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Geographic differences and variation of functional components of brown rice in 690 mini-core collections from global germplasms

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ABSTRACT

Objective: To understand the geographic differences and variations in the functional components of brown rice cores collected from global rice germplasms.
Methods: Four functional components, γ-aminobutyric acid (GABA), resistant starch (RS), total flavonoids, and alkaloids, in brown rice from 690 mini-core collections from 31 countries from five continents and the International Rice Research Institute, were analyzed using a spectrophotometry colorimetric method, and the results were statistically validated.
Conclusion: The highest average amounts of functional components were obtained in Asian germplasms, except for GABA, and total flavonoids were highest in brown rice from Europe and Oceania, followed by Asia. The highest coefficient of variation for GABA was observed in Asia; that for RS and total flavonoids was observed in Africa, followed by Asia; and that for alkaloids was observed in America, followed by Asia. Overall, Asian countries were the most prominent and representative zones with the highest genotypic potential for functional components of brown rice. Forty-one rice accessions with enriched functional components originated mostly from biodiversity-rich areas in China, followed by those in the Philippines. Late sowing favored the enrichment of these components in brown rice. The current study provides a reference for rice breeding with enriched functional constituents, and guidelines for screening functional rice that could be used for human chronic disease research.

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1. Introduction

Rice is the most consumed cereal and a staple food for more than half of the world's population [1]. The consumption of whole grains has been a traditional practice in China for centuries and has been increasing at a faster rate in recent years, playing a vital role in altering dietary patterns and improving the health status of people [2]. Dietary patterns based on whole-grain rice not only provide sufficient energy, but also help avoid excessive intake of lipids and animal foods, which is of great significance for preventing the occurrence of related chronic diseases [3,4].

Brown rice belongs to the whole-grain category, containing a fiber-enriched outer bran layer, a nutrient-packed germ core, and a starchy middle endosperm layer. With rapid economic development, lifestyles and dietary habits have changed significantly. Regardless of the health benefits of brown rice, polished rice has become the staple diet of people in the modern era owing to advances in processing and milling technologies and its superior palatability [5]. More than 64 % of the nutrients and physiologically active ingredients in rice are concentrated in the embryo and inner seed coat, and are lost during processing [6]. A comparative study of the main nutrient contents of brown rice and polished rice revealed that losses of several major nutrients, such as fat, dietary fiber, vitamin B complex, iron, calcium, and γ-aminobutyric acid (GABA), were obvious after processing into polished rice; however, changes in the protein content were avoidable [7]. Nevertheless, switching the staple diet from brown rice to polished rice might be an intrinsic factor in the development of chronic diseases such as type II diabetes [8].

Brown rice contains ample amounts of naturally occurring bioactive components in the bran layer, which improve health and provide adjunctive therapy for some chronic diseases [9,10]. GABA, a key bioactive non-protein amino acid found in brown rice, helps nurture blood vessels, adjust insulin levels, optimize blood cholesterol, reduce the risk of stroke, improve kidney and liver functions, and prevent leukemic cell proliferation, hyperlipidemia, and chronic alcohol disease [5,11,12]. In addition, GABA can improve aging-induced deterioration of cerebral visual cortex function [13]. Liu et al. [14] concluded that GABA levels in brown rice increased dramatically after immersion in water. As a result, germinated brown rice not only has an enhanced flavor, but also significantly boosts health benefits compared to unprocessed brown rice. Resistant starch (RS) refers to starch and its degradants, which are not absorbed in the small intestine of healthy people, but are fermented and decomposed in the large intestine [15]. RS is a dietary fiber that occurs naturally in cereal crops. One of the most beneficial effects of RS-enriched rice consumption is the control of blood glucose levels and type II diabetes [16]. RS also has chronic effects on several metabolism-related diseases, such as obesity, coronary heart disease, colon cancer, hypertension, dyslipidemia, and diarrhea [17,18]. Flavonoids and alkaloids are two major classes of plant secondary metabolites. As natural antioxidants, flavonoids help eliminate free radicals from the human body, stimulate and enhance the function of the immune system, and inhibit cancer cell differentiation. Therefore, flavonoids are of great significance in the prevention of cardiovascular diseases, various tumors, osteoporosis, and other chronic diseases [19–22]. Although alkaloids are less abundant in plants, they are closely related to human health, most of which have significant physiological activities, such as anticancer, antioxidant, anti-aging, and antimicrobial activities, and can also play a highly efficient physiological role in the central nervous system [23–27].

There have been many reports on the functional components of grains. The functional constituents of food grains depend largely on intrinsic factors, including differences in genotype, origin, and growth environment [28]. Dong et al. [29] investigated the natural variations in several forms of secondary metabolites in the core collection of rice germplasms, and a highly differential accumulation was reported among the japonica and indica subspecies. Recently, comprehensive profiling and characterization of some rice germplasms based on variations in different functional components, including flavonols and GABA, revealed that only 3 % of the germplasms were superior cultivars containing higher health-promoting substances [30]. The zonal characteristics and variation of functional ingredients in 907 brown rice cultivars collected from 16 prefectures in five rice-growing areas of the Yunnan Province were

Table 1
The source of 690 mini-core collections of rice germplasms.

