



REVIEW ARTICLE

# Therapeutic rice (*Oryza sativa* L.): Crop breeding interventions to combat chronic diseases

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## ARTICLE HISTORY

Received: 27 September 2024

Accepted: 20 October 2024

Available online

Version 1.0 : 03 December 2024



## Additional information

**Peer review:** Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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## CITE THIS ARTICLE

Vaishnavi K, Geetha S, Mathankumar P, Ganesan KN, Boopathi NM, Uma D, Anandhi K. Therapeutic rice (*Oryza sativa* L.): Crop breeding interventions to combat chronic diseases. Plant Science Today.2024;11 (sp4):01-14. <https://doi.org/10.14719/pst.5331>

## Abstract

Rice (*Oryza sativa* L.) serves not only as a dietary staple worldwide but also harbours a rich array of bioactive compounds with therapeutic potential. This review explores various therapeutic traits in rice such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins, elucidating their profound health benefits. These compounds act as antioxidants and are crucial in reducing oxidative stress and the risk of type 2 diabetes, obesity, hypertension, high cholesterol, cardiovascular diseases and certain cancers, thereby offering notable nutraceutical benefits. Enhancing these compounds in rice is advantageous, as it is a staple food. The success of crop improvement programs depends on the availability of genetic diversity. This review extensively discusses the genetic diversity among rice genotypes for therapeutic traits and the potential for enhancing these traits through breeding initiatives. Molecular-based approaches are essential in breeding programs, as they enable accurate identification of genetic markers associated with desirable traits and Quantitative Trait Loci (QTLs), facilitating marker-assisted selection easier. These techniques also help to understand the genetic basis and expression of targeted traits. Advances in molecular markers and genomic tools are explored, with an emphasis on developing rice varieties enriched with health-promoting compounds. Furthermore, genome editing and transgenic technologies are examined for their role in precisely modulating bioactive compound levels. This synthesis offers a comprehensive perspective for breeders aiming to enhance therapeutic traits in rice.

## Keywords

rice; therapeutic traits; health benefits; genetic diversity; marker-assisted selection; genomic tools

## Introduction

Antioxidants are substances capable of donating electrons to free radicals to neutralize them, thereby preventing cell damage. These free radicals are generally produced by various processes, such as metabolic activity in the body and exposure to environmental chemicals (1). Antioxidants in plants exist in both enzymatic and non-enzymatic forms. Superoxide dismutase

(SOD), catalase, peroxidases, glutathione and other proteins are examples of enzymatic antioxidants. They protect plant cells from oxidative stress generated by reactive oxygen species (ROS), functioning as a defense system. Non-enzymatic antioxidants include phenolic compounds, flavonoids, phenolic acids, other phenols, carotenoids and nitrogen compounds (2). These secondary metabolites are essential for seed defense against both biotic and abiotic stresses (3). For instance, trans-cinnamic acid (4), coumarins (5) and flavonoids (6) are involved in regulating seed dormancy and germination. Isoflavones such as daidzein, genistein and glycitein, which accumulate significantly in soybean and maize seeds, exhibit antifungal and antibacterial activities (7, 8). Additionally, proanthocyanidins and flavonols protect seed embryos and reserves against abiotic stresses, including desiccation, high temperatures and UV radiation (9). The intake of non-enzymatic antioxidants has shown potential health benefits, such as reducing the risk of cholesterol levels, oxidative stress, obesity, type 2 diabetes and cancer (10). These natural antioxidants found in cereal grains exert these benefits by acting as free radical scavengers, reducing agents and singlet oxygen quenchers (11).

Chronic diseases have a profound impact on global health, affecting millions of people worldwide. Notable among these conditions are metabolic disorders, diabetes, cardiovascular diseases, cancer and central nervous system (CNS) disorders. Diabetes is a chronic metabolic disease, characterized by increased blood glucose levels, which severely damages the heart, blood vessels, eyes, kidneys and nerves. Globally, the prevalence of diabetes has surged from 108 million in 1980 to 422 million in 2014, particularly in low- and middle-income countries. In 2019 alone, diabetes was directly responsible for 1.5 million deaths, with high blood glucose levels contributing to an additional 20 % of cardiovascular fatalities (12). Similarly, cardiovascular diseases (CVDs) significantly impact global health. In 2019, CVDs accounted for 17.9 million deaths (32 % of all global deaths), with 85 % of cases resulting from heart attack and stroke, primarily in low- and middle-income countries. Most CVDs can be prevented through lifestyle changes and early detection is crucial for effective management (13). Obesity, defined as a BMI over 30 (with over 25 considered overweight), is the accumulation of excessive fat that poses health risks. In 2022, it was reported that 1 in 8 people globally were obese, with 2.5 billion adults overweight and 890 million of them obese. From 1990 to 2022, adult obesity rates more than doubled and childhood obesity rates quadrupled, highlighting a significant and growing health concern (14). The primary behavioural risk factor for these chronic diseases is an unhealthy diet accompanied by a lack of physical activity, smoking and excessive alcohol consumption. While lifestyle modifications can prevent or manage most of these conditions, implementing such changes remains challenging in today's fast-paced world. In this context, the consumption of antioxidant-rich foods has gained significant attention. Cereal grains are rich in antioxidant molecules that have therapeutic and nutraceutical properties, especially rice.

*Oryza sativa* L. is a staple food consumed by more than half of the global population (15). It is estimated that to ensure food and nutritional security for a growing population, rice production must meet the demands of 9.7 billion people by 2050 (16). Epidemiological studies have proposed that the incidence of several chronic diseases in rice-consuming regions is low, which may be associated with the antioxidant contents present in rice (17). Interest in rice antioxidants began around the year 2000 (18). Research reported a strong relationship between the low incidence of heart disease and cancer in a population of rice-consuming Asians. Whole grain rice, commonly referred to as brown rice, is the unpolished version of white rice, retaining its bran, germ and endosperm. Consumption of whole-grain rice has been linked to various health benefits. Consequently, there has been considerable scientific interest in identifying and quantifying the bioactive compounds present in whole-grain rice (19). The rice phytochemicals include  $\gamma$ -oryzanol, phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols and phytic acid (17). In particular,  $\gamma$ -oryzanol and tocotrienols are abundant in rice bran oil, which is extracted from rice bran and rice hulls (20). These phytochemicals play a crucial role in the health benefits attributed to whole rice grains. The consumption of rice grains that are rich in antioxidants could be a less expensive option for overcoming lifestyle-related diseases and providing impressive health benefits in rice-consuming countries.

Improving these therapeutic traits in rice is the next uphill task for breeders, as it could revolutionize the therapeutic profile of staple foods. Considerable efforts have been made in recent years to improve therapeutic traits (21). Breeders have studied the genetic diversity of therapeutic traits to identify potential donor genotypes (22). Furthermore, the underlying genetic and environmental influences on these traits were studied to include modern breeding methods for the improvement of therapeutic traits (23). QTLs and linked markers play crucial roles in enabling precision plant breeding through marker-assisted selection (MAS), facilitating the accurate improvement of complex traits (24). Association mapping provides a genome-wide approach for breeders to dissect complex therapeutic traits, revealing potential genomic regions and associated markers (25). Genome editing offers wide yet largely underutilized potential for the exploration and enhancement of therapeutic traits. This review provides a comprehensive analysis of the health-promoting compounds in rice and recent advancements in breeding programs to enhance these traits, underscoring the crop's role in promoting global health.

