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THE DIVERSITY OF FREE AMINO ACIDS IN 14 REPRESENTATIVE VIETNAMESE RICE ACCESSIONS

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ABSTRACT

Rice (*Oryza sativa* L.) serves as the primary dietary staple for approximately half of the global population. Amino acids play a critical role in plant biology as they constitute the fundamental components of proteins. The free amino acid (FAA) content in rice holds nutritional significance due to its observable influence on the sensory qualities of cooked rice. In this study, 14 rice accessions from diverse ecosystems in Vietnam were selected for analysis of their FAA profiles to identify rice accessions possessing elevated levels of valuable FAA. High-performance liquid chromatography using the Biochrom 30+ system was employed for this purpose. The findings revealed substantial variability in FAA content among the rice accessions evaluated. Notably, the genotype G52 (BA TRANG HUONG) exhibited the highest concentration of FAA, indicating its potential utility as a genetic resource in breeding programs. Essential amino acids constituted approximately 36.1% of the total FAA content. Furthermore, significant differences in FAA, essential amino acids (EAA), and non-essential amino acids (NEAA) were observed between *Indica* and *Japonica* genotypes. Among the five amino acid families assessed, the glutamate and aspartate families demonstrated the highest concentrations. The outcomes of this study provide valuable insights that could inform future breeding efforts aimed at enhancing the FAA profile of rice varieties within the Vietnamese rice collection.

Keywords: amino acids, chromatography, diversity, *Oryza sativa* L.

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, particularly in Asia, and contributes significantly to daily nutritional requirements. The nutritional composition of rice varied significantly between accessions. *Indica* long-grain rice and *Japonica* round-grain rice are the two main varieties of rice (Ikehashi, 2009). While *Indica* rice is grown predominantly in Asia and is also known as aromatic rice, *Japonica* rice is primarily grown and consumed in Australia, China, and Taiwan (Awan et al., 2017). The major components of rice are carbohydrates, protein (amino acids), fats, fiber, minerals, and vitamins (Chaudhari et al., 2018).

Amino acids (AA) are essential nutrients for the growth of the human body and numerous biological processes, such as controlling gene expression, preventing the development of tumors, reducing obesity, and lowering blood pressure (Kowalska et al., 2022). On the other hand, the function, texture, consistency, and organoleptic characteristics of rice grains are significantly influenced by protein, where amino acids are the building components (Martin & Fitzgerald, 2002). Although there are over 300 amino acids in nature, only 20 are used as the building components of proteins in living things (Wu, 2009). In *O. sativa*, Asian rice exhibited the greatest overall variation in protein content (4–14%), while South American rice had the highest average protein content (7.9%). AAs are divided into two categories: nutritionally essential amino acids (EAAs) and non-essential amino acids (NEAAs). EAAs are histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val) while NEAAs are alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamic, glycine, proline, serine, taurine, and tyrosine (Hou et al., 2015). The majority of the essential amino acids, that must be supplied from the diet, are of plant origin (Galili et al., 2016). Malnutrition caused by the insufficient intake of AAs, especially the EAA, is a serious problem around the world. Small levels of free amino acids (FAA) are also present in rice grains that serve as their primary components, approximately 0.35–0.55% of the total amino acids (Yang et al., 2022). Despite being in small quantity, FAAs considerably increase the acceptability of rice grains by serving as a sensory-active flavoring ingredient in cooked rice. The taste rating of rice is directly correlated with the concentration of various FAA and soluble carbohydrates (Kasumyan, 2016). Isoleucine, leucine, lysine, and threonine all contribute to the aroma of cooked rice (Wong et al., 2008). Moreover, FAAs also play essential roles in plant growth, development, and responses to stress, and serve as important nutrients for human health (Yang et al., 2022).

Despite the wide variety of rice types in Vietnam, very few articles deal with as well as amino acid composition analysis. Therefore, it is essential to conduct further research on the amino acid content of different rice varieties and to offer more nutritional value for human health.

The present study has several objectives. First, the composition of FAAs in some representative Vietnamese rice accessions was determined. Second, the FAA composition, EAA, and NEAA content in *Indica* and *Japonica* genotypes were compared. The content of different AA families was also investigated. Lastly, among the studied rice varieties, the one with high EAA content was considered for breeding to improve the nutritional content of Vietnamese rice.

