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Functional rice: a new direction for sustainable development of rice production

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Abstract

Functional rice has a broad market prospect and represents one of the vital developmental directions for future rice production. This paper summarizes the types, breeding and cultivation technologies of functional rice, as well as prevention and control of pests and diseases. We conclude the following: (1) breeding for functional rice should focus on breeding rice varieties with an endosperm that is enriched with multiple active components and broad-spectrum resistance to pests and diseases; (2) moderate water stress and optimized fertilizer management practices of low nitrogen, low phosphorus, high potassium, high silicon, and moderate micronutrient fertilization, as well as timely and early harvest, are conducive to improving the yield and quality of functional rice. In addition, we stress the need to focus on the development and application of polymerization breeding technologies for the advancement of the functional rice industry, and future research in these areas should be reinforced.

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Introduction

With the development of the world economy, people's lifestyles have changed dramatically, and long-term high-intensity work has put many people's bodies in a sub-healthy state. The increasing incidence of various chronic diseases has not only put enormous pressure on society's healthcare systems but also caused endless suffering to people[1]. Therefore, people's demands on the functionality and safety of food are increasing, and it has become the consensus of people that 'not just eating enough, but more importantly eating well'.

Rice is the staple food for more than half of the world's population and the main economic source for a large number of rural people[2]. However, due to the rising cost of rice cultivation, farmers are gaining less and less economic benefits from growing rice, which seriously undermines their incentive to grow rice and poses a serious threat to world food security. Increasing the added value of rice not only helps to increase farmers' income but also helps to ensure world food security. The presence of a large number of functional ingredients in rice makes it possible to increase the added value of rice, and functional rice has therefore been widely noticed.

Functional rice refers to rice containing certain specific components that play a regulatory and balancing role in human physiological functions in addition to the nutrients necessary for human growth and development in the endosperm, embryo, and rice bran. They can increase human physiological defense mechanisms, prevent certain diseases, help recovery, delay aging, and boost physical strength and energy levels[3]. Rice is a staple food for more than half of the world's population[4], and its functional components have a great potential to be exploited for human welfare. Using functional rice as a carrier to address health problems and realize 'medicine-food homology' is an excellent motivation for promoting functional rice. The current typical functional rice is introduced in this paper. It also summarizes the breeding and cultivation technologies of functional rice.

Types of functional rice

High resistant starch rice

Rice has a high glycemic index. Its long-term consumption leads to obesity, diabetes, and colon disease in many people[5]. However, the consumption of rice rich in resistant starch (RS) can greatly reduce the risk of these diseases[6]. Therefore, breeding rice varieties with high RS content has attracted considerable attention from breeders in various countries. However, the variability of RS content between different rice varieties is low, and there are few germplasm resources available for selection, thus making it challenging to breed rice varieties with high RS content using traditional breeding methods. Combining traditional and modern molecular breeding techniques can greatly improve the successful production of high RS rice breeds. Nishi et al.[7] selected a high RS rice variety EM10 by treating fertilized egg cells of Kinmaze with N-methyl-N-nitrosourea. However, its yield was very low, and it was not suitable for commercial production. Wada et al.[8] crossed 'Fukei 2032' and 'EM129' as parents and selected Chikushi-kona 85, a high RS rice variety with a higher yield than EM10. Miura et al.[9] bred ultra-high RS Bel-BEIIB double mutant rice by crossing the Abe I and Abe IIB mutant strains, and the content of RS in the endosperm reached 35.1%. Wei et al.[10] found that the simultaneous inhibition of starch branching enzyme (SBE) genes *SBEIIb* and *SBEI* in Teqing by antisense RNA could increase the RS content in rice to 14.9%. Zhu et al.[11] used RNAi technology to inhibit the expression of *SBEI* and *SBEII* genes in rice, which increased the content of RS in rice endosperm from 0 to 14.6 %. Zhou et al.[6] found that rice RS formation is mainly controlled by soluble starch synthase (SSIIA). However, its regulation is dependent on the granule-bound starch synthase Waxy (Wx), and SSIIA deficiency combined with high expression of Wx^a facilitates the substantial accumulation of RS in the rice. The results of Tsuiki et al.[12] showed that BEIB deficiency was the main reason for the increased accumulation of RS in rice. Itoh et al.[13] developed new mutant rice lines with significantly higher levels of RS in rice by introducing genes encoding starch synthase and granule-bound starch synthase in the rice into the BEIB-deficient mutant line be2b.