### Health Benefits of Therapeutic Compounds in Rice

ROS and oxidative stress play significant roles in various non-communicable diseases, including metabolic disorders, cardiovascular diseases, cancer and central nervous system (CNS) disorders (26). Bioactive compounds found in pigmented rice offer numerous health benefits, such as anti-inflammatory and antidiabetic effects and the ability to inhibit new blood vessel formation (27). In studies involving C57BL/KsJ-db/db type 2 diabetic mice, oral ingestion of a phenolic acid-rich rice extract significantly improved glucose regulation. This improvement was evidenced by reduced

blood glucose levels and enhanced plasma insulin levels, linked to the restoration of hepatic glucokinase activity and increased glycogen storage (28). Ferulic acid, a major component of the extract, has been noted for its ability to inhibit nitric oxide and NF- $\kappa$ B, both proinflammatory cytokines, thereby demonstrating anti-inflammatory properties (29). Anthocyanins and proanthocyanidins abundant in pigmented rice, are recognized for their potential antidiabetic properties. Extracts from purple rice have been shown to lower blood sugar levels by inhibiting carbohydrate-digesting enzymes, reducing aldose reductase activity and mitigating retinal damage caused by high glucose levels in both humans and rats (30). Additionally, Cyanidin-3-glucoside, which is a major anthocyanin of black rice, suppresses obesity (31).

Rice phytochemicals help in the prevention of colorectal cancer, colon cancer, digestive cancer, breast cancer, liver cancer, ovarian cancer and prostate cancer (32). The hepatoprotective activity of rice bioactive compounds has been reported by many epidemiological studies (33, 34). The occurrence of hepatic steatosis is reduced after dietary intake of black rice extract (31). A study performed on hepatotoxic mice (induced by  $\text{CCl}_4$ ) illustrated that peonidin-3-glucoside and cyanidin-3-glucoside effectively promoted antioxidant activity by increasing the levels of SOD and glutathione (GSH) and reducing thiobarbituric acid reactive substances (TBARS) while enhancing cell viability (33). To demonstrate the neuroprotective effects of germinated brown rice and brown rice, research was conducted using neuroblastoma SH-SY5Y cells (35). Both the extracts of germinated brown rice and brown rice exhibited notable antiapoptotic potential by increasing cell viability in the presence of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Furthermore, extracts from the sebok rice variety, rich in polyphenols and flavonoids, exhibited anti-melanogenic activity. These extracts inhibited the activation of p-38 MAPK (Mitogen-Activated Protein Kinases) while stimulating phosphorylated ERK 1/2 (Extracellular Signal-Regulated Kinase) and Akt. This ultimately leads to the downregulation of MITF (Microphthalmia-associated Transcription Factor) and subsequent decline in tyrosinase and related proteins. This finding suggests the potential for rice seed extracts as a natural treatment for hyperpigmentation (36). Additionally, these results highlight the promising role of rice phytochemicals as natural remedies with diverse therapeutic benefits (Fig. 1). Therefore, enhancing these bioactive compounds holds significant potential for improving health, considering rice's global consumption.

### Genetic Diversity Among Rice Genotypes for Therapeutic Traits

While enhancing cereal crop yields to ensure access to affordable calories has long been a priority for breeders globally, the exploration of their therapeutic (relating to disease treatment and healing) and nutraceutical (food component that provides health benefits, including disease prevention) benefits has lagged. However, a growing awareness of this untapped potential is prompting a paradigm shift in breeding priorities, with a

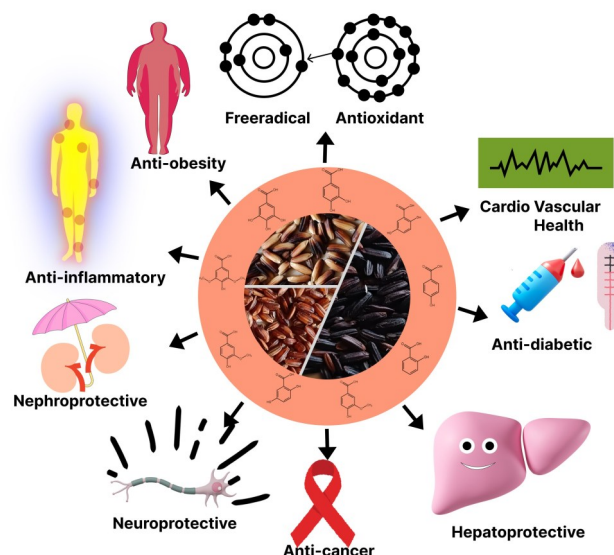


Fig. 1. Health benefits of therapeutic compounds in rice.

renewed focus on enriching these valuable properties. Recent investigations have revealed a wealth of genetic diversity within rice germplasm, particularly in the content of flavonoids, anthocyanins and polyphenols.

In grains with a light brown pericarp, the predominant phenolic acids are ferulic and p-coumaric. In contrast, rice varieties with red and black pericarp primarily contain the anthocyanins, cyanidin-3-O- $\beta$ -d-glucoside and peonidin-3-O- $\beta$ -d-glucoside (37, 38). In an analysis of pigmented rice, including 5 black and 2 red cultivars, the study examined flavonoid and carotenoid profiles. Positive correlations were found between all the phytochemicals. HPLC analysis of the five black rice cultivars identified kaempferol, quercetin, apigenin, cyanidin-3-O-glucoside, peonidin-3-O-glucoside,  $\beta$ -carotene, lutein and zeaxanthin. The predominant flavonols in black rice were quercetin and kaempferol, while carotenoid levels were highest in the black cultivars, followed by the red cultivars (39). In a study of 8 pigmented rice varieties, the chemical compositions and antioxidant properties were analyzed, revealing a positive correlation between antioxidant capacity and polyphenol content. Polyphenol levels, determined using the Folin-Ciocalteu method, ranged from 58 to 329 mg GAE (Gallic Acid Equivalent) per 100 g. In particular, rice varieties with dark purple grains exhibited higher polyphenol content compared to those with red-brown grains (40). A comparative analysis of 9 red and 3 black rice varieties revealed no significant differences in total phenolic content (TPC) or antioxidant properties between the 2 groups. The TPC was estimated by the Folin-Ciocalteu method and TAC by a protocol described by previous study (41). HPLC analysis in this black rice revealed that the dominant anthocyanins were cyanidin 3-glucoside (19.4-140.8 mg/100 g dry matter (DM)) and peonidin 3-glucoside (11.1-12.8 mg/100 g DM) and free phenolic acids in red varieties predominantly contained ferulic, protocatechuic and vanillic acids, while black varieties had higher protocatechuic acid concentrations. Ferulic acid was the most abundant bound form in both colors, followed by p-

coumaric and vanillic acids (42).

