



Research Status and Genetic Improvement of Important Characters in Purple Rice

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Abstract

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. Purple rice, as a special rice with important nutritional and health functions, has important scientific significance and application value for genetic improvement of purple rice and breeding of new varieties through in-depth study on its agronomic and related quality traits and products processed, and analysis of its genetic basis. In this paper, the new progress of purple rice in agronomic traits, quality traits, environmental impacts on purple rice and genetic basis of purple rice seed coat in recent years were reviewed and analyzed, and the application prospects of purple rice processing products and purple gene in rice genetic improvement were also prospected. Thus, our results will provide important information and reference for breeding new purple rice varieties with good quality and high yield.

Keywords: Purple rice; Quality traits; Heredity; Agronomic traits.

1. Introduction

Rice (*Oryza sativa* L.) is the staple food of more than 3 billion people in the world and provides about 25% of its energy. It is one of the most important food crops [1-3]. For most residents in Southeast Asia, rice provides more than 35% of their energy [4, 5]. In the next 30 years, the world population is expected to grow at a rate of 25% and reach 10 billion [6]. Colored rice is widely found in its ancestor wild rice. Because colored rice is closely linked to easy setting and low germination rate, it is gradually eliminated in production, and ordinary rice is selected as the main cultivated rice [7]. With the improvement of people's living standard, the demand for high-quality rice is increasing [8]. Compared with ordinary rice, colored rice contains more protein and trace elements, and has higher nutritional value than ordinary rice. In recent years, colored rice has been more and more popular.

Purple rice is one of the oldest rice varieties in China. It has a long history of planting and many varieties. It has high economic value. The accumulation of anthocyanins in the seed coat of rice resulted in purple rice [9, 10]. Anthocyanins have the functions of antioxidant and free radical scavenging. They also have many health functions, such as reducing the activity of enzymes and resisting mutation. They can replace benzoic acid in food to synthesize preservatives and be used as nutritional fortifiers and food colorants. Therefore, the control of purple rice seed coat gene research and its application in breeding has been widely concerned by rice genetics experts and breeding experts.

The yield of rice in the second half of the 20th century has been significantly improved, but since the 1980s, the yield of rice has been stagnating [2]. At present, the nutritional status, climatic factors, ecological environment and cultivation methods all affect the growth and development of rice, and ultimately affect the yield of rice, and seriously affect the quality of rice. According to the biological characteristics of purple rice, optimizing its planting methods and exploring the best sowing date can maximize the yield of Purple Rice under the existing conditions. However, the development of purple rice is limited to a certain extent by its own characteristics, such as low germination rate and easy collapse of high stalks. With the sustained growth of the world population and the steady improvement of living standards, traditional rice breeding can not meet the strong demand for high-quality and high-yield rice [11]. Aiming at the problems of the development of the purple rice, people need to use the gene of excellent quality gene and lodging resistance as the foundation, combine the gene of high yield and ideal plant type, use the concept and technology of the molecular module to design and breed, and cultivate a new variety of the high-quality, high-yield and anti-reverse purple rice. Therefore, this paper focuses on the new progress in agronomic traits, quality traits, effects of environment on purple rice seed coat and genetic basis of purple rice seed coat in recent years, and looks forward to the application prospect of purple rice processing products and purple gene in rice genetic improvement, in order to provide reference for the cultivation of new purple rice varieties with high quality and high yield.

2. Important Agronomic Characters of Different Purple Rice

The written records of purple rice have been recorded for more than 1500 years, distributed in Yunnan, Guizhou, Guangxi, Guangdong, Fujian, Jiangxi, Hunan, Sichuan, Jiangsu and other provinces, mainly planted in middle and high altitude areas. Purple rice has a long history of cultivation, wide distribution, complex geographical environment and great climate differences, thus forming the diversity of purple rice varieties [12]. Purple rice has abundant variety resources, including ordinary rice, upland rice, sticky rice, glutinous rice (including *indica* glutinous rice and *japonica* glutinous rice), and early, middle and late rice. Among them, *indica* glutinous rice and *japonica* glutinous rice are the most common, followed by late rice, and *japonica* rice is rare [13]. There are some purple rice varieties with unique flavor, such as Yunnan purple waxy, fragrant purple waxy. At present, purple rice is Yunnan, Guangxi, Guizhou and Guangdong according to the size of distribution area and the amount of planting area. There are great differences in agronomic characters among different purple rice varieties Table 1 [14].