MATERIALS AND METHODS

Plant materials

Fourteen Vietnamese rice accessions, representing diverse geographical regions from the North to the South and encompassing genetically distinct rice species (*Indica* and *Japonica*) (Table 1), were analyzed to determine their FAA content.

Sample preparation for free amino acid analysis

Rice seeds were dried at 50−60 ^{o}C for 72 hours. The seeds were then peeled and sieved through an 840 µm sieve after being ground with a pestle and mortar. Two hundred mg of each sample was mixed with 1.5 mL of sodium accelerated buffer pH 2 (Biochrom, Cambridge, UK) and stirred for an hour at 1,000 rpm on a shaker (Eppendorf, Germany). After that, the mixture was centrifuged for 10 minutes at 15,000 rpm before being filtered through a 0.2 µm syringe filter (Sartorius AG, Germany). The supernatant was collected and subjected to a high-performance liquid chromatograph analysis (Biochrom 30 +, Cambridge, UK) (Nguyen et al., 2023).

Table 1. Fourteen Vietnamese rice accessions used for the study

ID	Name	Province	Sub_population
G165	GIONG 90 NGAY	Kien Giang	*Indica*
G18	NEP QUYT HAI DUONG	Hai Duong	*Indica*
G158	VA TAI ANA ACU	Ninh Thuan	*Japonica*
G16	NEP VAN RUONG HOA BINH	Hoa Binh	*Japonica*
G161	BN1	An Giang	*Japonica*
G85	CHANH CHUI	Thanh Hoa	*Japonica*
G182	KHAU MO	Son La	*Indica*
G140	LUA BAY DANH	Ben Tre	*Indica*
G145	BA RIA	Ben Tre	*Japonica*
G204	PLE DO	Dien Bien	*Japonica*
G152	LOC SOM	Bac Giang	*Japonica*
G155	KHAU PE LANH	Son La	*Indica*
G10	TAM SON NAM DINH	Nam Dinh	*Indica*
G52	BA TRANG HUONG	Quang Nam	*Indica*

Amino acid profile analysis by high - performance liquid chromatography - Biochrom 30+

An amino acid analysis method based on high-performance liquid chromatography was utilized to provide both qualitative and quantitative composition analysis. An established post-column, ninhydrin derivatization, and acetate buffers were used. In each analysis, 1 µL of the extracted FAA sample was used. The total run time was 60 minutes. The amounts of FAAs identified were based on the peak area of each amino acid and the amino acid standard curve (Thermo Scientific).

Statistical analysis

The experiment was repeated three times. The statistical analysis was performed on GraphPad Prism version 8.3 at a 5% significance level. The t-test and one-way ANOVA were used to analyze the differences between parameters. The data were presented as a mean ± standard deviation.

RESULTS

The diversity of FAA composition in the rice accessions

There was a wide variation in total FAA content between different rice accessions. The total FAA ranged from 1.23 to 4.41 mg/g in each accession in the *Indica* group, and from 1.37 to 3.04 mg/g in each accession in the *Japonica* group (Fig. 1). Among 14 studied rice accessions, the rice accession G52 (4.41 ± 0.03 mg/g) had the highest total FAA content, while the accession G165 had the lowest total FAA content (1.23 ± 0.05 mg/g). Among the found amino acids, cysteine, threonine, and isoleucine had the lowest content while methionine, glutamic, and serine had the highest content. Proline and lysine were rarely found in these 14 studied rice accessions and also in the Vietnamese rice collection.

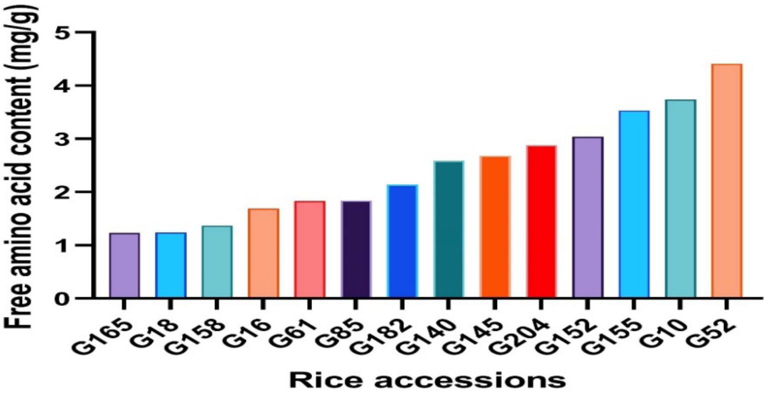

Figure 1. The total free amino acid content analyzed in 14 Vietnamese rice accessions