Colored rice

The accumulation of anthocyanins/proanthocyanidins in the seed coat of the rice grain gives brown rice a distinct color[14]. Most common rice varieties lack anthocyanins in the seed coat, and so far, no rice variety with colored endosperm in its natural state has been identified. However, Zhu et al.[15] bred rice with purple endosperm using transgenic technology. Red rice contains only proanthocyanidins, while black and purple rice contain anthocyanidins and proanthocyanidins[16]. Red seed coat of rice was found to be controlled by the complementary effects of two central effect genes *Rc* and *Rd*. The loss of function of the *Rc* gene prevented the synthesis of proanthocyanidins, while the *Rd* gene could enhance the effect of the *Rc* gene in promoting proanthocyanidins synthesis[17]. Purple seed coat color is controlled by two dominant complementary genes *Pb* and *Pp*. *Pb* determines the presence or absence of seed coat color, and *Pp* determines the depth of seed coat color[18]. In addition, phycocyanin synthesis is also regulated by transcription factors such as *MYB*, *bHLH*, *HY5*, and *WD40*[14], but the exact regulatory mechanism is not clear. Colored rice is rich in bioactive components, such as flavonoids, phenolic acids, vitamin E (V_E), glutelin, phytosterols, and phytic acid (PA). It also contains large amounts of micronutrients such as Ca, Fe, Zn, and Se[19], and has a much higher nutritional and health value than ordinary white rice. In addition, Zhu et al.[20] successfully developed rice with enriched astaxanthin in the endosperm by introducing the genes *sZmPSY1*, *sPaCrtI*, *sCrBKT*, and *sHpBHY*. This achievement has laid a solid foundation for the further development of functional rice industry.

Giant embryo rice

Giant embryo rice refers to rice varieties whose embryo volume is more than twice that of ordinary rice[21]. Rice embryo contains more nutrients than the endosperm; therefore, the nutritional value of giant embryo rice greatly exceeds that of ordinary rice. Studies have found that the levels of γ-aminobutyric acid (GABA), essential amino acids, V_E, γ-oryzanol, phenols, and trace elements in giant embryo rice are considerably higher than that in ordinary rice[21]. Satoh & Omura[22] used the chemical mutagen N-methyl-N-nitrosourea to treat the fertilized egg cells of the rice variety Kinmaze to obtain a 'giant embryo' mutant. The mutants' embryo occupied 1/4–1/3 of the rice grain volume and was 3–4 times larger than normal rice embryo[23]. Its GABA content increased dramatically after the rice was soaked in water. Maeda et al.[24] crossed the giant embryo mutant EM40 of Kinmaze with the high-yielding variety Akenohoshi to produce the giant embryo rice variety 'Haiminori'. The embryo size of 'Haiminori' is 3–4 times that of ordinary rice, and the GABA content of its brown rice is 3–4 times higher than that of 'Nipponbare' and 'Koshihikari' after soaking for four hours in water. A few genes that can regulate the size of rice embryos have been identified, and *GE* is the first identified rice giant embryo gene[25]. Nagasawa et al.[26] found that the loss of *GE* gene function resulted in enlarged embryos and smaller endosperm in rice. Lee et al.[27] found that the inhibition of *LE* gene expression by RNAi technology could lead to embryo enlargement in rice, but the regulatory mechanism remains to be investigated.