Source		No. of Accessions	Source		No. of Accessions
Continents	Countries		Continents	Countries	
Asia	China	488	Africa	Egypt	4
	Bangladesh	2		Ethiopia	1
	India	26		Central Africa	8
	Indonesia	6		Cote d'Ivoire	4
	Iran	3		Uganda	1
	North Korea	1			
	Malaysia	7	America	USA	6
	Myanmar	3		Brazil	3
	Sri Lanka	5			
	Vietnam	14	Europe	Portugal	1
	Philippines	24		Hungary	2
	Pakistan	4		Russia	2
	Japan	22		Romania	1
	Thailand	2		Bulgaria	1
	Nepal	2		Italy	1
	South Korea	4		France	1
	IRRI	33			
			Oceania	Australia	8

studied, revealing distinct zonal characteristics in the content of three functional components: total flavonoids, GABA, and RS [31]. The zonal features and genetic diversity of these health-promoting functional ingredients were also evident in the grains of 629 barley accessions collected from four continents [32].

Rice is widely consumed in both China and globally, and its functional components have the potential to positively impact human health if they are present in abundance. In recent years, significant progress has been made globally in morphological traits and genome-wide association analysis of the rice core germplasm [33,34], whereas reports on zonal differences and variations in functional components of the global rice core germplasm are rare. This study aimed to investigate the global rice core germplasm, and 690 mini-core collections of rice from 31 countries and the International Rice Research Institute (IRRI) were sown and harvested in Yunnan Province and used for functional component analysis. The geographic differences and amplitude of variations in the content of the four functional components of brown rice among the five continents, 31 countries plus the IRRI, and two sowing dates were investigated to provide a reference for rice breeding with enriched functional constituents and guidelines for screening functional rice that could be used as a material for human chronic disease research.

2. Materials and methods

2.1. Plant materials

A mini-core collection of 690 rice cultivars was collected from 31 countries from five continents (646 from Asia, 18 from Africa, nine from America, nine from Europe, and eight from Oceania) and from the International Rice Research Institute (Table 1). The test materials were provided by the China Agricultural University.

2.2. Cultivation method

Seeds of 690 rice germplasms were planted in Yanhe Town (altitude 1638 m) of Yuxi City in the Yunnan Province on two sowing dates. The first seeding was performed on March 26, 2015, transplanted on May 19, and harvested on September 20. The second seeding was performed on April 10, 2015, transplanted on June 2, and harvested on September 20. A randomized complete block design was used for all experiments. Seedlings were transplanted into a 20 × 10 cm grid pattern under flooded conditions with two replicates. Recommended management practices used to raise crops. After harvesting and drying, 20 g of dehulled brown rice from each sample was ground for the functional component analysis.

2.3. Estimation of functional components in brown rice

2.3.1. Estimation of GABA content

The GABA content was determined using a colorimetric method [35]. Details of estimation procedures of GABA content in brown rice are available in the authors' other published article [34] and supplementary data (Table S1).

2.3.2. Estimation of RS content

The estimation of RS content in brown rice for our present study was performed following the Goñi method [36], with minor modifications. Details of estimation protocols of RS content in brown rice are available in the authors' other published articles [34,37] as well as in supplementary data (Table S1).

2.3.3. Estimation of total flavonoids content

The total flavonoid content was determined using the $NaNO_2$–$Al(NO_3)_3$ method [38]. The estimation of the total flavonoids content in brown rice for the current study has been discussed in detail in the authors' other published article [34] and has also been made available in supplementary data (Table S1).

2.3.4. Estimation of alkaloids content

The acid dye colorimetry method [39] was used for the estimation of alkaloids content in brown rice for the current study [39]. Details of estimation procedures of alkaloids content have been discussed in the authors' other published article [34,37] and are available in supplementary data (Table S1).

2.4. Data analysis

R software, version 3.4.2, was used for the descriptive statistical analysis, frequency distribution, one-way ANOVA, and difference significance analysis. Multiple comparisons of the means were performed using Tukey's test [40]. A graph of the geographical distribution of the four functional components of brown rice from 31 countries was created using the StatPlanet software [41].

3. Results

3.1. Geographic difference and variation of functional components in brown rice from five continents

The mean and other related parameters of GABA, RS, total flavonoid, and alkaloid contents in brown rice genotypes from Asia, Africa, the Americas, Europe, and Oceania are shown in Tables 2 and 3. All four functional components of brown rice from the five continents exhibited differences and wide variations. The hierarchy in average GABA content (mg·100 g^{-1}) in brown rice was Europe (10.43 ± 2.62) > Asia (10.40 ± 3.08) > America (9.73 ± 2.54) > Africa (9.49 ± 2.80) > Oceania (8.71 ± 2.17), the order of coefficient of variation for GABA was Asia > Africa > America > Europe > Oceania, ranged from 24.98 to 29.61 %. However, there were no significant differences between the groups. The order based on the average RS content (%) in brown rice was Asia (1.65 ± 0.58) > Europe (1.61 ± 0.40) > America (1.53 ± 0.46) > Africa (1.40 ± 0.53) > Oceania (0.98 ± 0.31), the order of coefficient of variation for RS was Africa > Asia > Oceania > America > Europe, ranged from 25.11 to 37.65 %. The difference in RS content between germplasm lines from Asia and Oceania was highly significant ($p \leq 0.01$). The average content of total flavonoids (mg·100 g^{-1}) o brown rice was Oceania (138.18 ± 18.43) > Asia (136.18 ± 37.76) > America (134.50 ± 21.49) > Africa (126.79 ± 56.82) > Europe (122.10 ± 17.31), and the order of the coefficient of variation for total flavonoids was Africa > Asia > America > Europe > Oceania, ranging from 13.33 % to 44.81 %. There was a highly significant difference in the total flavonoid content between Asia and Africa ($p \leq 0.01$). Considering the average content of alkaloids (mg·100 g^{-1}) in brown rice, the order was Asia (37.70 ± 10.45) > Africa (36.62 ± 8.84) > Europe (33.34 ± 8.79) > America (32.72 ± 10.07) > Oceania (31.11 ± 6.07), the order of the coefficient of variation for alkaloids was America > Asia > Europe > Africa > Oceania, ranging from 19.51 % to 30.77 %. However, the differences were not statistically significant.