The pigmented varieties, Kala Namak were found to have high levels of TPC ( $43.19 \pm 0.54$  mg/g), total flavonoid content ( $7.18 \pm 0.52$  mg/g) (TFC) and Mapillai Samba had high total anthocyanin content ( $42.21 \pm 0.28$  mg/g) (TAC). The Folin-Ciocalteu method, aluminum chloride colorimetric method and pH differential method were employed to estimate the TPC, TFC and TAC respectively. These findings suggest that pigmented landraces are valuable sources of these beneficial compounds (43). IG6, Kavuni and Mikkuruvai have been identified as rich sources of flavonoids and anthocyanins. TFC estimation was performed by the protocol (44) and the pH differential method was used to estimate TAC. Kavuni was notable for containing both flavonoids and anthocyanins, while Mikkuruvai and IG6 were particularly high in flavonoid content (45). Tyagi studied nine Korean-grown rice varieties (one red and eight brown rice) and reported that DM29 had the highest TPC ( $395.85 \pm 1.23$  mg gallic acid Equiv/100 g, DW), TFC ( $224.14 \pm 1.81$  mg Catechin Equiv/100 g, DW) and TAC ( $317.29 \pm 1.86$  mg Cyanidin-3-O-glucoside Equiv/100 g, DW) levels along with high free radical scavenging ability of ABTS ( $295.17 \pm 2.02$  mg Trolox Equiv/100 g, DW) and DPPH (2,2-diphenyl-1-picrylhydrazyl) ( $291.88 \pm 1.31$  mg Trolox Equiv/100 g, DW). TPC, TFC and TAC were estimated by following the protocols from previous studies (46-48). UHPLC Q-TOF-MS/MS (Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry) was used to identify eight phenolic compounds and of these, DM29 was found to contain quercetin, ferulic acid, p-coumaric acid, ascorbic acid, caffeic acid and genistein. All these compounds are known to have potential health benefits (49).

It was reported that the higher phenolic content (12.70 mg GAE/g) and higher anthocyanin content (9.13 mg cyanidin-3-glucoside/g) were observed in black rice (50). Red rice had the highest flavonoid content of 6.12 mg catechin/g and exhibited DPPH inhibition of 92.62 %. The Folin-Ciocalteu method was employed to estimate TFC, while spectrophotometric methods for TFC and TAC were used as described by previous studies (44, 51). Additionally, phenolic acids and anthocyanins were analyzed using an HPLC system, which exclusively quantified two soluble free phenolic acids, protocatechuic and vanillic acid in black rice (52). It was observed that conjugated protocatechuic acid was present in black rice while 2,5-dihydroxybenzoic acids was found in red rice but absent in non-pigmented rice. In red rice, the predominant bound phenolic acids were ferulic acid, p-coumaric acid and 2,5-dihydroxybenzoic acids, whereas black rice contained protocatechuic and vanillic acids. It was noted that soluble-conjugated phenolics, TFC and anthocyanins decreased as rice grains became lighter, while proanthocyanidins increased with a deeper red color. This highlights the link between grain color and bioactive compound content in rice. TPC was measured using the Folin-Ciocalteu assay, while TFC was assessed following the method described by previous research (53). Total proanthocyanidin content (TPAC) was determined using the vanillin assay (52).

Chakhao, an aromatic and glutinous rice from Manipur, received protection under a geographical indication

in India in 2019, recognizing its exceptional nutraceutical qualities. A notable characteristic is its wide range of pericarp pigmentation, which varies from white to deep purple or black. Through LC-MS (Liquid Chromatography-Mass Spectrometry) analysis, researchers identified 2 anthocyanin compounds, cyanidin-3-O-glucoside and peonidin-3-O-glucoside, in pigmented rice varieties. Among these pigmented genotypes, TAC ranged from 29.8 to 275.8 mg per 100 g of DW. TPC varied from 66.5 to 700.3 mg GAE per 100 g DW, while radical scavenging activity was observed to range between 17.7 % and 65.7 %. TPC was measured using the Folin-Ciocalteu method, while TAC was determined following the protocol outlined in previous studies (42, 54).

Using gas chromatography with time-of-flight mass spectrometry (GC-TOFMS) and tertbutyldimethylsilyl (TBDMS) derivatization, researchers identified phenolic acids in rice. Pigmented rice showed a higher TPC (168–368  $\mu$ g/g) relative to non-pigmented rice (146–172  $\mu$ g/g), with free-form phenolic acids comprising less than 10 % of the total soluble phenolic acids. In these samples, ferulic and sinapic acids were the dominant phenolic acids, with significant variation in salicylic acid content among different genotypes (55).

In a study analyzing seven brown rice varieties and the red rice variety Luotiangongmi, researchers examined their phenolic profiles using both HPLC and LC-MS. The study identified eleven phenolic compounds in the brown rice samples, including protocatechuic acid, gallic acid, vanillic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, chlorogenic acid, ferulic acid, p-coumaric acid, vanillin and trans-3-hydroxycinnamic acid. TPC in brown rice was estimated using the Folin-Ciocalteu assay, with values ranging from 92.32 to 196.54 mg of GAE per 100 g. The study found significant variations in phenolic acid composition among the different rice genotypes, with specific phenolic acids being unique to certain genotype. For instance, chlorogenic acid was detected only in Luotiangongmi, syringic acid was specific to Ezhong No.5 and no gallic acid was detected in Guangliangyouxiang-66. The analysis also revealed that the levels of free, bound and total phenolic compounds in Luotiangongmi (red rice) were significantly higher than those in the brown rice varieties (56). These studies highlight the significant influence of genotype on phenolic acid composition in rice.

Several studies have reported a tendency for japonica rice varieties to have higher levels of phytochemicals compared to indica varieties (57, 58). Specifically, it was utilized clustering analysis of single-nucleotide polymorphisms (SNPs) in phenolic compound pathways and successfully categorized rice varieties into indica and japonica subspecies. Their analysis of 10 rice varieties revealed a statistically significant difference in the TPC of rice between the 2 subspecies, reaching up to 100 mg GAE/100 g. Further supporting this trend, another study (59) reported average TFC in japonica rice (6 varieties) was 25 % higher than that of indica rice (8 varieties). This study also found elevated anthocyanin levels and oxygen radical absorbance capacity (ORAC) values in japonica varieties, making them promising candidates for breeding programs aimed at enhancing therapeutic traits. In addition, it was analyzed phenolic levels