Table-1. Representational Purple Rice Variety Types

Category	Plant height	Thickness of culms	Tiller number	Leaf color	Leaf hair	Grain shape	Grain length
Viscous Type of Late Rice	Medium height	Thin	Medium	Green	dense	Ellipse	Medium
Indica waxy type of late rice	Tall	Thin	Medium	Green	dense	Ellipse	Long
Japonica glutinous type of late rice	Medium height	thick	Few	Dark green	Medium	Broadly ovate	Medium
Indica glutinous type of Upland Rice	Medium short	Thin	Few	Green	Sparse	Ellipse	Medium
Japonica glutinous type of Upland Rice	Medium short	Medium coarse	Few	Green	/	Broadly ovate	Medium
Indica Waxy Type of Upland Late Rice	Medium high	Thin	Few	Green	Medium	Ellipse	Medium

Purple rice is one of the ancient local varieties of rice. Its planting area is closely related to the local ecological environment and people's living habits. Purple rice is adapted to the climate conditions in mountainous areas, so it is planted more in mountainous areas and less in plain or hilly areas [14]. With the development of the new variety of hybrid rice, the planting area of the purple rice is gradually reduced, but a more mature industrial chain of the purple rice has been developed in the Mojiang County of Yunnan Province. Because of its unique light, heat, water, soil, climate and other resource conditions, and the north-to-back line passes through the unique location condition from the middle of the Mojiang County, it is very suitable for the growth of the purple rice of the Mojiang River. In 2007, Mojiang county was honored as the "hometown of purple rice in China". In 2009, Yunnan Mojiang purple rice obtained the "Mojiang purple rice" geographical indication trademark issued by the State Administration for Industry and commerce [15]. Therefore, the purple rice industry is relatively mature. At the same time, purple rice has a certain commercial value, and is worthy of extensive application and popularization. Through the combination of government, enterprises and farmers, the purple rice industry can be planned reasonably, so that the industrialization and integration of purple rice from planting to marketing can be realized, and then the rapid development of local agricultural economy can be promoted.

Among the purple rice investigated and collected, whether it belongs to the black waxy of indica rice subspecies or japonica rice subspecies, their performance is high pole, sparse grain arrangement on panicle, less grain number per panicle, lighter 1000 grain weight, lower yield and longer growth period, all of which are late ripening varieties in late season [12]. The common characteristic of purple rice is that the seed coat of rice is purple and black, except the seed coat, glutinous rice is milky white and sticky rice is white and transparent. So far, no varieties with purple seed coat and endosperm have been found. At present, the technology of genetic engineering has been used to breed new purple endosperm germplasm and develop new purple rice varieties with high yield. Therefore, from the point of view of popularization and development of purple rice, high-yield purple endosperm rice has broad application prospects and is expected to become the target of cultivation of new varieties of special rice in the future.

3. Development and Application of Purple Rice Quality Characters and Processing Products

The quality of rice mainly includes appearance quality, grinding quality, cooking and eating quality and nutritional quality. The most important index to measure the quality of the grinding and processing of rice is the ratio of the fine rice, the higher the rate of the fine rice, and the better the grinding quality. The important factor affecting the milled rice rate and milled rice rate of purple rice is sowing date [16]. The control of the sowing time is an important part of the improvement of the whole fine rice rate of the purple rice. In general, rice is cooked before eating, and can be used in a variety of methods such as fast cooking, pressurization, and steaming, depending on the consumer's preferences and expectations for sensory quality (aroma, taste, and texture). Pressurized rice cooker is widely used as a simple and rapid cooking method. It is noteworthy that the phenolic compounds in colored rice decreased by 54% after cooking in an electric cooker [17]. The traditional cooking method will greatly reduce the antioxidant activity in purple rice. Grains that come into contact with the edge of the container during processing may be subjected to a higher heat load than the grains inside the container, so it is more beneficial to use a larger electric cooker to reduce the surface area of rice. Especially in terms of commercial value, in order to increase the content of phenols and other water-soluble compounds in purple rice, it is necessary to carry out further research on the design of rice cookers and the physical and chemical properties of rice. In addition, the degradation of antioxidants is also related to the oxidative decomposition during storage [18]. Under certain storage conditions, the content of phenolic compounds will decrease after rice husk hulling, while the phenolic compounds [19], which accumulate as the main substance in the bran cortex, will be directly exposed to oxygen in the atmosphere. The