Low glutelin rice

Protein is the second most crucial nutrient in rice, accounting for 7–10% of the grain weight, and glutenin accounts for 60%–80% of the total protein content in rice grains[28]. Compared to other proteins, glutenin is more easily digested and absorbed by the body[29]. Therefore, higher glutenin content in rice can improve its nutritional value. However, people with renal disease (a common complication of diabetes) have impaired protein metabolism, and consumption of rice with lower glutelin content can help reduce their protein intake and metabolic burden[30]. Japanese breeders treated Nihonmasari with the chemical mutagen ethyleneimine and selected the low-glutelin rice mutant NM67[31]. Iida et al.[31] developed a new rice variety LGC-1 (Low glutelin content-1) with a glutelin content of less than 4% by backcrossing the NM67 mutant with the original variety 'Nihonmasari'. According to Miyahara[32], the low glutelin trait in LGC-1 is controlled by a single dominant gene *Lgc-1* located on chromosome 2. Subsequently, Nishimura et al.[33] produced two rice varieties, 'LGC Katsu' and 'LGC Jun' with lower glutelin content by crossing LGC1 with a mutant line Koshikari (γ-ray induction) lacking 26 kDa globulin (another easily digestible protein).

Golden rice

Vitamin A (V_A) is one of the essential nutrients for the human body[34]. However, rice, a staple food, lacks V_A, leading to a V_A deficiency in many people. β-carotene is a precursor for V_A synthesis and can be effectively converted into V_A in the human body[35]. Therefore, breeding rice varieties rich in β-carotene has attracted the attention of breeders in various countries. Ye et al.[36] simultaneously transferred phytoene synthase (*psy*), phytoene desaturase (*crt I*), and lycopene β-cyclase (*lcy*) genes into rice using the *Agrobacterium*-mediated method and produced the first generation of golden rice with a β-carotene content of 1.6 $\mu g \cdot g^{-1}$ in the endosperm. However, due to the low content of β-carotene in rice, it is difficult to meet the human body's demand for V_A. To increase β-carotene content in rice, Paine et al.[37] introduced the phytoene synthase (*psy*) gene from maize and the phytoene desaturase (*crt I*) gene from Erwinia into rice. They obtained the second generation of golden rice with 37 $\mu g\ g^{-1}$ of β-carotene in the endosperm, with nearly 23-fold increase in β-carotene content compared to the first generation of golden rice.

Fe and Zn fortified rice

Fe and Zn are essential trace elements for human beings. The contents of Fe and Zn in common rice are about 2 $\mu g \cdot g^{-1}$ and 16 $\mu g \cdot g^{-1}$, respectively[38], which are far from meeting human needs. In 2004, to alleviate micronutrient deficiencies among underprivileged people in developing countries, the Consultative Group on International Agricultural Research launched the HarvestPlus international collaborative program for improving

Fe, Zn, and β-carotene levels in staple crops, with breeding targets of 13 $\mu g \cdot g^{-1}$ and 28 $\mu g \cdot g^{-1}$ for Fe and Zn in rice, respectively. Masuda et al.[39] found that expression of the nicotianamine synthase (NAS) gene *HvNAS* in rice resulted in a 3-fold increase in Fe and a 2-fold increase in Zn content in polished rice. Trijatmiko et al.[38] overexpressed rice *OsNAS2* gene and soybean ferritin gene *SferH-1* in rice, and the Fe and Zn content in polished rice of rice variety NASFer-274 reached 15 $\mu g \cdot g^{-1}$ and 45.7 $\mu g \cdot g^{-1}$, respectively. In addition, it has been found that increasing Fe intake alone does not eliminate Fe deficiency but also decreases the amount of Fe absorption inhibitors in the diet or increases the amount of Fe absorption enhancers[40]. The negatively charged phosphate in PA strongly binds metal cations, thus reducing the bioavailability of Fe and Zn in rice[41], while the sulfhydryl group in cysteine binds Fe, thereby increasing the absorption of non-heme Fe by the body[42]. To improve the bioavailability of Fe and Zn, Lucca et al.[40] introduced a heat-tolerant phytase (*phyA*) gene from Aspergillus fumigatus into rice and overexpressed the cysteine-rich protein gene (*rgMT*), which increased the content of phytase and cysteine residues in rice by 130-fold and 7-fold, respectively[40].