Essential amino acid composition in Vietnamese rice accessions

Table 2 lists the essential amino acid (EAA) composition, average content, and minimum and maximum content for each of our Vietnamese rice types. In our study, there were eight essential amino acids in rice grains. The leucine was hydrolyzed in acidic conditions, therefore it was not reported. Of all the FAA, EAA content makes up roughly 36.1% of the total. Three essential amino acids (methionine, isoleucine, and phenylalanine) were presented in the highest concentrations in G52, G10, and G155, respectively.

Table 2. The average, minimum, and maximum content of essential amino acids in rice accessions

Essential amino acids	Average	Min (mg/g)	Name	Max(mg/g)	Name
Histidine	0.035 ± 0.002	0.0115	G16	0.082	G155
Phenylalanine	0.036 ± 0.0011	0.02	G165	0.085	G155
Lysine	0.044 ± 0.001	0.006	G18	0.082	G204
Methionine	0.63 ± 0.005	0.17	G165	1.28	G52
Threonine	0.03 ± 0.002	0.014	G165	0.06	G155
Isoleucine	0.028 ± 0.004	0.013	G61	0.054	G10
Tryptophan	0.048 ± 0.0091	0.01	G61	0.27	G52
Valine	0.048 ± 0.0009	0.006	G16	0.063	G85

Family of amino acid composition

According to Horovitz & Paşca (2017), AA was classified into five families, which are the pyruvate family, serine family, aromatic family, aspartate family, and glutamate family, as shown in Figure 2. In the pyruvate family, alanine had the highest FAA content (0.164 ± 0.023 mg/g), followed by valine (0.048 ± 0.002 mg/g). Leucine was not found because of being degraded in water.

The aromatic family includes tryptophan (0.048 ± 0.0023 mg/g), tyrosine (0.083 ± 0.0015 mg/g), and phenylalanine (0.036 ± 0.0034 mg/g).

The glutamate and aspartate were the two families with the highest content. The glutamate family, which includes proline (0.28 ± 0.034 mg/g), arginine (0.1 ± 0.005 mg/g), histidine (0.035 ± 0.0036 mg/g), and glutamic acid (0.6 ± 0.078 mg/g). Glutamic acid had the greatest concentration in the glutamate family. The Aspartate family includes isoleucine, threonine, lycine, and methionine and its derivatives. Very low concentrations were obtained in isoleucine, threonine, and leucine; however, methionine and its derivatives had a very high content at 0.63 ± 0.12 mg/g.

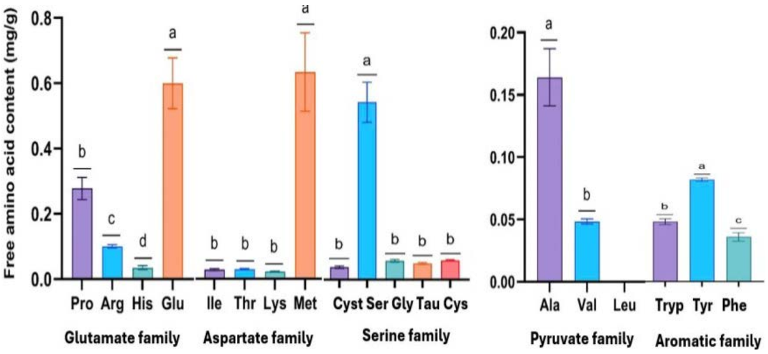

Figure 2. Amino acid content in five different families. Different letters indicate significant differences in concentration between amino acids in the same family

Lastly, the serine family has the highest serine (0.49 ± 0.05 mg/g). The four left amino acids including cysteic acid, glycine, taurine, and cysteine had relatively low content.