presence of oxygen can accelerate the degradation of phenolic compounds by direct oxidation mechanism [20], which will adversely affect the nutritional value of purple rice.

Colored rice varieties have higher protein content, relatively balanced amino acid composition, better glycemic index, and higher fat, fiber and vitamin E content [21]. Purple rice, as a kind of colored rice, contains water-soluble purple pigments in its seed coat, glume shell and leaves. Purple rice pigment is a compound flavonoids composed of anthocyanins and Mallow anthocyanins. It can change enzyme activity in human body, improve microcirculation, improve body immunity, antioxidant and anti-aging [22, 23]. Among them, the most antioxidant chemical component is anthocyanin. Anthocyanin is a kind of natural anthocyanin soluble in water, which has the ability of scavenging DPPH radical and is also lacking in rice. With the increase of anthocyanin concentration in purple rice, the scavenging rate of DPPH radical tends to increase gradually [24]. Due to the deterioration of the environment and the change of people's way of life, people pay more and more attention to their own nutrition and health. Purple rice contains strong ability to eliminate DPPH free radicals, which can effectively prevent atherosclerosis, coronary heart disease, hyperlipidemia, cancer and other chronic diseases [25]. Therefore, the improvement of the anthocyanin in the purple rice can greatly improve the nutritional value. One way to improve the nutritional value of purple rice is to identify rice genotypes that can accumulate higher levels of nutrients in endosperm. In view of this research direction, Zhu Qinlong [26] and others have used efficient transgenic system to make the biosynthesis of anthocyanin in the endosperm, and develop the "purple endosperm rice", which will meet the demand of people for higher quality rice to a certain extent.

The purple rice has a high economic value because of its many excellent characteristics, and has been widely used, for example, the purple rice is often used as an auxiliary material or a fortified food for the medicine. For a long time, purple rice has been used as a nutritional supplement for blood and bone in many areas of our country. In many areas, purple rice is used as a female postpartum lunar eclipse or as a gift to guests. As early as the Ming Dynasty, it was recorded in Compendium of Materia Medica that purple rice had the effects of tonifying qi, invigorating spleen and liver, nourishing yin and tonifying kidney, etc., and mainly treated body deficiency, night sweat and thirst. The clinical results of Guizhou Institute of traditional Chinese Medicine have confirmed that the wine made from purple rice or purple rice is very effective in the treatment of deficiency sweat, anemia, neuroweakness, chronic gastritis, food accumulation and so on. In many areas, purple rice is used as a single-to-one, for treating edema, anemia, and rice-rice-rice root-water treatment for treating deficiency and cough, and is used for external use for treating the allergic dermatitis of the macula. Therefore, purple rice mixed with a variety of different uses of drugs made of functional pharmaceutical wine, in the domestic and foreign markets more popular.

Nowadays, there are few deep processed products related to purple rice in the market, and the added value of the products is also relatively low, but the fermented food on the market is more and more favored by consumers. Therefore, the development of new fermented food with purple rice as raw material will have great development potential. Purple rice contains gamma-aminobutyric acid (GABA), GABA with diuretic, liver-invigorating [27], strengthening kidney, reducing blood pressure, enhancing brain function and long-term memory, relieving insomnia and other physiological activities [28]. As a component of new functional food, it will have a broad development prospect. As far as the diet of modern people is concerned, daily food intake can not meet the physiological needs of the human body for GABA [36]. In the previous study, GABA, ammonia ground state nitrogen, titratable acidity, and reducing sugar content in the process of using fungi to ferment purple rice increased continuously with the fermentation time [13]. Therefore, the use of safe filamentous fungi can develop more nutritious purple rice food, such as health food. These nutrients not only increased the physiological activity of purple rice, increased the nutritional value of rice, but also enhanced the taste of purple rice, and increased the digestibility of purple rice food in human body.