Breeding technology of functional rice

The functional quality of rice is highly dependent on germplasm resources. Current functional rice breeding mainly adopts transgenic and mutagenic technologies, and the cultivated rice varieties are mainly enriched with only one functional substance and cannot meet the urgent demand by consumers for rice enriched with multiple active components. The diversity of rice active components determines the complexity of multifunctional rice breeding. In order to cultivate multifunctional rice, it is necessary to strengthen the application of different breeding technologies. Gene polymerization breeding is a crop breeding technology that can polymerize multiple superior traits that have emerged in recent years, mainly including traditional polymerization breeding, transgenic polymerization breeding, and molecular marker-assisted selection polymerization breeding.

Traditional polymerization breeding

The transfer of beneficial genes in different species during traditional polymeric breeding is largely limited by interspecific reproductive isolation, and it is challenging to utilize beneficial genes between different species effectively. Gene transfer through sexual crosses does not allow accurate manipulation and selection of a gene and is susceptible to undesirable gene linkage, and in the process of breed selection, multiple backcrosses are required[43]. Thus, the period of selecting target plants is long, the breeding cost is high, and the human resources and material resources are costly[44]. Besides, it is often difficult to continue the breakthrough after a few generations of backcrossing due to linkage drag. Thus, there are significant limitations in aggregating genes by traditional breeding methods[45].

Transgenic polymerization breeding

Transgenic technology is an effective means of gene polymerization breeding. Multi-gene transformation makes it possible to assemble multiple beneficial genes in transgenic rice breeding rapidly and can greatly reduce the time and workload of breeding[46]. The traditional multi-gene transformation uses a single gene transformation and hybridization polymerization method[47], in which the vector construction and transformation process is relatively simple. However, it is time-consuming, laborious, and requires extensive hybridization and screening efforts. Multi-gene-based vector transformation methods can be divided into two major categories: multi-vector co-transformation and multi-gene single vector transformation[47]. Multi-vector co-transformation is the simultaneous transfer of multiple target genes into the same recipient plant through different vectors. The efficiency of multi-vector co-transformation is uncertain, and the increase in the number of transforming vectors will increase the difficulty of genetic screening, resulting in a reduced probability of obtaining multi-gene co-transformed plants. Multi-gene single vector transformation constructs multiple genes into the T-DNA region of a vector and then transfers them into the same recipient plant as a single event. This method eliminates the tedious hybridization and backcrossing process and solves the challenges of low co-transformation frequency and complex integration patterns. It can also avoid gene loss caused by multi-gene separation and recombination in future generations[47]. The transgenic method can break through the limitations of conventional breeding, disrupt reproductive isolation, transfer beneficial genes from entirely unrelated crops to rice, and shorten the cycle of polymerizing target genes significantly. However, there are concerns that when genes are manipulated, unforeseen side effects may occur, and, therefore, there are ongoing concerns about the safety of transgenic crops[48]. Marker-free transgenic technology through which selective marker genes in transgenic plants can be removed has been developed. This improves the safety of transgenic crops, is beneficial to multiple operations of the same transgenic crop, and improves the acceptance by people[49].

Molecular marker-assisted selection polymerization breeding

Molecular marker-assisted selection is one of the most widely used rice breeding techniques at present. It uses the close linkage between molecular markers and target genes to select multiple genes directly and aggregates genes from different sources into one variety. This has multiple advantages, including a focused purpose, high accuracy, short breeding cycle, no interference from environmental conditions, and applicability to complex traits[50]. However, few genes have been targeted for the main effect of important agronomic traits in rice, and they are mainly focused on the regulation of rice plant type and the prevention and control of pests and diseases, and very few genes related to the synthesis of active components, which can be used for molecular marker-assisted selection are very limited. Furthermore, the current technical requirements and costs for analyzing and identifying DNA molecular markers are high, and the identification efficiency is low. This greatly limits the popularization and application of functional rice polymerization breeding. Therefore, to better apply molecular marker-assisted selection technology to breed rice varieties rich in multiple active components, it is necessary to construct a richer molecular marker linkage map to enhance the localization of genes related to functional substance synthesis in rice[51]. Additionally, it is important to explore new molecular marker technologies to improve efficiency while reducing cost.