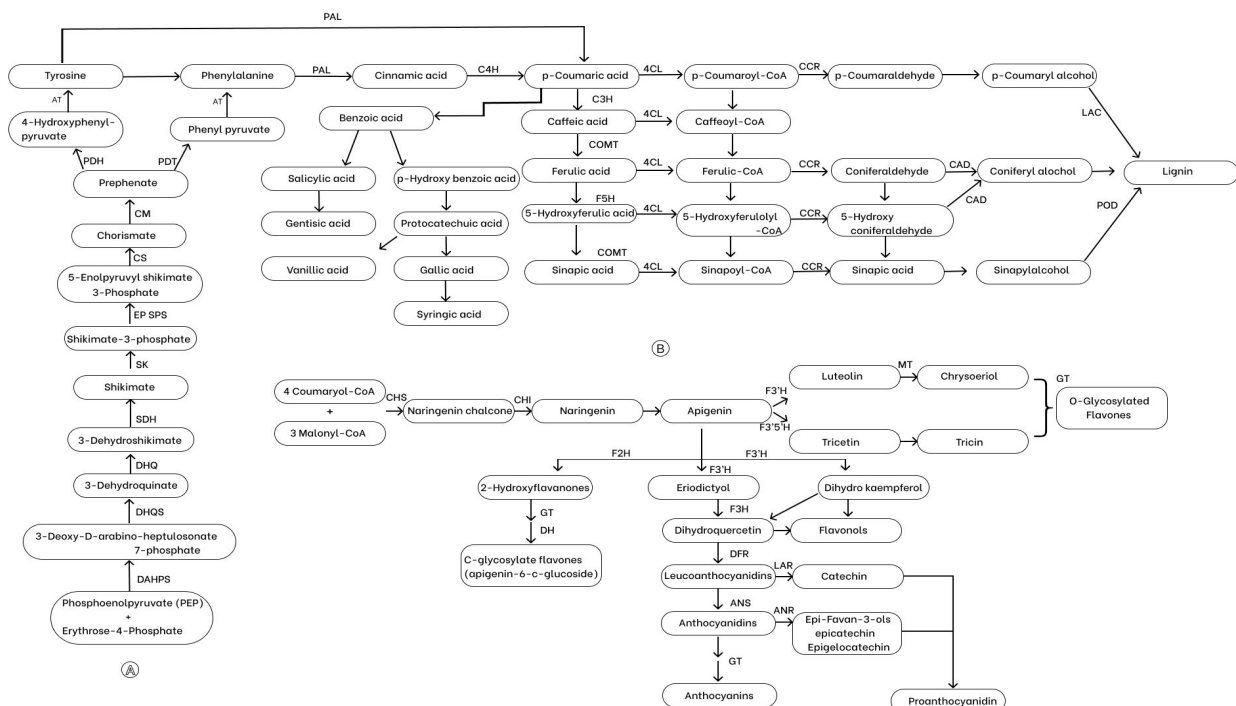
and antioxidant properties in 15 advanced breeding lines derived from a cross between white and black rice (I132 B/ Yunanheixiannuo) though they did not assess flavonoids (60). No free phenolic acids were detected in the soluble fraction; however, p-coumaric acid, isoferulic acid, ferulic acid and vanillic acid were observed in the insoluble bound fractions. Additionally, phenolic dehydrodimers were exclusively found in the insoluble bound fractions. Line YF53 exhibited the highest TPC (23.3 mg ferulic acid equiv/g), TAC (2.07 mg cyanidin-3-glu equiv/g) and antioxidant activities. Identifying potential donors is a critical yet tedious and prolonged process. Studies have highlighted specific rice genotypes with exceptional levels of bioactive compounds, indicating potential for targeted breeding to augment these traits. The therapeutic donor traits identified in various studies are summarized in Table 1.

Phenolic compounds, a broader category encompassing both flavonoids and anthocyanins, share a common precursor molecule, phenylalanine and their biosynthesis (Fig. 2) is often regulated by similar environmental and genetic factors (66). This suggests that enhancing the overall phenolic content in rice could lead to a concomitant increase in flavonoid and anthocyanin levels. Further, a strong positive correlation between flavonoid and anthocyanin content in a diverse set of rice genotypes has been reported (65). Similarly, positive associations have been observed between phenolic compounds, flavonoids and anthocyanins in different rice varieties (49, 67). Another study also noted a positive association among the studied phytochemicals (39).

**Table 1.** Donors for therapeutic traits in rice.

Traits	Potential donors	References
High flavonoids	Heuginjubyeo	(39)
High carotenoids	Heugseolbyeo	
High TPC	Bahng Gawk (red Thai rice)	(42)
High antioxidant activity	Mapillai samba, Iluppapoo samba	(61)
Total phenols, Anthocyanins, Antioxidant activity	Kattuyanam	(62)
Phenolic content	Kalanamak	(43)
Antioxidant properties		
Phenolic content TFC	Mapillai samba	(45)
Antioxidant activity		
Total Anthocyanin Content	Kavuni	(63)
High flavonoid		
High flavonoid	RPHP 93	(64)
Anthocyanin		
High TFC	Ac. 43670, Ac. 43660, Ac. 43675	(65)
Lutein content		
High TAC	Ac. 44592, Ac. 43670, Ac. 4460, Ac. 44595, Ac. 44588, Ac. 43660, Ac. 43738, Ac. 43732	(65)
High ABTS		
High TFC	Ac. 43670, Ac. 43660, Ac. 44646, Ac. 44592, Ac. 44595, Ac. 43737, Ac. 43738, Ac. 43676	(56)
Total Phenols	Luotiangongmi	(55)
	Josengheugchalbyeo	(55)

TPC- Total Phenolic Content; TFC- Total Flavonoid Content; TAC- Total Anthocyanin Content; ABTS- 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)



**Fig. 2.** Biosynthesis pathway of bioactive compounds. **A- Phenolic acids biosynthesis-** DAHP synthase: 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, DHQS: 3-dehydroquininate synthase, DHQ: 3-dehydroquininate dehydratase, SDH: shikimate dehydrogenase, SK: shikimate kinase, EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase, CS: chorismite synthase, CM: chorismite mutase, PDH: prephenate dehydrogenase, PDT: prephenate dehydratase, PAL: phenylalanine ammonia lyase, C4H: cinnamate 4-hydroxylase, AT: amino transferase, CCR: cinnamoyl-CoA reductase, 4CL: 4-coumarate CoA ligase, COMT: caffeic acid O-methyltransferase, F5H: ferulate 5-hydroxylase, CAD: cinnamyl alcohol dehydrogenase, POD: peroxidase, LAC: laccase; **B- Flavonoid biosynthesis-** CHS: chalcone synthase, CHI: chalcone isomerase, F3'H: flavone 3-hydroxylase, F2H: flavanone 2-hydroxylase, F2H: flavanone 2-hydroxylase, GT: glucosyltransferase, DFR: dihydroflavonol 4-reductase, ANS: anthocyanin synthase, ANR: anthocyanin reductase, LAR: leucoanthocyanidin reductase.

The simultaneous improvement of flavonoids, anthocyanins and polyphenols in rice grains through breeding offers a promising avenue for enhancing the bioactive profile and functional value of this staple crop. By targeting key genes involved in their biosynthesis and regulatory pathways, breeders can potentially develop rice varieties with elevated levels of these health-promoting compounds. Furthermore, understanding the environmental factors that influence their accumulation can help optimize cultivation practices to maximize their content. Total phenolic, anthocyanin and flavonoid contents in rice grains are also significantly influenced by environmental factors, as evidenced by studies demonstrating interactions with growing conditions (68, 69). Moreover, storage conditions such as temperature and duration, also interact with these compounds, highlighting the dynamic nature of their accumulation and stability in rice grains (70).

Studies consistently demonstrate that pigmented rice varieties contain higher levels of polyphenols and flavonoids than non-pigmented rice (Fig. 3). These compounds exhibit strong antioxidant properties, which offer notable health benefits. Interestingly, IG6, a variety rich in flavonoids as reported earlier (45), is non-pigmented, highlighting the diversity of therapeutic compounds across different rice types. This diversity provides significant opportunities for breeders aiming to develop rice with enhanced therapeutic potential. Figures 4a, 4b and 4c illustrate the structures of selected phenolic acids, flavonoids and anthocyanin compounds.

After harvest, rice is referred to as paddy or rough rice (17). Rough rice undergoes threshing to separate the husk from the brown rice. Brown rice, derived from rough rice, consists of bran layers (6-7 %), an embryo (2-3 %) and an endosperm (approximately 90 %) (71). Further processing of brown rice produces polished rice or white rice, which is achieved by removing the bran through milling. There is a difference in antioxidant intake between white rice, which consists mainly of the endosperm and brown rice, which retains the whole grain. The bran layers of rice are rich in antioxidant compounds. Therefore, whole grain or bran rice is preferable for maximizing antioxidant consumption (17). However, a drawback of brown rice is its longer cooking time due to lower water absorption and it is often regarded as inferior in eating quality compared to white rice (72). Additionally, the primary challenge in using rice bran as a food ingredient is its susceptibility to hydrolytic rancidity (73).