3.2. Geographic difference and variation of functional component contents in brown rice from different countries

The geographical distribution of the four functional components based on 690 mini-core collections of brown rice from 31 countries is shown in Fig. 1. The means and other related parameters of the four functional components in brown rice from 31 countries and the IRRI are provided in supplemental data (Table S2). All four functional components of brown rice from different genotypes from 31 countries and from the IRRI showed differences and variation. The average GABA content of brown rice was highest in a sample from North Korea (16.95 mg·100 g^{-1}) and was lowest in a sample from Uganda (6.46 mg·100 g^{-1}). The order of countries for average GABA content was North Korea > Indonesia > Italy > Thailand > Russia > Philippines > Nepal > Brazil > Portugal > India > Bulgaria > Myanmar > China > Central Africa > Iran > Malaysia > IRRI > Vietnam > France > Cote d'Ivoire > Japan > the USA > Bangladesh > Australia > Sri Lanka > Egypt > Ethiopia > South Korea > Romania > Pakistan > Hungary > Uganda. The coefficient of variation ranged from 1.22 % (Hungary) to 53.90 % (Myanmar). There were significant differences in the GABA content among samples originating from Indonesia, China, IRRI, Japan, and Australia ($p \leq 0.05$).

The average RS content was lowest (0.70 %) in the brown rice from Uganda, whereas brown rice from Myanmar was found to have the highest RS enriched germplasms (2.36 ± 0.36 %). The average RS content in brown rice was as follows: Myanmar > South Korea > Portugal > Philippines > Bulgaria > Ethiopia > Hungary > Iran > Malaysia > Thailand = North Korea > IRRI= Pakistan > India > Sri Lanka > Central Africa > USA= China > Indonesia > Vietnam > Italy > Japan > Russia > Egypt > Brazil > France > Nepal > Romania > Australia > Cote d'Ivoire > Bangladesh > Uganda. The coefficient of variation ranged from 0.31 % (Nepal) to 58.82 % (Egypt). There were significant differences between the Philippines and Australia ($p \leq 0.05$).

The average content of total flavonoids was maximum (180.37 ± 113.99 mg·100 g^{-1}) in brown rice accessions of Cote d'Ivoire, while it was least (103.53 ± 3.58 mg·100 g^{-1}) in Thailand accessions. The average content of this functional component in brown rice was ranked in the following order: Cote d'Ivoire > Vietnam > Philippines > Iran > Australia > China > France > USA > Sri Lanka > Pakistan > Nepal > North Korea > IRRI > Brazil > South Korea > Malaysia > Portugal > India > Japan > Hungary > Russia > Indonesia > Italy > Bulgaria > Bangladesh > Central Africa > Ethiopia > Romania > Uganda > Egypt > Myanmar > Thailand. The coefficient of variation of total flavonoids in brown rice ranged from 3.10 % (South Korea) to 63.20 % (Cote d'Ivoire). However, there were no significant differences between the groups.

The average content of alkaloid in brown rice from 31 countries and IRRI ranges from 23.56 mg·100 g^{-1}(Italy) to 45.78 mg·100 g^{-1}(Bulgaria). The order of countries with high to low average alkaloid contents was Bulgaria > Philippines > Iran > North Korea > Central Africa > Myanmar > Pakistan > Ethiopia > Sri Lanka > IRRI > Portugal > Indonesia > China > India > Hungary > Nepal >

Table 2

Coefficient of variation and differences of GABA and RS content in brown rice from five continents.

Origin	Accessions	GABA (mg·100g^{-1})			RS (%)		
		Mean±*SD*	*CV%*	Range	Mean±*SD*	*CV%*	Range
Asia	646	10.40 ± 3.08A	29.61	5.30–27.04	1.65 ± 0.58A	35.26	0.33–3.48
Africa	18	9.49 ± 2.80A	29.46	4.72–14.51	1.40 ± 0.53AB	37.65	0.57–2.50
America	9	9.73 ± 2.54A	26.07	7.15–14.56	1.53 ± 0.46AB	30.03	1.20–2.70
Europe	9	10.43 ± 2.62A	25.17	7.34–15.08	1.61 ± 0.40AB	25.11	1.12–2.28
Oceania	8	8.71 ± 2.17A	24.98	7.26–13.77	0.98 ± 0.31B	31.31	0.42–1.49

Note: Mean values without the same capital letter indicated a significant difference at the level of 0.01.

Table 3
Coefficient of variation and differences of total flavonoids and alkaloids in brown rice from five continents.

Origin	Accessions	Total flavonoid ($mg\cdot100g^{-1}$)			Alkaloid ($mg\cdot100g^{-1}$)		
		Mean±*SD*	*CV%*	Range	Mean±*SD*	*CV%*	Range
Asia	646	136.18 ± 37.76A	27.73	90.61–435.91	37.70 ± 10.45A	27.72	15.96–60.78
Africa	18	126.79 ± 56.82B	44.81	93.57–347.78	36.62 ± 8.84A	24.13	22.41–52.18
America	9	134.50 ± 21.49AB	15.98	104.02–161.26	32.72 ± 10.07A	30.77	20.71–52.81
Europe	9	122.10 ± 17.31AB	14.18	93.29–150.46	33.34 ± 8.79A	26.35	23.56–45.78
Oceania	8	138.18 ± 18.43AB	13.33	107.00–169.31	31.11 ± 6.07A	19.51	24.02–38.94

Note: Mean values without the same capital letter indicated a significant difference at the level of 0.01.