It is worth noting that the effects of gene aggregation are not simply additive. There are cumulative additive effects, greater than cumulative epistatic effects, and less than cumulative epistatic effects among the polymerization genes, and the effects are often smaller than the individual effect. Only with a clearer understanding of the interaction between different QTLs or genes can functional rice pyramiding breeding be carried out reasonably and efficiently. Except for RS and Se, other active components of rice mainly exist in the rice bran layer, and the content of active components in the endosperm, the main edible part, is extremely low. Therefore, cultivating rice varieties with endosperm-enriched active components have broad development prospects. In addition, because crops with high quality are more susceptible to pests and diseases[52], the improvement of rice resistance to pests and diseases should be considered during the polymerization breeding of functional rice.

Cultivation and regulation technology of functional rice

The biosynthesis of active components in rice is influenced by rice varieties but also depends on cultivation management practices and their growth environment.

Effect of light on the accumulation of active components in rice

Environmental conditions have a greater effect on protein content than genetic forces[53]. Both light intensity and light duration affect the synthesis and accumulation of active components in rice. Low light intensity in the early stage of rice growth is not conducive to the accumulation of glutelin in rice grains but favors the accumulation of amylose, while the opposite is true in the late stage of rice growth[54]. Low light intensity during the grain-filling period reduces the accumulation of total flavonoids in rice[55] and decreases Fe ions' movement in the transpiration stream and thereby the transport of Fe ions to rice grains[56]. An appropriate increase in light intensity is beneficial to the accumulation of flavonoids, anthocyanins, and Fe in rice, but the photostability of anthocyanins is poor, and too much light will cause oxidative degradation of anthocyanins[57]. Therefore, functional rice is best cultivated as mid-late rice, which would be conducive to accumulating active components in rice.

Effect of temperature on the accumulation of active components in rice

The temperature has a great influence on the synthesis of active components in rice. An appropriate increase in the temperature is beneficial to the accumulation of γ-oryzanol[58] and flavonoids[59] in rice. A high temperature during the grain-filling period leads to an increase in glutelin content in rice[60], but an increase in temperature decreases the total phenolic content[61]. The results regarding the effect of temperature on the content of PA in rice were inconsistent. Su et al.[62] showed that high temperatures during the filling period would increase the PA content, while Goufo & Trindade[61] reported that the increase in temperature would reduce the PA content. This may be due to the different growth periods and durations of temperature stress on rice in the two studies. The synthesis of anthocyanins/proanthocyanidins in colored rice requires a suitable temperature. Within a certain range, lower temperatures favor the accumulation of anthocyanins/proanthocyanidins in rice[63]. Higher temperatures will lead to degradation, and the thermal stability of proanthocyanidins being higher than that of anthocyanins[64]. In addition, cold or heat stress facilitates GABA accumulation in rice grains[65]. Therefore, in actual production, colored rice and low-glutelin rice are best planted as late rice, and the planting time of other functional rice should be determined according to the response of its enriched active components to temperature changes.

Effect of water management on the accumulation of active components in rice

Moderate water stress can significantly increase the content of glutelin[66] and GABA[67] in rice grains and promote the rapid transfer of assimilation into the grains, shorten the grain filling period, and reduce the RS content[68]. Drought stress can also induce the expression of the phytoene synthase (*psy*) gene and increase the carotenoid content in rice[69]. Soil moisture is an important medium in Zn diffusion to plant roots. In soil with low moisture content, rice roots have low available Zn, which is not conducive to enriching rice grains with Zn[70]. Results from studies on the effect of soil water content on Se accumulation in rice grains have been inconsistent. Li et al.[71] concluded that flooded cultivation could significantly increase the Se content in rice grains compared to dry cultivation. However, the results of Zhou et al.[72] showed that the selenium content in rice grains under aerobic and dry-wet alternative irrigation was 2.44 and 1.84 times higher than that under flood irrigation, respectively. This may be due to the forms of selenium contained in the soil and the degree of drought stress to the rice that differed between experiments[73]. In addition, it has been found that too much or too little water impacts the expression of genes related to anthocyanin synthesis in rice, which affects the accumulation of anthocyanins in rice[74]. Therefore, it is recommended to establish different irrigation systems for different functional rice during cultivation.