4. Effects of Environment and Cultivation Methods on Purple Rice

The yield of rice is not only determined by its own gene, but also has an important influence on the yield of the purple rice. The adaptive environment of different varieties of purple rice was different [13]. The types of upland rice are generally distributed in the hillside or foot slope with high altitude, low wind and gentle slope, and the suitable growth temperature is not more than 45 °C. The fertile land of the soil is more beneficial to the growth of land-and-rice. The growth of purple rice is not only affected by climate and ecological environment, but also by its cultivation methods. However, at this stage, there is no perfect cultivation system for how to increase the yield of purple rice. At present, there are the following imperfections in the cultivation system of purple rice: The main results are as follows: (1) The cultivation technology system of purple rice is lack of unified standard, and there are great differences in the cultivation process of different varieties of purple rice in different regions, such as seed use, growth period, seedling density and so on; (2) the best planting season of purple rice is not clear, and the planting mode of purple rice is one season a year in most areas, but in fact, the optimum planting time of different varieties of purple rice is different, and there are purple rice varieties suitable for planting in almost every season; (3) Current cultivation techniques can not effectively improve the yield and quality of purple rice, and exploring new cultivation techniques is a problem worthy of attention at present; (4) There is also a lack of the necessary national standards for large-scale marketing of purple rice [16]. Therefore, the establishment of scientific and rational cultivation system is beneficial to the application and popularization of purple rice.

The existing studies have shown that cultivation technology is one of the most important environmental factors affecting the yield of purple rice. The master of the cultivation techniques is often the most direct and convenient aspect from the control of the reasonable planting density, the sowing time and the application of nitrogen. Reasonable density can coordinate the relationship between individuals and populations, promote the organic

combination of various yield components, so that the yield can reach the maximum [29]. There is a great correlation between density and rice yield. The high yield population can be obtained by controlling the planting density of rice to increase its yield. The yield of rice increases with the increase of cultivation density, but decreases after exceeding the suitable density [30]. The selection of sowing date affected the growth period of purple rice. With the delay of sowing date, the whole growth period of purple rice was obviously shortened, and the environmental factors such as temperature and light in different stages of the growth process of purple rice were affected. Early sowing will lead to redundant growth period of purple rice, and the quality of rice tends to deteriorate. Therefore, it is of great significance to control the sowing date for yield and quality.

The type and dosage of the fertilizer have an important influence on the yield of rice to a certain extent. Purple rice belongs to special rice, and its yield level is low [31], and there are significant differences among different varieties [32]. Nitrogen is a necessary basic element for the synthesis of amino acids, amides, proteins, nucleic acids, enzymes, chlorophyll and other compounds, while phenylalanine ammonia-lyase is the key enzyme in the phenylalanine pathway, which is involved in the synthesis of a variety of phenolic compounds, including flavonoids and anthocyanins. The growth and yield of purple rice were affected by the application of nitrogen fertilizer. The yield and quality characters of purple rice samples were statistically analyzed by setting the proportion of nitrogen application rate with different gradients and organic fertilizer. It was found that with the increase of nitrogen application rate, the tiller, panicle formation rate, leaf area, material accumulation and yield of purple rice increased significantly [33]. Proper application of nitrogen fertilizer and organic fertilizer can increase the ratio of brown rice and milled rice, and the content of amylose in purple rice decreases with the increase of nitrogen application or the proportion of organic fertilizer. Therefore, planting purple rice should not only choose the suitable place according to the type of purple rice, but also master the appropriate cultivation technology. Therefore, reasonable close planting, proper sowing date and the amount of nitrogen fertilizer applied are beneficial to the improvement of yield and quality of purple rice.

