genetic breeding, all important alleles can be hybridized to one or more alleles. However, compared with traditional breeding or MAS, genome design and whole genome selection based on backcross progeny are two major components of genome breeding, which are characterized by high throughput, accuracy and time saving. At present, some researchers are committed to the cultivation of green super rice, using genomics technology and genetic resources to cultivate higher quality rice. Based on the genetic, functional and phenotypic information of genes, high-throughput detection of DNA polymorphism is used as a detection method, and selection is made at the whole genome level according to breeding objectives, nontarget genes and genetic background, so as to obtain high-quality traits [42]. The newly discovered CRISPR / cas9 system opens up a new way for life science research through reverse inheritance of related protein (cas9) (Fig. 2) [43]. At first, genome editing was mainly based on the mechanism of DNA double strand break and nonhomologous end joining. Therefore, some negative genes can be knocked out by gene editing, thus weakening the synthesis or accumulation of target nutrients in the required organs. For example, the inhibition of CRISPR / cas9 on SBEIIb resulted in the increase of RS content in rice.

Heredity is controlled by environment and gene. Based on the influence of genetic factors, the breeding of excellent varieties needs continuous hybridization and backcross to aggregate a large number of favorable genes, After generations of breeding, some relatively stable traits can be obtained, so as to screen out good rice characters and keep the excellent characters stably, This also laid a genetic foundation for our future genetic modification. With the different needs of people, more and more new rice has been developed and used, which has been affirmed by

researchers. For a long time, because of its high decomposition rate, RVA profile can screen out rice with soft texture and good eating quality, which has been favored in rice quality breeding [44]. The black rice with the highest nutritional value is taken as an example. The peel and seed coat are rich in anthocyanin, phenolic acid and other antioxidant substances, which are more nutritious than pure rice. Through agrobacterium tumefaciens mediated transformation of rice, specific synthesis of anthocyanins in endosperm of Japonica Rice (ZH11) and indica rice (HG1) was realized, and purple endosperm rice "Amethyst rice" with high antioxidant activity was created for the first time [41]. Due to the successful cultivation of golden rice and the recognition of many people, selenium rich rice is also in full swing, and according to people's pursuit of nutritional value, the corresponding rice rich in trace elements is also in continuous research.

Fig-2. Gene mutation is related to rice quality, potential genes for improving grain quality of rice varieties through CRISPR / cas9 editing; red down arrow (↓) indicates decrease of traits, while green up arrow (↑) indicates increase / improvement of traits when their genes are mutated [43]

Traditional breeding is mainly through cross and backcross breeding, the test cycle is long. Compared with traditional breeding, gene editing technology can improve rice quality efficiently and quickly for multiple quality traits, and accelerate the breeding process of high-quality rice varieties. Liu chunming research group of Institute of plant research of Chinese Academy of Sciences, in cooperation with the Australian Federal scientific and industrial organization, has established a semi grain seed screening system, and used this system to screen nearly 30000 rice seeds, and obtained a rice line ta2 with thickened paste layer, The aleurone layer increased from 1 layer of wild type rice cells to 4-10 layers, which improved the nutritional factors of rice such as protein, fatty acid, vitamins, trace elements and dietary fiber. For example, compared with ordinary whole rice, zhongzi 4 increased by 13% in total protein, 16% in total fat, 55% in iron, 5.5% in zinc, 94% in dietary fiber, 56% in vitamin A, 80% in vitamin B2 and 550% in vitamin B6, it is the only fully nutritious rice in the real sense. This is a new way to improve the nutritional quality of rice for the first time in the world, and provides a new genetic material for breeding high nutritional rice. Through gene cloning, the researchers found that the aleurone layer thickening phenotype was caused by a dominant

negative mutation of a DNA demethylase gene *OsROS1*. The research group also obtained a batch of new materials with *OsROS1* gene allele mutation through the rice TILLING platform independently developed, and their nutritional quality was improved to varying degrees [45]. Some excellent traits can also be introduced into black rice and purple rice. Now, a new rice variety "kefujing 9" with "high yield and good taste" has been bred by using molecular design breeding technology to "assemble" the control genes of high yield and good quality.