Effect of fertilizer application on the accumulation of active components in rice

Both the amount and method of nitrogen application affect the accumulation of glutelin. Numerous studies have shown that both increased and delayed application of nitrogen fertilizer can increase the accumulation of lysine-rich glutelin to improve the nutritional quality of rice (Table 1). However, this improvement is not beneficial for kidney disease patients who cannot consume high glutelin rice. Nitrogen stress can downregulate the expression of *ANDs* genes related to the anthocyanins biosynthesis pathway in grains, resulting in a decrease in anthocyanins synthesis[55]. Increased nitrogen fertilizer application can also increase the Fe, Zn, and Se content in rice[75,76]. However, some studies have found that increased nitrogen fertilizer application has no significant effect on the Fe content of rice[77], while other studies have shown that increased nitrogen fertilizer application will reduce the Fe content of rice[78]. This may be influenced by soil pH and the form of the applied nitrogen fertilizer. The lower the soil pH, the more favorable the reduction of Fe^{3+} to Fe^{2+}, thus promoting the uptake of Fe by rice. Otherwise, the application of ammonium fertilizer can improve the availability of soil Fe and promote the absorption and utilization of Fe by rice. In contrast, nitrate fertilizer can inhibit the reduction of Fe^{3+} and reduce the absorption of Fe by rice[79].

Table 1. Effect of nitrogen fertilizer application on glutelin content of rice.

Sample	N level (kg ha^{-1})	Application time	Glutelin content (g 100 g^{-1})	References
Rough rice	0		5.67	[66]
	270	Pre-transplanting : mid tillering : panicle initiation : spikelet differentiation = 2:1:1:1	6.92	
	300	Pre-transplanting : mid tillering : panicle initiation : spikelet differentiation = 5:2:2:1	6.88	
Brown rice	0		5.35	[83]
	90	Pre-transplanting : after transplanting = 4:1	6.01	
		Pre-transplanting : after transplanting = 1:1	6.60	
	180	Pre-transplanting : after transplanting = 4:1	6.53	
		Pre-transplanting : after transplanting = 1:1	7.29	
	270	Pre-transplanting : after transplanting = 4:1	7.00	
		Pre-transplanting : after transplanting = 1:1	7.66	
Rough rice	0		5.59	[84]
	187.5	Pre-transplanting : after transplanting = 4:1	6.47	
		Pre-transplanting : after transplanting = 1:1	6.64	
	300	Pre-transplanting : after transplanting = 4:1	7.02	
		Pre-transplanting : after transplanting = 1:1	7.14	
Polished rice	0		3.88	[85]
	90	Pre-transplanting : tillering : booting = 2:2:1	4.21	
	180	Pre-transplanting : tillering : booting = 2:2:1	4.43	
	270	Pre-transplanting : tillering : booting = 2:2:1	6.42	
	360	Pre-transplanting : tillering : booting = 2:2:1	4.87	
Brown rice	0		9.05	[86]
	120	Flowering	22.14	

Appropriate application of phosphorus fertilizer is beneficial in promoting the translocation of Fe and Zn from leaves to rice grains, thus increasing the content in rice grains[80]. However, the excessive application of phosphate fertilizer will reduce the availability of Fe and Zn in soil, resulting in less uptake by the roots and a lower content in the rice grains[81]. The content of PA in rice increased with a higher phosphorus fertilizer application rate[80]. Increasing the phosphorus fertilizer application rate would increase the values of [PA]/[Fe] and [PA]/[Zn] and reduce the effectiveness of Fe and Zn in rice[80]. Currently, there are few studies on the effect of potassium fertilization on the synthesis of active components in rice. Available studies report that increased application of nitrogen fertilizer can increase the Zn content in rice[82]. Therefore, the research in this area needs to be strengthened.