Given these challenges, most research on phytochemical attributes has focused on pigmented rice varieties. To gain a comprehensive understanding of these characteristics, it is essential to study segregating populations. This approach would provide insights into the genetic variability and inheritance patterns of therapeutic compounds, which is vital for breeding programs aimed at improving these traits. While pigmented varieties are of scientific interest for their higher antioxidant content, consumer preference strongly favors white rice. Therefore, future research should aim to develop white rice varieties that combine high levels of therapeutic compounds with the desirable qualities of white rice, bridging the gap between health benefits and consumer preferences. Additionally, advances in molecular techniques will accelerate the development of rice varieties with improved bioactive compounds.

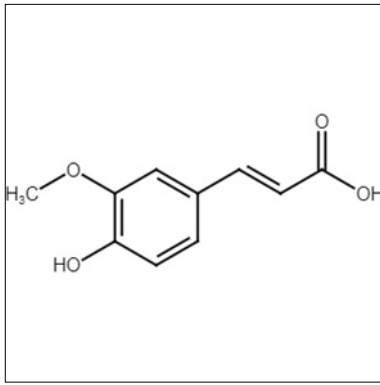
### Molecular Advances for Improving Therapeutic Traits in Rice

Molecular-based approaches have significant importance for the enhancement of therapeutic compound levels in rice breeding programs. As traditional breeding methods are labor-intensive and time-consuming, molecular techniques have increasingly supplemented these approaches. The identification of DNA markers and quantitative trait loci (QTLs) has facilitated MAS, enabling selection at the DNA or gene level rather than through phenotyping. Numerous studies have highlighted the substantial impact of environmental factors on these traits, underscoring the significant advantage that molecular markers offer in the breeding process. Recent molecular advances in rice, including the identification of molecular markers, the mapping of QTLs associated with therapeutic traits and gene expression studies, are examined in terms of their implications for enhancing therapeutic properties of rice.

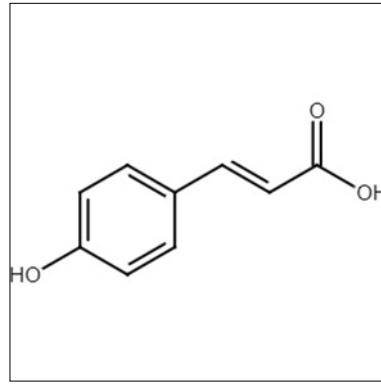
Recent studies have pinpointed several key genomic regions associated with important therapeutic traits. Associations between the rice SSR markers RM339 (Chromosome 8) and RM316 (Chromosome 9) were identified with respect to the combined presence of phenolics, flavonoids and antioxidant capacity. Additionally, RM228 (Chromosome 10) was found to specifically influence flavonoid content and antioxidant capacity. The genes *Ra* (also known as *Prp-b*) and *Rc* were confirmed to be major loci contributing to rice grain color and nutritional quality traits (74). The marker-trait associations identified RM452 on



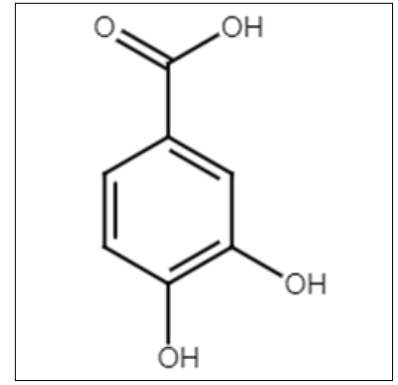
**Fig. 3.** Pigmented rice (a, b, c) and non-pigmented rice (d).