Malaysia > Brazil > Cote d'Ivoire > Egypt > Russia > USA > Japan > Australia > Vietnam > France > South Korea > Bangladesh > Thailand > Romania > Uganda > Italy. The coefficient of variation ranged from 8.33 % (Iran) to 51.26 % (Brazil). There were highly significant differences in the alkaloid content of the Philippines compared with those of Vietnam and Japan ($p \leq 0.01$).

3.3. *Genotypic differences and variation of functional component contents in brown rice at two sowing times*

The average content and related parameters of the four functional components in the 690 mini-core collections of brown rice at the two sowing times were analyzed (Table 4). In this study, the growing season and environmental factors had greater effect on the functional components of brown rice, and the content of all functional components in brown rice was higher at late sowing than at early sowing. However, the difference between sowing dates was not statistically significant for GABA content.

Successional variations in GABA, RS, total flavonoid, and alkaloid content in brown rice at the two sowing times are shown in Fig. 2. Table 4 shows the variation ranges for GABA content at sowing times I and II were 2.58–23.21 $mg\cdot100\ g^{-1}$ and 2.13–31.42 $mg\cdot100\ g^{-1}$, the coefficient of variation ranged from 39.18 % to 28.45 %, respectively. As shown in Fig. 2(a and b), there was a peak in GABA content at 7–11 $mg\cdot100\ g^{-1}$ with a frequency of 55 % and 115 % at sowing times I and II, respectively. Normal distribution patterns were observed for sowing time I, whereas skewed distribution patterns were observed for sowing time II. Based on the average GABA content at both sowing times, the Chinese cultivar Zhejing22 had the highest amount (27.04 $mg\cdot100\ g^{-1}$), and the African cultivar NERICA-L-1 had the lowest amount (4.72 $mg\cdot100\ g^{-1}$), wherein the GABA content of three rice germplasms was above 20 $mg\cdot100\ g^{-1}$, and two accessions were from China and one from the Philippines (Table S3).

The RS content at sowing times I and II varied between 0.32–3.75 % and 0.32–3.46 %, respectively, and the coefficient of variation was 43.33 %–38.42 %, respectively (Table 4). As shown in Fig. 2(a and b), the RS content was 0.9–1.6 % with a frequency of 45 and 40 % at sowing times I and II, respectively. RS content appeared to be normally distributed at sowing times I and II. Therefore, RS content presented a wide range of genetic variations and genotypic differences in the 690 mini-core collections of brown rice. Based on the average RS content at both sowing times, the Chinese cultivar Hemeizhan had the highest amount (3.48 %), and the Chinese cultivar Dandongludao had the lowest (0.33 %). The RS content of nine rice germplasms was above 3 %, seven accessions were from China, and one was from the Philippines and India (Table S3).

The total flavonoid content at sowing times I and II varied between 71.40 and 437.20 $mg\cdot100\ g^{-1}$ and 89.14–448.54 $mg\cdot100\ g^{-1}$, respectively, and the coefficient of variation was 29.76 %–30.96 %, respectively (Table 4). As shown in Fig. 2(a and b), there was a peak in total flavonoid content in 100–140 $mg\cdot100\ g^{-1}$ with the frequency of 115 % and 135 % at sowing times I and II, respectively and the total flavonoid content appeared to have a skewed distribution at sowing times I and II. Based on the average performance of total flavonoids contents at both sowing times, the Chinese cultivar Yanshuichi had highest amount (435.91 $mg\cdot100\ g^{-1}$), and the Chinese cultivar Tieganwuhad lowest amount (90.61 $mg\cdot100\ g^{-1}$), wherein the total flavonoids content of 17 rice germplasms were above 250 $mg\cdot100\ g^{-1}$, 13 accessions were from China, one from each of IRRI, the Philippines, Vietnam and Côte d'Ivoire (Table S3).

The alkaloid content at sowing times I and II varied between 11.45 and 63.29 $mg\cdot100\ g^{-1}$ and 15.03–60.92 $mg\cdot100\ g^{-1}$, respectively, and the coefficient of variation was 29.98 %–27.11 %, respectively (Table 4). Fig. 2(a and b) shows a peak of alkaloid content at 30–40 $mg\cdot100\ g^{-1}$ with a frequency of 55 and 50 % at sowing times I and II, respectively. Alkaloid content appeared to have a normal distribution at both sowing times. Therefore, the alkaloid content presented a wide range of genetic and genotypic differences in the 690 mini-core collections of brown rice. Based on the average performance of alkaloid content at both sowing times, the Chinese cultivar Xianghui91269 had the highest amount (60.78 $mg\cdot100\ g^{-1}$), and the Chinese cultivar LCH-135 had the lowest amount (15.96 $mg\cdot100\ g^{-1}$), and the alkaloid content of 12 rice germplasms was above 58 $mg\cdot100\ g^{-1}$, in nine accessions from China, two from the Philippines, and one from the IRRI (Table S3).