Because the iron in soil mainly exists in the insoluble form Fe^{3+}, the application of iron fertilizer has little effect on rice biofortification[87]. There are different opinions about the effect of Zn fertilizer application methods. Phattarakul et al.[88] believed that foliar spraying of Zn fertilizer could significantly improve the Zn content in rice grains. Jiang et al.[89] concluded that most of the Zn accumulated in rice grains were absorbed by the roots rather than from the reactivation of Zn in leaves. In contrast, Yuan et al.[90] suggested that soil application of Zn fertilizer had no significant effect on Zn content in rice grains. The different results may be affected by the form of zinc fertilizer applied and the soil conditions in the experimental sites. Studies have found that compared with the application of ZnEDTA and ZnO, zinc fertilizer in the form of $ZnSO_4$ is most effective for increasing rice's Zn[70]. In addition, the application of zinc fertilizer reduces the concentration of PA in rice grains[70].

The form of selenium fertilizer and the method and time of application will affect the accumulation of Se in rice grains. Regarding selenium, rice is a non-hyperaccumulative plant. A moderate application of selenium fertilizer can improve rice yield. However, the excessive application can be toxic to rice, and the difference between beneficial and harmful supply levels is slight[91]. Selenite is readily adsorbed by iron oxide or hydroxide in soil, and its effectiveness in the soil is much lower than selenite[92]. In addition, selenate can migrate to the roots and transfer to rice shoots through high-affinity sulfate transporters. In contrast, selenite is mainly assimilated into organic selenium in the roots and transferred to the shoots in smaller amounts[93]. Therefore, the biological effectiveness of Se is higher in selenate-applied soil than in selenite application[94] (Table 2). Zhang et al.[95] found that the concentration of Se in rice with soil application of 100 g Se ha^{-1} was only 76.8 μg·kg^{-1}, while the concentration of Se in rice with foliar spray of 75 g Se ha^{-1} was as high as 410 μg·kg^{-1}[73]. However, the level of organic selenium was lower in rough rice with foliar application of selenium fertilizer compared to soil application[96], while the bioavailability of organic selenium in humans was higher than inorganic selenium[97]. Deng et al.[73] found that the concentrations of total selenium and organic selenium in brown rice with selenium fertilizer applied at the full heading stage were 2-fold higher than those in brown rice with selenium fertilizer applied at the late tillering stage (Table 2). Although the application of exogenous selenium fertilizer can rapidly and effectively increase the Se content of rice (Table 2), it can easily lead to excessive Se content in rice and soil, which can have adverse effects on humans and the environment. Therefore, breeding Se-rich rice varieties is a safer and more reliable way to produce Se-rich rice. In summary, functional rice production should include the moderate application of nitrogen and phosphorus fertilizer and higher levels of potassium fertilizer, with consideration to the use of trace element fertilizers.

Effect of harvesting time on the accumulation of active components in rice

The content of many active components in rough rice is constantly changing during the development of rice. It was

Table 2. Effect of selenium fertilizer application on the selenium content of rice.

Sample	Se level (g Se ha^{-1})	Selenium fertilizer forms	Application method	Se content (μg·g^{-1})	References
Rough rice	0			0.002	[98]
	18	Selenite	Foliar spray at full heading	0.411	
Polished rice	0			0.071	[99]
	20	Selenite	Foliar spray at full heading	0.471	
	20	Selenate	Foliar spray at full heading	0.640	
Rough rice	75	Selenite	Foliar spray at late tillering	0.440	[73]
	75	Selenite	Foliar spray at full heading	1.290	
	75	Selenate	Foliar spray at late tillering	0.780	
	75	Selenate	Foliar spray at full heading	2.710	
Polished rice	0			0.027	[100]
	15	Selenite	Foliar spray at full heading	0.435	
	45	Selenite	Foliar spray at full heading	0.890	
	60	Selenite	Foliar spray at full heading	1.275	