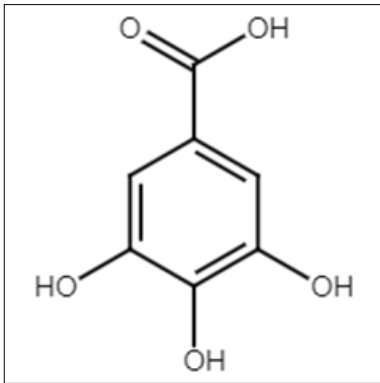
Ferulic acid



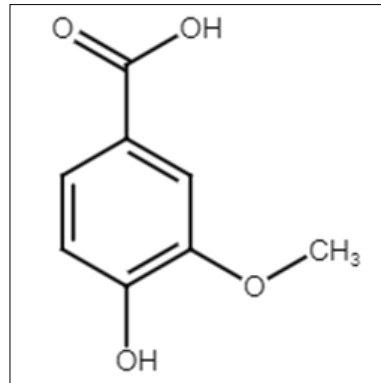
p-Coumaric acid



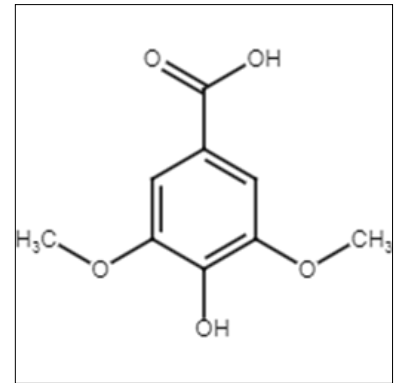
Protocatechuic acid



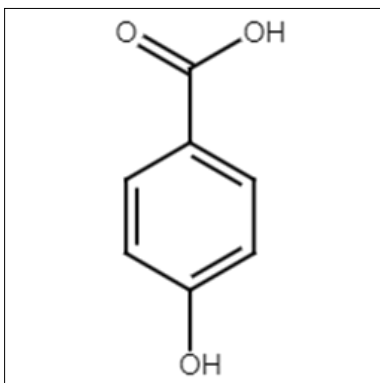
Gallic acid



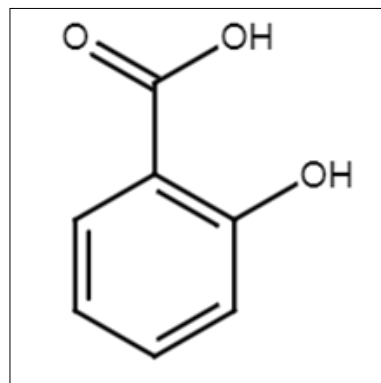
Vanillic acid



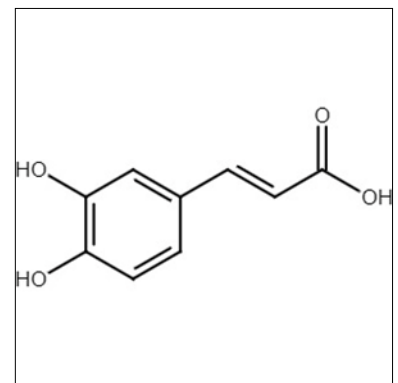
Syringic acid



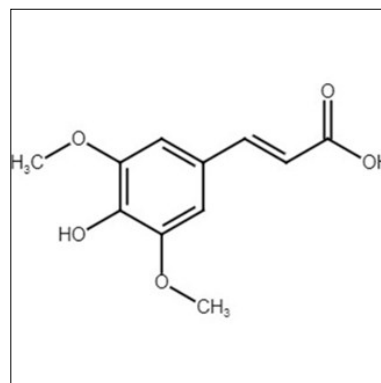
4-hydroxybenzoic acid



Salicylic acid

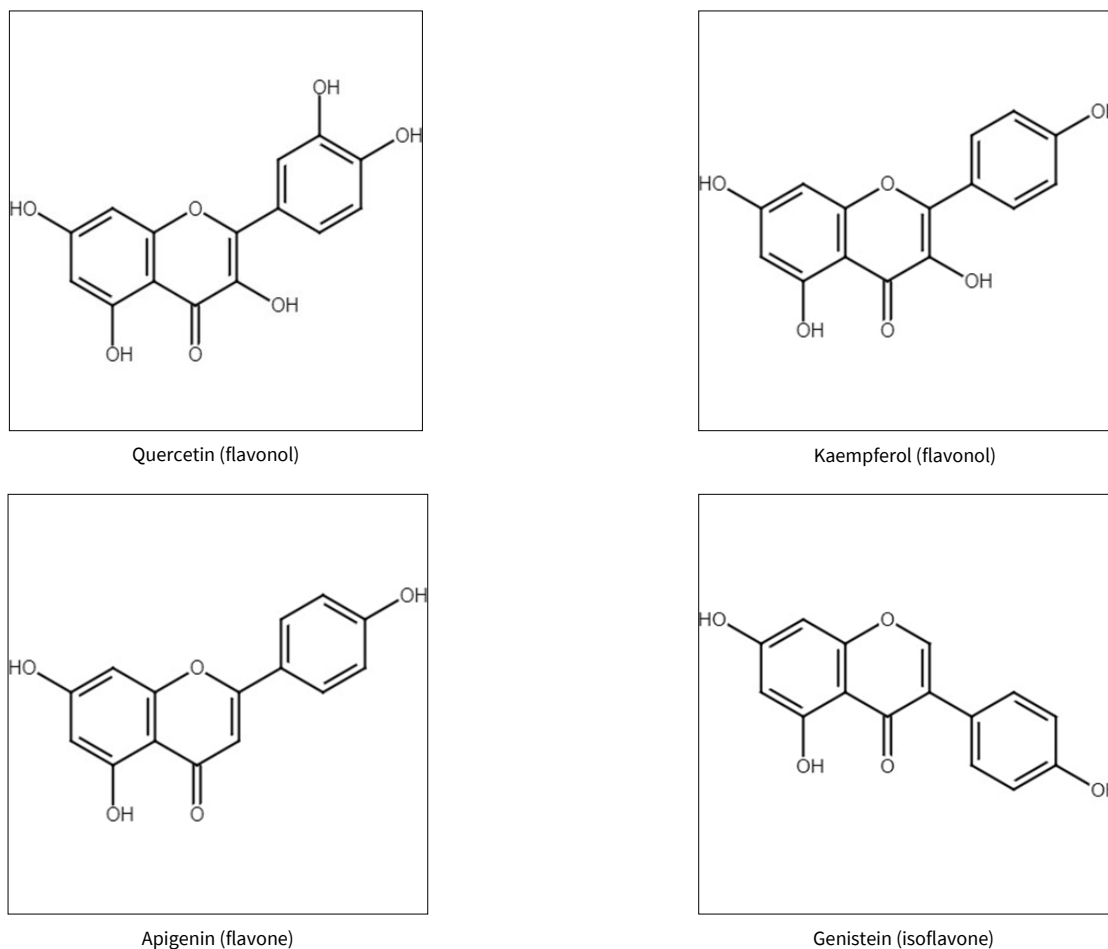


Caffeic acid

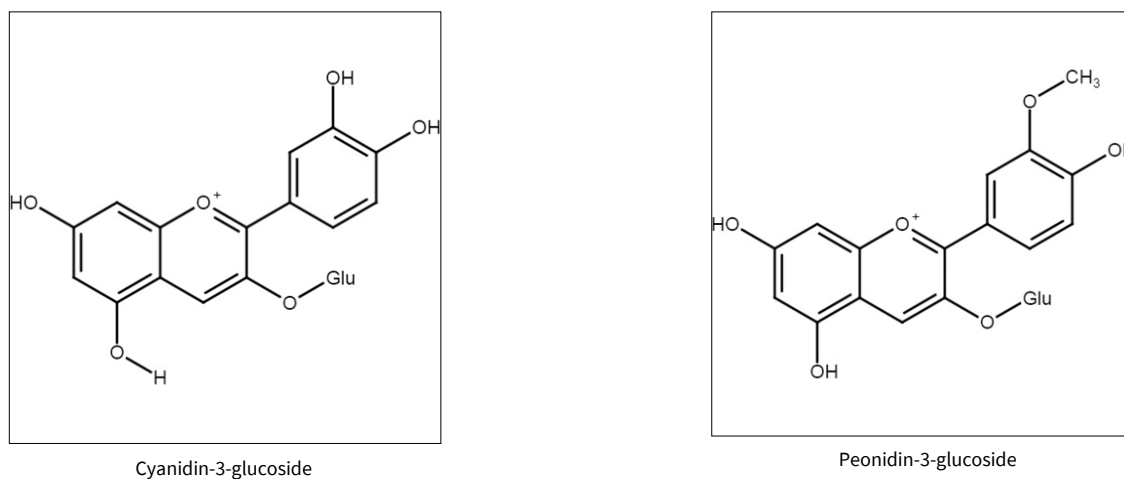


Sinapic acid

**Fig. 4a.** Chemical structures of some phenolic acids.



**Fig. 4b.** Chemical structures of some flavonoids.



**Fig. 4c.** Chemical structures of some anthocyanin compounds.

Chromosome 2 and RM2197 on Chromosome 12 were linked to TFC. As well, RM287 on Chromosome 11 and RM19358 on Chromosome 6 were associated with TAC. Further, RM10916 (Chromosome 1), RM5963 (Chromosome 6) and RM559 (Chromosome 4) were found to be linked with both TFC and TAC. The markers RM287 and RM19358 have been identified as being closely associated with the candidate genes LOC9266684:14.28 (which encodes probable 2-oxoglutarate-dependent dioxygenase ANS) and LOC43401082: 2.394-2.397 Mb (which encodes anthocyanin 5-aromatic acyltransferase) respectively. These genes are crucial for the biosynthesis of TFC and TAC (63).

Furthermore, Fathima (75) reported three associated

SSR markers, RM228 on chromosome 10 for both flavonoid ( $R^2 = 0.8431$ ) and anthocyanin content ( $R^2 = 0.140$ ), RM5348 ( $R^2 = 0.1478$ ) on chromosome 10 for flavonoid content and RM297 ( $R^2 = 0.0932$ ) on chromosome 1 for anthocyanin content. Considerably, RM5348 located in the genomic region of the gene encoding the enzyme flavonoid 3'-monooxygenase. Similarly, it was identified a genomic region on chromosome 6, spanning 3.0 to 3.7 megabases, containing four linked markers (RM197, RM204, RM225 and RM19442) associated with lutein content accounting for genetic variation of 35.9% (64). It was also employed bulk segregant analysis in the  $F_2$  population derived from ADT 48/Kavuni and RILs derived from CO 50/Kavuni respectively (64, 75).