4. Discussion

Brown rice contains bioactive components that improve health and can be used as adjunctive therapy for several chronic diseases [9,10]. However, the differences in cultivated types and variations in the functional components of international rice resources remain largely unknown. In this study, geographic differences and variations in the functional components of brown rice in 690 mini-core collections from global germplasms were analyzed. The conclusions revealed for the first time that Asian countries had the most prominent and representative zones with higher genotypic potential for functional components in brown rice, and had several rice accessions with enriched functional components that were screened. This study provides a reference for functional rice breeding and

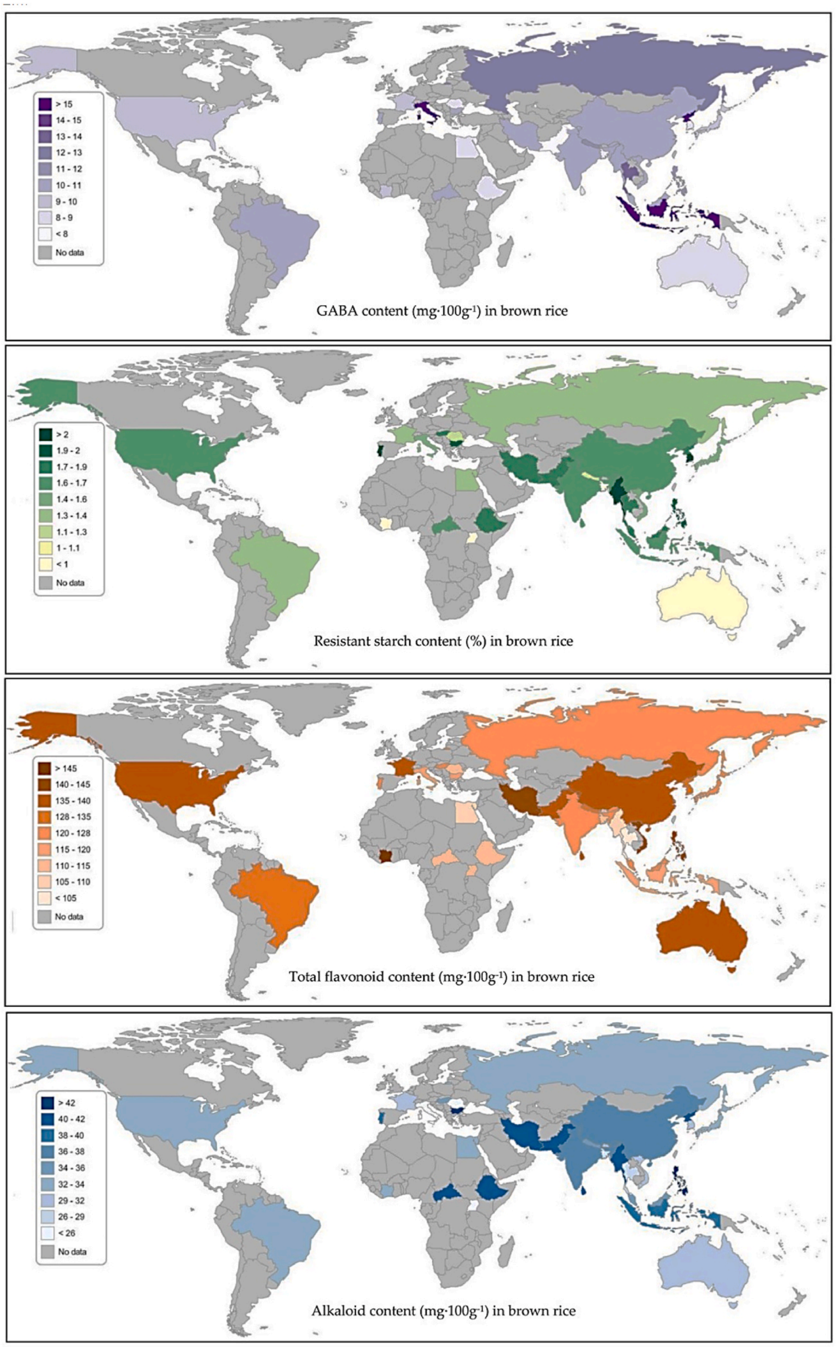

Fig. 1. Geographical distribution of four functional components based on 690 mini-core collections of brown rice from 31 countries. The colors purple, green, orange and blue indicate GABA, Resistant starch, Total flavonoids and Alkaloid content, respectively. The deeper the color, the higher the functional component content of brown rice in this region; conversely, the lighter the color, the lower the functional component content of brown rice in this region. Gray indicates that there is no data in the region. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

functional food development to prevent chronic diseases in humans.

4.1. Regional differences and variations of four functional components in brown rice from five continents

The results of this study indicate that all four functional components in brown rice of different genotypes from the five continents

Table 4
Coefficient of variation and differences in functional components content of brown rice at two sowing times.

Functional Component	Sowing time	*CV%*	Range	Mean ± *SD*	95 % confidence interval		*P* value
					Lower limit	Upper limit	
GABA ($mg{\cdot}100g^{-1}$)	I	39.18	2.58–23.21	10.26 ± 4.02	−0.19	0.56	0.33
	II	28.45	2.13–31.42	10.44 ± 2.97			
	Mean	29.52	4.72–27.04	10.35 ± 3.06			
RS (%)	I	43.33	0.32–3.75	1.50 ± 0.65	0.21	0.35	0.00**
	II	38.42	0.32–3.46	1.77 ± 0.68			
	Mean	35.48	0.33–3.48	1.64 ± 0.58			
Total flavonoids ($mg{\cdot}100g^{-1}$)	I	29.76	71.40–437.20	131.65 ± 39.18	3.84	12.57	0.00**
	II	30.96	89.14–448.54	139.85 ± 43.30			
	Mean	27.87	90.61–435.91	135.75 ± 37.84			
Alkaloids ($mg{\cdot}100g^{-1}$)	I	29.98	11.45–63.29	36.72 ± 11.01	0.38	2.63	0.01**
	II	27.11	15.03–60.92	38.22 ± 10.36			
	Mean	27.68	15.96–60.78	37.47 ± 10.37			

Note: ** indicated a significant difference at the level of 0.01.