5. Genetic Study on Purple Seed Coat of Rice

Although there have been some detailed reports on the inheritance of pigment in purple rice seed coat, the conclusion is not consistent because of the difference of genetic structure and color division standard of the tested materials. The deposition of anthocyanins in rice seed coat makes rice purple. The genetic results of different research groups on rice purple seed coat were not consistent, some studies suggested that rice purple seed coat was controlled by a pair of dominant genes, others showed that rice purple seed coat was controlled by two pairs of dominant complementary genes, and the results showed that rice purple seed coat was controlled by 3 pairs of genes and belonged to quantitative trait inheritance [34]. Further studies in the later stage showed that the purple seed coat of rice was a dominant inheritance controlled by a single gene, which was determined by the maternal genotype and was a typical delayed inheritance, and the glumes of purple seed coat and purple of rice were controlled by different genes.

With regard to the study of genes related to purple seed coat of rice, Hsieh found that purple seed coat was dominant and white seed coat was recessive character [35]. When the *Pb* gene is not present, the seed coat is white; when the *Pb* gene exists separately, the seed coat is brown; when the gene *Pb* and the *Pp* are simultaneously present, the seed coat is in the purple [36]. The *Pb* gene, which controls the color of rice seed coat, exists on chromosome 4 of rice. By co-isolation and analysis of polymorphism DNA probe and seed coat color, it was found that the gene was linked to DNA markers RG329 and RG214 on chromosome 4 of rice, and the genetic distance between the gene and RG329 and RG214 was 18.9 cM and 26.3 cM [37], respectively. However, some studies have also shown that *Pb* gene is located on chromosome 1 [38, 39]. In order to determine the exact position of *Pb* gene on chromosome, the *Pb* gene was carefully mapped and the candidate genes were analyzed in depth [40]. Furthermore, the *Pb* gene was located downstream of RM3820 molecular markers on chromosome 4. By designing high density InDel primers and CAPS primers in the candidate region, the *Pb* gene was carefully mapped within the range of 25 kb between two InDel markers RID3 and RID4 [40], indicating that *Pb* gene and *Ra* gene were the same gene, and it was speculated that the purple seed coat traits of rice might be caused by GT deletion in exon 7 of *Ra* gene. The results of CAPS marker analysis showed that purple seed coat gene could be cut open, but purple glumes could not be cut open. Sequencing results showed that purple seed coat material had GT deletion in exon 7 compared with white seed coat material [41]. Therefore, the CAPS molecular marker of purple seed coat gene can be used as a functional molecular marker for new purple rice varieties.

6. Conclusions

With the progress and development of modern science and technology, the quality of the purple rice and the inheritance of the purple seed coat, the function of the gene and the regulation of its regulation are gradually clear. At present, the genetic research on the purple seed coat of the rice has shown that the purple is the reason that the purple rice seed coat has a GT deletion in the exon 7 compared with the common rice [41]. The CAPS molecular marker of the purple seed coat gene can be used as the gene function mark of the purple rice breeding. Using the gene knockout strategy, it can create the purple endosperm rice, which is a great progress in the field of the study of the purple rice. However, there are still some obstacles to the promotion of purple rice. The yield of wild purple rice varieties is generally low and the planting range is small, which can't meet the needs of promotion as the main crops, and the developed purple endosperm rice has not been used in agricultural production at present. People's lack of understanding of purple rice is also one of the important reasons that limit the development of purple rice. Therefore,

while cultivating new varieties of purple rice with high quality and high yield, it is necessary for local governments to strengthen the application and popularization of purple rice.

Purple rice is a kind of natural green food, which is rich in anthocyanin and other nutrients that ordinary rice does not have. Its nutritional health function and medicinal value are the core elements of purple rice competitiveness in the market. Nowadays, with the continuous growth of the world population and the continuous improvement of people's living standards, purple rice with nutritional and health care function will be favored by more and more consumers in the market. Therefore, in view of the genetic improvement of purple rice and the continuous rapid development of breeding technology, purple rice will provide more choices for the healthy diet of future consumers.

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