Identifying and mapping QTLs (Quantitative Trait Loci) are critical for crop improvement, particularly when utilizing molecular markers. This approach facilitates the incorporation of desirable genes into advanced cultivars through marker-assisted selection (MAS), aids in map-based cloning of tagged genes and enhances understanding of the genetic basis of complex traits. Two main approaches used for QTL mapping are linkage analysis and association mapping (76). With the advent of next-generation sequencing technologies, research on QTL analysis has advanced significantly, enabling the discovery of molecular markers that are closely linked or even located within the gene/QTL controlling traits of interest. These advancements have also propelled efforts in cloning gene/QTLs and identifying potential candidate genes associated with specific traits (77). Reported QTLs of therapeutic traits are listed in Table 2.

Genome-Wide Association Studies (GWAS) use genetic diversity present in a population to identify genomic regions linked to traits, revealing associations between genetic variants and phenotypic traits. They depend on linkage disequilibrium (LD), which refers to the non-random association of alleles at various loci, to uncover the genetic basis of complex traits. Using GWAS, it was found that 32 and 16 SNPs significantly linked to anthocyanin and flavonoid contents respectively (81), with 44 co-occurring genes predicted to influence the biosynthesis pathways of these compounds in black rice. It was identified 23 marker-trait associations (MTAs) for flavonoid, phenol and antioxidant capacity, with markers RM484, RM162 and RM5371 consistently associated with all 3 traits, suggesting potential pleiotropic effects or linkage to a common regulatory region (82). Additionally, a GWAS identified over 280 SNPs associated with bioactive compounds and color-related traits in rice, with many influencing multiple traits. Remarkably, 67 SNPs within 52 candidate genes were linked to 24 traits, with haplotypes in genes *Rc/bHLH17* and *OsIPT5* playing key roles in regulating phenolic compounds impacting pericarp color (83).

An enzyme, flavonoid 3'-hydroxylase (F3'H) is responsible for the 3'-hydroxylation of flavonoids, a critical modification in the biosynthetic pathway. In a study on gene expression, cDNA clones of 2 F3'H genes, *CYP75B3* and *CYP75B4*, were isolated from Korean rice varieties with white, black and red grains. Sequence analysis revealed allelic variants within these genes, marked by 1 or 2 amino acid substitutions. *CYP75B3* exhibited a strong preference for kaempferol and a lower affinity for dihydrokaempferol, whereas *CYP75B4* showed a higher specificity for apigenin. Interestingly, the *CYP75B3* gene from black rice demonstrated a two-fold increase in enzymatic activity for both naringenin and dihydrokaempferol, compared to white and red rice. The *CYP75B3* showed higher F3'H activity than *CYP75B4*. Analysis of gene expression showed that *CYP75B3*, *CYP75B4* and many other genes in the flavonoid pathway were primarily active in the developing seeds of black rice, with limited expression observed in white and red rice (84). In another investigation (85), the expression of eight biosynthetic genes in pigmented and non-pigmented

rice varieties was studied across various tissues, including root, leaf, stem and seedling. These genes included phenylalanine ammonia lyase, 4-coumarate CoA ligase, chalcone synthase, chalcone flavone isomerase, flavanone 3-hydroxylase, dihydroflavonol 4-reductase, leucoanthocyanidin reductase and anthocyanin synthase. The results indicated that transcript levels were higher in pigmented rice varieties, which demonstrates the genetic basis for increased flavonoid content in rice varieties with color pigmentation.

Additionally, HPLC and transcriptome analysis were employed (86) to investigate variation in anthocyanin levels and gene expression in rice during three developmental stages: milky, dough and mature. These stages are the critical phase of grain development. In the first 5 days after anthesis, the caryopsis is entirely green, indicating low anthocyanin levels. By 7 days after flowering (DAF) at the milky stage, a faint purple tint appears, signifying the initial accumulation of anthocyanins, which becomes more noticeable by 11 DAF. During the dough stage (15–20 DAF), the caryopsis turns dark purple as anthocyanin levels increase and this color deepens further as the grain matures to the fully ripe stage. They focused on 8 key anthocyanin genes: one early biosynthetic gene (*OsF3'H*), 3 late biosynthetic genes (*OsANS*, *OsDFR* and *OsGT*), 3 transcription factors (*OsMYB3*, *OsKala4* and *OsWD40-50*) and 1 transporter (*OsGSTU34*). The study found that differential expression occurred during caryopsis development, with peak expression at the dough stage, which corresponded to increased anthocyanin levels. The analysis identified 6 main contributors responsible for anthocyanin peak at this stage: *OsANS*, *OsDFR*, *OsGSTU34*, *OsMYB3*, *OsbHLH015* and *OsWD40-50*.

Recent molecular advancements offer crucial tools for enhancing rice's therapeutic value through MAS, significantly enhancing the efficiency and precision of breeding efforts for therapeutic traits. Markers with high  $R^2$  values, or a combination of such markers, can be utilized in MAS. They enable the efficient reduction of large breeding populations to a more manageable size, facilitating the downstream breeding process. The findings of gene expression studies provide valuable insights into understanding the regulation and behaviour of target genes that are involved in the biosynthesis of phytochemicals and lay the groundwork for future breeding strategies focused on improving the health benefits of rice through targeted nutraceutical compound enrichment.

### Genome Editing and Transgenic Approach for Improving Therapeutic Compounds

Cutting-edge genetic technologies provide new opportunities for increasing bioactive compounds in rice. One such technology is CRISPR/Cas9 genome editing, which has revolutionized plant molecular biology, allowing for precise modifications of target genes. A CRISPR-based genome knockout approach was used to investigate the function of the *OsCOP1* gene on Chromosome 2 in rice. The knockout of this gene resulted in rice seeds with a yellow pericarp, while