Cultivation technology has no direct relationship with the nutritional quality of rice, but good cultivation methods can make each quality of rice relatively improved, so as to meet the needs of people for rice. In the process of cultivation, some pests can also improve the nutritional quality of rice. Research shows that insects are very smart. They not only know how to "eat", but also make the food more "nutritious and delicious" [46]. The rice with *CYP71A1*gene knockout is "rejected" by pests. However, after ingesting more serotonin, the growth and development of the insect are accelerated and the body is more "robust", which is particularly obvious in the body of the borer.

5. Challenges and Prospects of Rice Nutrition Quality Improvement

At present, people's demand for food is more and more close to energy, nutrition, delicious and fun. Moreover, the nutrition concept has been deeply rooted in the hearts of the people. The requirements for nutritional quality are higher and higher, and more attention is paid to food safety. We are in an era of rich and diverse food, but rice, as one of the staple foods, still occupies a large consumer market. Consumers prefer rice varieties with high whiteness, strong aroma, low bitterness, compact texture, soft and non sticky [3]. It is known that *OsBADH2* is an aroma determining gene encoded by *frg* gene, which mainly encodes BADH protein. It inhibits the synthesis of 2AP by consuming precursors of 2-AP, 4-aminobutyraldehyde, thus making rice lose its flavor. However, as a genetic trait, aroma is still too complex to be used in genetic research and breeding. Considering the daily nutritional needs of human beings, rice is not complete in nutrition.

In rice breeding, it is difficult to combine quality, yield and abiotic and biological resistance, and many high-qualities rice also have corresponding defects. It is known that many molecular markers have been developed for rice breeding. The contents of vitamin A, zinc, iron, anthocyanins and lysine can be increased by transgenic breeding, and the contents of heavy metals and allergens can be reduced by mutation breeding. Mutation is considered to be the ultimate cause of variation. Without mutation, plant breeding is impossible. In order to start the breeding program, breeders must find a suitable genotype from the existing germplasm resources. If the required genes cannot be found in nature, they must create variation, mutation breeding is a process of producing new alleles. Once a suitable mutant allele (whether natural mutation or target induced mutation) is determined, the whole genome selection technology based on DNA microarray can integrate the target allele into the required receptor varieties accurately and efficiently. At present, through the research of functional genomics, rice can be improved by T-DNA incorporation, transposon labeling and chemical mutagenesis (knockout, overexpression, ectopic expression).

Nutritional quality is very important, but it is the appearance quality of rice that restricts the consumption and produces benefit. Therefore, it is necessary to unify the appearance quality and nutritional quality. Rice quality is a complex quality trait. Appearance quality is reflected in the ability to attract customers, including color, shape, chalkiness and transparency, which are mainly determined by grain shape and endosperm transparency (or chalkiness)[47, 48]. Rice grain shape related traits include grain length, grain width, grain thickness and length width ratio [4]. Chalkiness can be divided into white belly, white nucleus and white back according to their position on or inside the endosperm. The grain length and grain width of cultivated rice are mainly determined by *GS3* and *GW5* [41] , in addition to *GS3* and *GW5*, it also includes *GS2/GL2*, *GL3.1*, *GS5*, *GW2*, *GW8*, *GL7/GW7*, *GLW7* and *GL3.3* [49-54]. Through the analysis of the mechanism of gene action, five regulatory pathways have been revealed, among which genes such as *GW5*, *D2*, *D11*, *TGW6* and *TGW3* participate in plant hormone pathways to regulate grain morphology [29, 49, 55]. *LGY3* plays a role in the G protein pathway by interacting with *DEP1* and *GS3* [56]. In order to meet the needs of consumers, we can edit and improve the genes controlling the appearance of rice while improving the nutritional quality, so that it can complement the nutritional quality and create higher economic benefits, which had become a problem that researchers need to pay attention to. With the continuous improvement of people's living needs, the comprehensive quality of rice will eventually be put on the agenda.

The genetic improvement of rice nutritional quality is facing new challenges: (1) the genes controlling the formation of black rice pigment are cloned and confirmed at the molecular level. (2) High nutritional quality rice was selected by molecular marker technology. (3) To develop new rice with multiple functions. (4) All kinds of good quality of rice should be gathered together to make it more economical. According to the current evolutionary point of view, some transgenic plants from rice are not safe for the environment, and some transgenic plants without major selection advantages may cause some or potential environmental problems. However, it is very important to improve the understanding of the factors affecting the nutritional components of new varieties. These newly developed rice varieties should be evaluated for environmental benefits and risks such as total nutrition test, impact on human health, environmental assessment and public accessibility, so as to determine the criteria for considering the impact of new rice varieties.

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