found that the content of total flavonoids in brown rice increased continuously from flowering stage to dough stage and then decreased gradually[101]. The γ-oryzanol content in rice decreased by 13% from milk stage to dough stage, and then gradually increased to 60% higher than milk stage at full maturity[101]. The results of Shao et al.[102] showed that the anthocyanin content in rice reached its highest level at two weeks after flowering and then gradually decreased. At full ripeness, and the anthocyanins content in brown rice was only about 50% of the maximum level. The content of total phenolics in rice decreased with maturity from one week after flowering to the fully ripe stage, and the loss of total phenolics reached more than 47% by the fully ripe stage. In contrast, the content of total phenolics in black rice increased with maturity[102]. Moreover, RS content in rough rice decreases during rice maturation[68]. Therefore, the production process of functional rice should be timely and early harvested to obtain higher economic value.

Prevention and control of pests and diseases for functional rice

Pests and diseases seriously impact the yield and quality of rice[103]. At present, the two most effective methods to control pests and diseases are the use of chemical pesticides and the planting of pest and disease-resistant rice varieties. The use of chemical pesticides has greatly reduced the yield loss of rice. However, excessive use of chemical pesticides decreases soil quality, pollutes the environment, reduces soil biodiversity[104], increases pest resistance, and aggravates the adverse effects of pests and diseases on rice production[105]. It also increases residual pesticide levels in rice, reduces rice quality, and poses a severe threat to human health[106].

Breeding pest and disease-resistant rice varieties are among the safest and effective ways to control rice pests and diseases[107]. In recent years, many pest and disease resistance genes from rice and microorganisms have been cloned[47]. Researchers have used these genes to breed rice varieties resistant to multiple pests and diseases through gene polymerization breeding techniques. Application in production practices delivered good ecological and economic benefits[108].

Green pest and disease control technologies must consider the synergies between rice and water, fertilizer, and pest and disease management. In this regard, the rice-frog, rice-duck, and other comprehensive rice production models that have been widely used in recent years are the most representative. These rice production models significantly reduced chemical pesticide usage and effectively controlled rice pests and diseases[109]. The nutritional imbalance will reduce the resistance of rice to pests and diseases[110]. Excessive application of nitrogen fertilizer stimulates rice overgrowth, protein synthesis, and the release of hormones, increasing its attractiveness to pests[111]. Increased soluble protein content in rice leaves is more conducive to virus replication and increases the risk of viral infection[112]. Increasing the available phosphorus content in the soil will increase crop damage by pests[113], while insufficient potassium supply will reduce crop resistance to pests and diseases[114]. The application of silica fertilizer can boost the defense against pests and diseases by increasing silicon deposition in rice tissue, inducing the expression of genes associated with rice defense mechanisms[115] and the accumulation of antifungal compounds in rice tissue[116]. The application of silica fertilizer increases the release of rice volatiles, thereby attracting natural enemies of pests and reducing pest damage[117]. Organic farming increases the resistance of rice to pests and diseases[118]. In addition, rice intercropping with different genotypes can reduce pests and diseases through dilution and allelopathy and changing field microclimate[119].

In conclusion, the prevention and control of rice pests and diseases should be based on chemical and biological control and supplemented by fertilizer management methods such as low nitrogen, less phosphorus, high potassium and more silicon, as well as agronomic measures such as rice-aquaculture integrated cultivation, organic cultivation and intercropping of different rice varieties, etc. The combined use of multiple prevention and control measures can improve the yield and quality of functional rice.

Conclusions

Functional rice contains many active components which are beneficial to maintaining human health and have high economic and social value with broad market prospects. However, the current development level of the functional rice industry is low. The development of the functional rice requires extensive use of traditional and modern polymerization breeding techniques to cultivate new functional rice varieties with endosperm that can be enriched with multiple active components and have broad-spectrum resistance to pests and diseases. It is also important to select suitable planting locations and times according to the response characteristics of different functional rice active components to environmental conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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