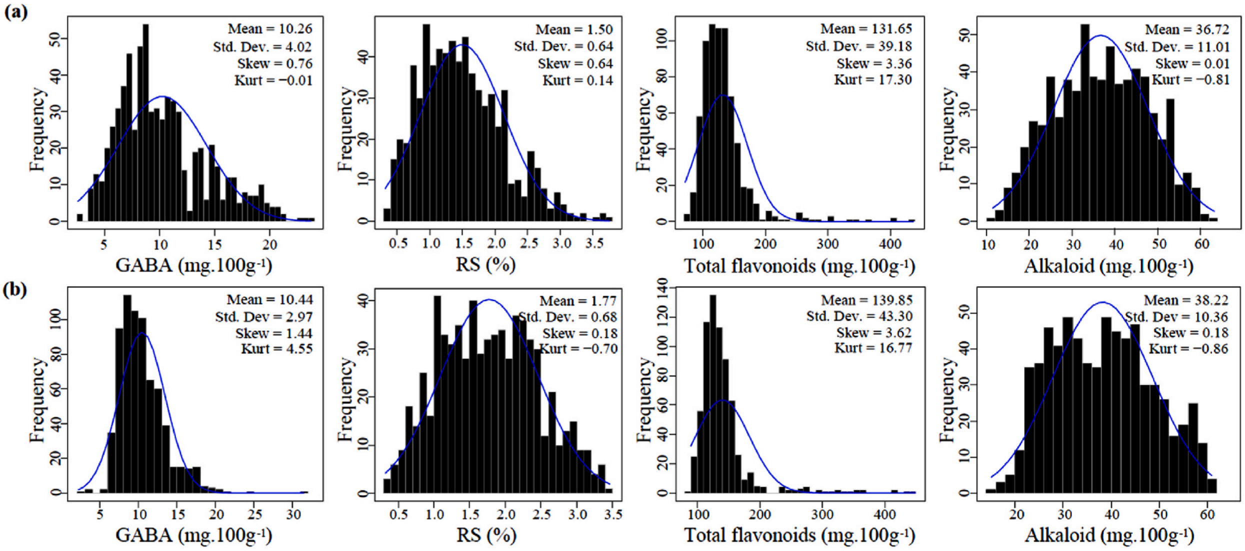

Fig. 2. Frequency distribution of 690 mini core collection of brown rice based on four functional component contents at the two sowing times. (a) Represents sowing time I; (b) represents sowing time II. Mean, Std. Dev, Skew and Kurt represent the average, standard deviation, skewness and kurtosis values of the distribution curves obtained, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

have certain differences and wide variations. The overall results of the variation coefficients and ranges of the four functional components of brown rice in the five continents indicated that Asia was the most prominent and representative zone, with the highest genotypic potential for functional components in brown rice. Crop species and the diversity between and within them have significant socioeconomic and heritage value [42]. Asia has four out of eight Vivilov centers of crop origin and diversity [43], with 379 crops and more than 40 anticancer crop species, leading to the lowest prevalence of cancer and the lowest mortality in the world [8]. Zeng et al. [44] suggested that the migration of early humans from Africa to Asia and then to Eurasia was related to the physiological activity of modern human chronic diseases. Social determinants, such as sustaining healthy eating behavior, may contribute to 80–90 % of the health outcomes of a population, and ancient Asian people consume more than 90 % of brown rice [45,46].

In this study, brown rice from Asia was superior to that from other continents with respect to the average content of functional components, except for GABA and total flavonoid content, which were the highest in brown rice from Europe and Oceania. The RS content (%) in brown rice of Asian germplasms (1.65 ± 0.58) was significantly higher than that in rice germplasms (0.98 ± 0.31) from Oceania. The total flavonoid content ($mg{\cdot}100\ g^{-1}$) in brown rice from Asian germplasms (136.18 ± 37.76) was significantly higher than that from African rice germplasms (126.79 ± 56.82). The extraction yield of total flavonoids can reach a theoretical value of 80 $mg{\cdot}100\ g^{-1}$ [47]. However, the differences between the other continents were not statistically significant. Therefore, to a certain extent, there was a significant difference ($p < 0.01$) in the RS and total flavonoid content in brown rice between the eastern and western germplasms. Previous reports have shown that the zonal characteristics of functional components of global barley germplasms, and Zeng et al. [32] found significant differences between western barley accessions from the Fertile Crescent (Europe and America) and southern barley lines from Central to Far East Asia. The total flavonoid and GABA content (mg·100 g^{-1}) in grains of Asian barley lines (123.09 ± 29.56 and 9.49 ± 4.34, respectively) were significantly higher than those in American barley lines (103.85 ± 22.33 and 7.38 ± 3.59, respectively), supporting similar genotypic differences and geographic characteristics in the content of functional components in brown rice in this study. Similarly, the RS, total flavonoid, and GABA content in brown rice from the core collection of Yunnan local rice [31] and its mineral elements [48] also showed obvious zonal characteristics. In addition, the difference in GABA and alkaloid content in brown rice was not significant among global germplasms from Asia, Africa, America, Europe, and Oceania, which might be because the 690 mini-core collection cultivars were mostly crossbred offspring across the five continents. The rice samples collected from Africa, America, Europe, and Oceania in this study were relatively limited and may not be representative, influencing the results of this study.

4.2. *Regional differences and variations of four functional components in brown rice from different countries*

The results of this study indicate that all four functional components of brown rice of different genotypes from 31 countries and IRRI have differences and variations. In this study, considering the top half of the countries with high GABA, RS, total flavonoids, and alkaloids in brown rice, more than two thirds were in Asia. North/South Korea, Myanmar, Indonesia, Vietnam, and the Philippines ranked among the top three countries with the highest averages for the four functional components in brown rice. To some extent, these findings may support the belief that crop origin centers are the richest hubs for available functional food crops because Asia has four crop origin centers with the highest biodiversity and origin of ample anticancer crops in the world.