Free amino acid composition in *Indica* and *Japonica* rice subgroups

Among 14 rice accessions from the North to the South of Vietnam, there are seven accessions that belong to *Indica* and seven accessions that belong to *Japonica* subgroups. Table 3 shows the FAA compositions of these two subgroups, in which there were statistically significant differences between the two groups, in terms of taurine, serine, proline, tyrosine, phenylalanine, tryptophan, lysine, and arginine ($p < 0.01$ and 0.05). In contrast, no significant difference was obtained in the cysteic acid, methionine, and its derivatives, threonine, glutamic acid, glycine, alanine, cysteine, valine, isoleucine, and histidine concentration between the two subgroups ($p > 0.05$). Both *Indica* and *Japonica* accessions had high levels of methionine, serine, and glutamic acid, which help enhance the flavor quality of rice.

The difference in FAA content between the two subgroups was further analyzed not only in EAA but also in total FAA and NEAA. The results of Figure 3 showed that the *Indica* subgroup had a significantly higher total FAA (18.88 ± 0.35 mg/g) than the *Japonica* subgroup (16.13 ± 0.12 mg/g) ($p < 0.05$). The EAA and NEAA in the *Indica* subgroup were also higher than in the *Japonica* subgroup.

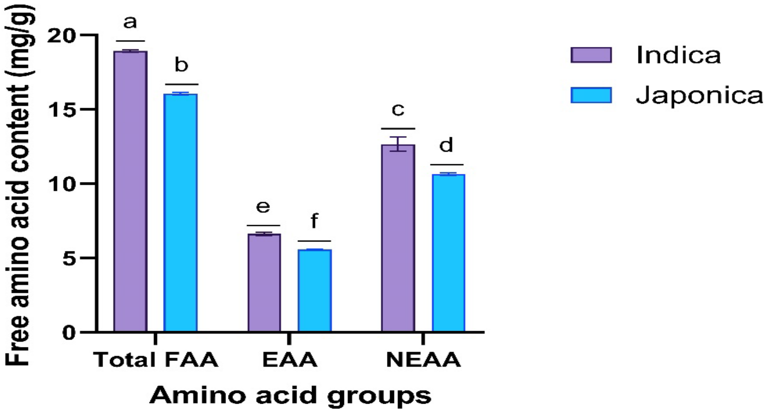

Figure 3. Variation in total free amino acid, essential, and non-essential amino acid between two rice subgroups. Different letters indicate significant differences in concentration between amino acids in the same category

Table 3. Comparison of free amino acid content in *Indica* and *Japonica* subgroups

Free amino acid	Content in subgroups (mg/g)	
Amino acid	*Indica*	*Japonica*
Cysteic acid	0.036 ± 0.002	0.032± 0.003
Taurine	**0.059 ± 0.001****	**0.03 ± 0.006**
Methionine and its derivatives	0.67 ± 0.023	0.6 ± 0.015
Threonine	0.031 ± 0.002	0.028 ± 0.001
Serine	**0.57 ± 0.06***	**0.41 ± 0.03**
Glutamic acid	0.6 ± 0.02	0.55 ± 0.11
Proline	**0**	**0.028 ± 0.001****
Glycine	0.048 ± 0.0008	0.055 ± 0.001
Alanine	0.16 ± 0.007	0.17 ± 0.008
Cysteine	0.053 ± 0.0007	0.054 ± 0.001
Valine	0.048 ± 0.005	0.048 ± 0.001
Isoleucine	0.033 ± 0.002	0.023 ± 0.001
Tyrosine	**0.075 ± 0.001**	**0.09 ± 0.007***
Phenylalanine	**0.043 ± 0.0009****	**0.029 ± 0.001**
Histidine	0.038 ± 0.005	0.033 ± 0.002
Tryptophan	**0.075 ± 0.009****	**0.022 ± 0.007**
Lysine	**0.006 ± 0.001**	**0.083 ± 0.002****
Arginine	**0.12 ± 0.006***	**0.082 ± 0.009**

Note: (*) and (**) indicate significant differences in amino acid concentration between the two subgroups with $p < 0.05$ and 0.01, respectively.

DISCUSSION

The diversity of FAA composition in the rice accessions

The amino acid composition of rice grains is a critical determinant of their nutritional quality. Enhancing the amino acid content in rice grains is a key objective in breeding programs aimed at improving the overall nutritional value of rice (Huang et al., 2019). The present study investigated the diversity of FAA content in a Vietnamese rice collection where we obtained a variation in the total FAA content in the 14 investigated rice accessions. The total FAA composition observed in rice was significantly lower than that reported for other grains such as wheat, barley (Nagaoka, 2005; Kowalska et al., 2022) and maize (Culea et al., 2015). Therefore, this marked difference in total FAA composition allows the separation of rice from other grains based on total FAA composition. However, the total FAA in our study was significantly higher than that in the consumed rice in Sri Lanka where the highest total FAA level was observed in Keeri Samba accession (0.73 ± 0.18 mg/g) (Mahanama et al., 2020).