**Table 2.** List of QTLs associated with therapeutic traits in rice.

Therapeutic traits	Mapping population	Genes/QTL	Linkage Group	Flanking markers/ associated marker/ peak marker	R <sup>2</sup>	Reference
Phenolic content	DH population of Zhai-Ye-Qing 8 (indica rice) x Jing-Xi 17 (Japonica rice)	<i>qPH-2</i> ,	2	CT87-G1234	16.91	(67)
		<i>qPH-4</i> ,	4	G177-CT206	6.07	
		<i>qPH-12</i>	12	RG463-G148	10.43	
	20 rice accessions including cultivars, germplasm lines and landraces	<i>qPC1</i> ,	1	-	-	(78)
		<i>qPC4</i> ,	4			
		<i>qPC12</i>	12			
	Panel population (120 genotypes) including landraces and cultivars	<i>qTFC6.1</i>	6	RM3701	6.61	(65)
		<i>qTFC11.1</i>	11	RM235	8.7	
		<i>qTFC12.1</i>	12	RM494	5.6	
Total flavonoids content (TFC)	DH population of Zhai-Ye-Qing 8 (indica rice) x Jing-Xi 17 (Japonica rice)	<i>qFL-2-1</i> ,	2	CT87-G1234	12.71	(67)
		<i>qFL-2-2</i> ,	2	G1327-CT16	7.25	
		<i>qFL-11</i>	11	PTA818-RG2	6.94	
	20 rice accessions including cultivars, germplasm lines and landraces	<i>qFC1-1</i> ,	1	-	-	(78)
		<i>qFC1-2</i> ,	1			
		<i>qFC2</i> ,	2			
	Panel population (120 genotypes) including landraces and cultivars	<i>qTAC1.1</i>	1	RM440	6.6	(65)
		<i>qTAC5.1</i>	5	RM5638	7.8	
				RM253	7.4	
Anthocyanin	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qANC1-1</i> ,	1	RM580-RM562	7.9-8.8	(79)
		<i>qANC3</i> ,	3	RM411-RM448	17.6-34.8	
		<i>qANC2-1</i> ,	2	RM13844-RM1367	9.3	
	Panel of 156 rice accessions	<i>qANC2-2</i> ,	2	RM530-RM166	10.4	(80)
		<i>qANC10</i> ,	10	RM24992-RM474	11.2	
		<i>qANC1-2</i> ,	1	RM582-RM580	11.9	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qANC7</i>	7	RM1364-RM501	7.1-9.6	(79)
		<i>qAntC<sub>1.1</sub></i>	1	S1_33820736	14.5	
		<i>qAntC<sub>10.1</sub></i>	10	S10_13377773	13.1	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qPAC7</i> ,	7	RM445-RM432	3.8	(79)
		<i>qPAC2-1</i> ,	2	RB1367-RM1379	11.4	
		<i>qPAC2-2</i> ,	2	RM1379-RM1347	13.7	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qPAC8-1</i> ,	8	RM281-RM23065	10.9	(79)
		<i>qPAC12-4</i> ,	12	RM1302-RM1310	14-23.3	
		<i>qPAC1-1</i> ,	1	RM11273-RM1282	7	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qPAC1-2</i> ,	1	RM294-RM5	5.7	(79)
		<i>qPAC2-3</i> ,	2	RM530-RM166	13.1	
		<i>qPAC12-1</i> ,	12	RM1261-RM309	14	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qPAC10</i> ,	10	RM24992-RM474	10.1	(79)
		<i>qPAC12-2</i> ,	12	RM1337-RM511	4.7	
		<i>qPAC12-3</i> ,	12	RM1300-RM1246	8.5	
	RILs of Hongxiang no.1 x Song 98-131 (Japonica varieties)	<i>qPAC8-2</i> ,	8	RM80-RM1345	7.3	(79)
		<i>qPAC8-3</i>	8	RM339-RM342	4.9	
		<i>qPAC1-1</i> ,	1			
Proanthocyanidin	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC1-2</i> ,	1	-	-	(78)
		<i>qPAC1-3</i> ,	1			
		<i>qPAC2-1</i> ,	2			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC2-2</i> ,	2	-	-	(78)
		<i>qPAC3</i> ,	3			
		<i>qPAC4</i> ,	4			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC4-1</i> ,	4	-	-	(78)
		<i>qPAC4-2</i> ,	4			
		<i>qPAC4-3</i> ,	4			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC6-1</i> ,	6	-	-	(78)
		<i>qPAC6-2</i> ,	6			
		<i>qPAC6-3</i> ,	6			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC6-4</i> ,	6	-	-	(78)
		<i>qPAC7-1</i> ,	7			
		<i>qPAC7-2</i> ,	7			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC7-3</i> ,	7	-	-	(78)
		<i>qPAC8</i> ,	8			
		<i>qPAC9</i> ,	9			
	Twenty rice accessions comprising cultivars, germplasm lines and landraces	<i>qPAC11</i> ,	11	-	-	(78)
		<i>qPAC12-1</i> ,	12			
		<i>qPAC12-2</i>	12			

its overexpression in a mutant background restored the original pericarp color. This study highlighted the critical role of the *OsCOP1* gene in early embryogenesis and flavonoid biosynthesis, providing valuable insights into its functional significance (87). Similarly, it was successfully edited 3 key genes involved in anthocyanin biosynthesis (*OsF3'H*, *OsDFR* and *OsLDOX*) in the Heugseonchal and Sinmyungheugchal rice varieties, demonstrating the potential of CRISPR technology to enhance the therapeutic benefits of rice (88).

While there is significant diversity in bioactive compounds in rice, further increasing their accumulation remains beneficial. Enhancing the levels of these compounds can improve the health-promoting properties of rice, making it a more valuable dietary staple. C1/R-S rice, a transgenic rice cultivar of *Oryza sativa* spp. *japonica* cv. Hwa-Young, is a flavonoid enriched cultivar (89). Transgenic rice plants were developed to produce multiple flavonoid classes in seeds by introducing genes for enzyme synthesis from phenylalanine to produce flavonoids. The introduction of genes for PAL (phenylalanine ammonia-lyase) and CHS (chalcone synthase) enabled naringenin accumulation. Additionally, genes for PAL and CHS, along with genes encoding flavonol synthase, flavone synthases and isoflavone synthase, produced kaempferol, apigenin and genistein respectively. The accumulation of triclin was achieved through hydroxylase and methyltransferase introduction (90). This represents a promising approach to enriching diets with beneficial compounds, though ethical concerns about genetic modification remain relevant. Genome editing should be conducted with precision to avoid unintended side effects, ensuring that modifications integrate seamlessly with the plant's existing metabolic pathways and associated traits.

Integrating molecular markers, QTLs, CRISPR/Cas9 genome editing and transgenic technology into rice breeding programs accelerates the development of therapeutically superior varieties. These efforts are essential for meeting global health demands and improving food security through crops rich in therapeutic traits. The advancements in molecular breeding and genome editing provide a robust framework for the continuous improvement of rice cultivars, ensuring their adaptation to future agricultural challenges and their contribution to human health. As the field progresses, further research and collaboration will be crucial to unlocking the full potential of these molecular tools for sustainable agricultural development.

## Conclusion

Consuming whole grain rice offers numerous health benefits, but its consumption can be challenging. Future research should focus on developing white rice varieties that integrate therapeutic compounds, such as phenolic acids and flavonoids, along with the preferred qualities of white rice. This approach can align the health benefits of therapeutic compounds with consumer preferences for white rice. Further, incorporating value-added products from pigmented rice varieties, such as puffed rice, poha, rice flour and health mixes etc, provides a practical alternative. These products allow individuals to access the

unique phytonutrients and antioxidants of colored rice in diverse and convenient forms. Collaborations between value-added product manufacturers and agricultural research sectors can create more health-beneficial processed foods by sourcing nutraceuticals-enhanced rice varieties. Integration of multi-omics approaches, including genomics, transcriptomics, and metabolomics, will provide deeper insights into the regulatory networks governing biosynthesis pathways. Moreover, addressing environmental stresses and climate change impacts on rice production will be critical, as these factors can influence the synthesis and efficacy of therapeutic compounds. Collaborative efforts between researchers, breeders, policymakers and industry stakeholders will be essential to translate scientific advancements into tangible benefits for global food security and public health. In conclusion, while challenges persist, the convergence of traditional breeding practices with cutting-edge biotechnological tools offers immense potential to harness the inherent diversity of rice for enhanced human health outcomes. By continuing to innovate and collaborate, we can pave the way for a future where rice not only nourishes billions but also serves as a powerful ally in combating prevalent chronic diseases worldwide.

## Acknowledgements

The authors acknowledge the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, for providing library and web source facilities for the review paper and S Kavinesh (B. Sc., TFD and UI/UX), who assisted in creating figures illustrating the health benefits of therapeutic compounds and their biosynthesis pathways. The authors also acknowledge DST, New Delhi for providing financial support under the SERB project (SPG/2022/000058).

## Authors' contributions

KV collected the literature and drafted the original manuscript. SG led the conceptualization, secured funding, conducted the investigation, provided project administration and supervision and contributed to the review and editing of the manuscript. PM assisted with manuscript review and editing, while KNG, NMB, DU and KA offered guidance throughout the process. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare

**Ethical issues:** None.

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