In this study, the GABA content (mg·100 g^{-1}) in brown rice of Indonesia germplasms (15.29 ± 3.32) was higher than that of China (10.42 ± 3.09), IRRI (10.03 ± 3.27), Japan (9.41 ± 2.13), and Australia (8.71 ± 2.17). The RS content (%) in brown rice from the Philippines germplasms (1.95 ± 0.69) was higher than that in brown rice from Australia (0.98 ± 0.31). The alkaloid content (mg·100 g^{-1}) in brown rice from the Philippines germplasms (45.13 ± 9.32) was higher than that of Japan (32.19 ± 7.17) and Vietnam (30.53 ± 7.01). However, the differences between the other countries were not significant. Similarly, the GABA content (mg·100 g^{-1}) in barley accessions from China (9.99 ± 4.59) was higher than that from the United States (8.31 ± 2.17) [49] and the GABA content in Qinghai-Tibet Plateau hull-less barley grains was higher than that of ICAR in the dry areas [50]. While preliminarily studying the functional ingredients in brown rice from different regions and different types of varieties, Du et al. [51] found that GABA and total flavonoid contents in brown rice were significantly different between different types of varieties originating from China, Japan, Korea, and the IRRI. The hierarchy of the average GABA content in brown rice was China (0.0180 ± 0.0069 %) > South Korea (0.0170 ± 0.0042 %) > IRRI (0.0165 ± 0.0070 %) > Japan (0.0161 ± 0.0020 %), and there were significant differences in the GABA content of IRRI when compared with that of China, South Korea and Japan. The average total flavone content of the countries was as follows: China (0.429 ± 0.212 %) > Japan (0.383 ± 0.099 %) > IRRI (0.368 ± 0.059 %) > South Korea (0.313 ± 0.112 %). However, there was no significant difference among them, supporting similar genotypic differences and geographic characteristics in the content of functional components in brown rice in this study.

The average RS content was the highest in the brown rice from Myanmar, which might be because a substantial proportion of their rice cultivars are traditional rice varieties that generally have higher RS than high-yielding varieties, and the rice processing technology is more rudimentary. The adoption rate of modern high-yielding varieties of rice in Myanmar is relatively low and rather sluggish compared to other rice-growing countries in Asia because of several socioeconomic factors and the popularity of traditional rice varieties with better adaptability to abiotic stresses [52,53], which has resulted in the stagnation of rice production in the country and down gradation to a minor player in the global rice trade from the largest rice exporter in the world [54]. The functional components of brown rice are mainly concentrated in the cortex and embryos, which make up approximately 10 % of the weight of brown rice. Polished rice as a staple food is more likely to cause chronic diseases than brown rice [31]. Interestingly, the abundance of RS in traditional rice landraces may be associated with a lower prevalence of diabetes in Myanmar. According to the International Diabetes Foundation, Myanmar has the lowest national prevalence of diabetes (4 % against 8.8 % of the global prevalence) among 22 IDF member countries in the Western Pacific region and six IDF member countries in Southeast Asia [55].

Bioactive compounds in brown rice, such as GABA, flavonoids, and alkaloids, also have the potential to prevent cardiovascular diseases (CVDs) [5,27]. CVD is a leading cause of diet-related deaths worldwide. Systemic analyses of the causes of chronic human diseases revealed that CVD was the leading cause of global health loss and mortality, and ischemic heart disease followed by stroke was identified as the major cause of CVD-associated health loss [56,57]. In 2015, the all-age mortality rates of both sexes due to ischemic heart disease were 51.48, 78.66 and 91.47 per million and those due to ischemic stroke was (18.56, 52.7 and 17.34 million^{-1}) in Cote d'Ivoire, Vietnam, and the Philippines, which were the top three countries with the highest average flavonoid content in brown rice but lower mortality rates (<100 per million) [57]. There may be a correlation between the high total flavonoid content and hepatitis treatment in Yunnan, China, where wild rice (with a total flavonoid concentration of 0.751 %) and herbal medicines are used to treat hepatitis and other diseases [51]. Therefore, the functional component content of brown rice, along with other determinants, may be related to the prevalence of chronic diseases in the countries studied. However, despite the health benefits of the bioactive compounds in brown rice, it is often difficult to conclude a direct causal relationship between the functional component content of brown rice and the prevalence of chronic diseases in a country. The health benefits of these phytochemicals depend not only on the quantity of brown rice but also on the level of brown rice intake as a daily diet by a given population. Many other dietary and livelihood determinants also play an important role in the prevalence of chronic diseases.

Previous reports have shown that the zonal features of brown rice for functional components have been studied previously, and significant variations were also reported for all four functional components of 905 rice germplasms collected from 16 prefectures of Yunnan, showing that the functional components in brown rice had regional traits, and they originated from the richest biodiversity areas of Wumeng Mountain, Ailao Mountain, Hengduan Mountain, and Gaoligong Mountain, and the basins of the Jinsha River, Pearl River, Red River, Lancang River, Nujiang River, and Irrawaddy River [31]. The zonal characteristics of brown rice regarding the mineral constituents were related to several factors, such as biodiversity centers, enrichment areas of mineral resources, origin of mankind, and distribution of rivers and mountains [48,58]. In this study, RS and GABA contents in brown rice were higher than those reported by Zeng et al. [31], whereas the total flavonoid content was lower than that reported by Zeng et al. [31], which may be due to the use of different varieties. Additionally, the number of brown rice accessions from different countries varied greatly, which may have affected the results of this study.