Notably, lysine, an essential and limiting amino acid in rice, exhibits the highest concentration among grains, followed by proline (Amankwah, 2015). The elevated FAA content in our collection suggests a high likelihood of increased lysine levels.

Essential amino acid composition in Vietnamese rice accessions

EAA content observed in our study which accounted for about 36.1% was comparable to the findings made by Kamara et al. (2010), Amankwah (2015), Amrinola et al. (2022) for rice accessions found in Asia, but lower than that found in Nerica-1 local rice, Quarshie soybean, and Sinkarzieand F-Mix groundnut accession in Ghana (Amankwah, 2015). Among the three amino acids with the highest concentrations in our study, the levels of isoleucine and phenylalanine were lower than those reported by Braspaiboon et al. (2020).

However, the concentrations of methionine and its derivatives were higher compared to the findings of the same study.

Family of amino acid composition

In our study, the concentration of the pyruvate family ranged from 0.048 to 0.164 mg/g. The aromatic amino acid family exhibited relatively low concentrations. However, aromatic amino acids play a dual role, contributing to the aromatic profile of cooked rice while also serving as precursors for various secondary metabolites essential for protein biosynthesis. (Kasumyan, 2016). In the study of Nguyen et al. (2023), aromatic family content was increased three times in response to abiotic stress from the environment.

The Glutamate family was one of the families having the highest concentration. Interestingly, glutamic acid adds to the distinctive flavor of food. L-glutamate, especially if it is in the form of Na-glutamate, can give food an intensely savory (umami) flavor (Kawai et al., 2009). Umami flavoring has been employed to enhance the global appeal of food. The polarity, structure, or configuration (L or D) of the amino acid has an impact on how much it contributes to flavor (Dajanta et al., 2011). Therefore, our rice collection has be potential for purifying glutamic acid for the food industry.

In the aspartate family, only methionine and its derivatives were found at a high concentration which is a very promising result since methionine is often limited in the seed of cereals (Yang et al., 2022). In the serine family, serine had the highest content. L-serine has been known to play an important role in plant metabolism, signalling, and responses to both biotic and abiotic stresses (Mishra et al., 2022). Thus, the high-containing serine rice plants can have more chances to survive and develop under stress conditions.

Free amino acid composition in *Indica* and *Japonica* rice subgroups

A statistically significant difference in the taurine, serine, proline, tyrosine, phenylalanine, tryptophan, lysine, and arginine concentration was found between the two *Indica* and *Japonica* subgroups. Significant differences in some AA content were also found between *Indica*- and *Japonica*-type in 49 rice accessions in the study of Kamara et al. (2010). In some studies, the FAA composition was used to distinguish the *Indica* and *Japonica* subgroups (Hu et al., 2014; Yang et al., 2014). Moreover, a very high concentration of glutamic acid was found in both the *Indica* and the *Japonica* subgroups which is similar to Mahanama et al. (2020) who performed the analysis in the consumed rice in the capital city of Sri Lanka.

Furthermore, the EAA and NEAA content in the *Indica* subgroup was higher than in the *Japonica* subgroup, which indicated that in terms of protein quantity, *Indica* is superior to *Japonica* in our rice collection. However, the total AAs and EAA in the *Japonica* subgroup exhibited a greater abundance than the *Indica* rice subgroup consumed in Portugal (Rego et al., 2018).

CONCLUSION

Our findings revealed a promising result in terms of FAA concentrations across different rice accessions. Among the genotypes analyzed, G52 (BA TRANG HUONG) exhibited the highest FAA content, suggesting its potential as a valuable genetic resource for breeding programs. Notably, lysine, the most nutritionally essential amino acid, was present at a high concentration within our rice collection. Furthermore, significant statistical differences were observed in the FAA, EAA, and NEAA content between the *Indica* and the *Japonica* genotypes. Among the five amino acid families, the glutamate and aspartate families were the most abundant. This study provides a foundation for further research on a larger rice collection to identify accessions with high concentrations of essential amino acids, which could be utilized in future breeding programs.

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