4.3. Genotypic differences and variation of four functional components in brown rice at two sowing times

In general, sowing time defines the environmental conditions to which crop plants are exposed throughout their life cycle, affecting the maturity or grain-filling stage. Previous studies have suggested that advanced or delayed sowing of cereal crops has a remarkable impact on different bioactive compounds and groups of phytochemicals [59–62]. Sowing of ten corn genotypes in the early season, characterized by the prevalence of relatively high temperatures in the temperate region, revealed that in corn grains, the concentration of secondary metabolites such as phenolic compounds and β-cryptoxanthin increased by 5 % and 23 %, respectively, and that of lutein and flavonoids (total anthocyanin) decreased by 18 % and 21 %, respectively [62]. In this study, the content of four functional components in brown rice was higher at late sowing than at early sowing, and the differences were highly significant, except for GABA. The effect of the growing environment on the content of these four active components in brown rice was evident in our study, and late sowing was more suitable for the enrichment of these components in brown rice.

Yang et al. [63] conducted field experiments in Xinping, Yuxi and Xundian to investigate the functional components and their environmental variation among different rice genotypes including 14 cultivars from Yunnan. The GABA content was influenced only by the growing environment, whereas the content of total flavonoids and alkaloids was influenced by genotype, and growing environment and genotype interaction, and RS was influenced only by genotype. The secondary metabolism of plants can produce stress-induced changes owing to changes in environmental temperature [64,65]. The variation in RS in field crops has been described as a consequence of environmental conditions, such as temperature, regulating the activity of the starch biosynthetic enzyme [66]. Alkaloid accumulation in seeds in response to variable environmental factors has been reported, and the synthesis of alkaloids was found to be accelerated by high ambient temperatures [67,68], which might act as a heat shock defense against adverse environments [69]. At late planting, the higher alkaloid content in brown rice in this study might indicate high-temperature stress at the grain-filling stage. Differential influences of environmental conditions on total flavonoid content have also been reported in plants, and in most cases, increased accumulation of flavonoids have been reported when plants were exposed to crucial abiotic stresses [70]. The total flavonoid content in brown rice at the two sowing times in this study was in contrast with a previous observation where the flavonoid content in rice brans of 64 germplasms at late planting was 827 mg·100 g^{-1}, which was 5.51 times higher than that at early planting [71]. In this study, except for the sowing dates, the planting site and its soil type, soil fertility, water and fertilizer management, and experimental materials were the same; therefore, the environmental impact was mainly reflected in the temperature. Our results were consistent with those previously reported.

The GABA, RS, total flavonoid, and alkaloid contents in this study showed a wide range of variation and genotypic differences. However, the functional component content of certain brown rice germplasms in the 690 mini-core collection was significantly higher than that of the Yunan rice landrace, which was analyzed and reported earlier [31]. Among the rice germplasms in the present study, three had a GABA content of more than 20 mg·100 g^{-1}, nine had RS content above 3 %, 17 had high total flavonoid content (above 250 mg·100 g^{-1}) and 12 had flavonoid content above 58 mg·100 g^{-1}. Therefore, the exploration and utilization of the functional components of the above mini-core collection from global rice germplasms has great prospects. In general, among all the tested varieties, 41 accessions with high functional components were screened, of which 31 accessions were from China, five from the Philippines, two from IRRI, and one from India, Vietnam, and Cote d'Ivore, which could be used as parents for breeding high functional component cultivars or directly for functional rice development and utilization.

5. Conclusions

Remarkable geographic differences in the four functional components of brown rice from five continents were evident from the larger variation coefficients and ranges of the four functional component contents in the 690 mini-core collections of brown rice in the present study. Overall, the Asian germplasms have more genotypic variation as well as higher average functional component contents compared to other continents. Europe and Oceania were associated with the lowest average functional components and minor genetic variability in their germplasms. The functionally enriched germplasms mostly originated from biodiversity-rich areas of China. To the best of our knowledge, this is the first study to reveal the regional characteristics of functional components in the core collection of global brown rice germplasms and their relationship with the incidence and prevalence of chronic diseases, such as diabetes and cardiovascular diseases. The growing season and environmental factors also significantly influenced the content of functional components in the mini-core collection of global brown rice germplasms, and late sowing was associated with substantial enrichment of RS, alkaloids, and flavonoids in brown rice. With the process of globalization and the rapid development of the food trade, functionally enriched rice germplasms can be used as important genetic resources for the development of high-yielding rice varieties with rich nutrition and functional components for domestic and foreign markets, which may play an important role in the treatment of chronic human diseases and malnutrition. The present study not only has theoretical significance in revealing the origin, evolution, and diversity of rice varieties with functional components, but also provides a reference for functional rice breeding, variety distribution, and industrialization. However, due to limited conditions, the number of samples tested in this study was relatively small, especially for foreign cultivars; therefore, further studies should be conducted with a proportionately higher sample number.

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Data included in article/supplementary material/referenced in the article.

CRediT authorship contribution statement

Xiaomeng Yang: Conceptualization, Writing – review & editing, Writing – original draft. **Md. Siddikun Nabi Mandal:** Methodology, Writing – original draft, Writing – review & editing. **Henan Diao:** Methodology, Formal analysis, Writing – original draft. **Juan Du:** Methodology, Project administration. **Xiaoying Pu:** Formal analysis, Investigation, Visualization. **Xia Li:** Formal analysis, Visualization. **Jiazhen Yang:** Investigation, Visualization. **Yawen Zeng:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Zichao Li:** Conceptualization, Resources. **Jianbin Li:** Supervision. **Akbar Hossain:** Writing – review & editing. **Muhammad Kazim Ali:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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