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Investigating the diversity of resistant starch in Vietnamese rice collection

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Abstract:

The rapid increase of obesity and type 2 diabetes has recently posed an enormous burden on the healthcare system worldwide. Resistant starch (RS) in rice can escape digestion by enzymes in the small intestine, making its calories unavailable for cells to use. As a result, RS can be used by diabetes patients to prevent diabetes and for obese individuals who do not want extra energy. In our study, 75 Vietnamese rice accessions originating from diverse ecosystems were chosen as plant materials to investigate the diversity of RS content in this collection. The Megazyme kit was used to measure the amount of RS. The release of quinonimine was measured using a spectrophotometer at 510 nm. The results showed that approximately 70% of Vietnamese rice accessions had RS content ranging from 0.015 to 0.2% while only 4% of samples had RS content ranging from 0.6 to 0.8%. The *Indica* subgroups had significantly higher RS content than the *Japonica* subgroup. Higher RS content was found in medium- and short-grain rice rather than in long grain. Finally, rice plants grown in rainfed lowlands (RL) and irrigated ecosystems had higher RS content than those grown under mangrove and upland ecosystems. Our results firstly give information about the diversity of RS in Vietnamese rice and secondly may contribute to the field of nutrition by developing a suitable rice-based diet for patients with diabetes or obesity.

Keywords: **diabetes, diet, obesity, resistant starch, rice, starch.**

Classification numbers: **3.4, 3.5**

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for half of the world's population. Rice is also an excellent source of starch, which is then hydrolysed by enzymes and converted into glucose that cells directly use to produce energy for their activities [1]. However, if the energy supply exceeds the energy requirement, it will be converted into glycogen or fats. Nowadays, thanks to social development and increases in quality of life, overeating and lack of exercise has increased the number of people having obesity and type 2 diabetes. In 2017, approximately 462 million people were affected by type 2 diabetes corresponding to 6.28% of the world's population. The global prevalence of type 2 diabetes will increase to 7,079 individuals per 100,000 by 2030, which will pose a significant burden to the healthcare system [2]. Therefore, solving the problem of diabetes and obesity based on a healthier diet is a priority.

Based on enzymatic hydrolysis, starch is composed of a large digestible starch, which is classified into two types: rapidly digested starch, slowly digested starch, and a small amount of indigestible starch also known as RS [3]. Traditionally, it was assumed that starch was completely digested. It is now known that a portion of starch resistant to digestion by human enzymes in the small intestine will move into the large intestine where it may or may not be fermented by gut bacteria [4]. As a result, RS serves as a dietary fibre, lowers calorie intake, has a low glycaemic index, and gives various advantages to colon health. When consumed, it promotes gut health, reduces glucose and

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insulin reactions, and minimises cardiovascular disease risk factors [5]. The RS content of rice ranges from 0.1 to 25.4% [6]. Consuming 18-20% of RS per day is recommended.

RS is classified into five categories based on enzyme resistance [7]. RS1 is starch that is physically protected in cell or tissue structures and is not available for enzymatic hydrolysis in the small intestine. RS2 is present in dehydrated high-amylose starch granules and has a tight form that prevents digestive enzymes from accessing the starch. RS3 is a gelatinised and retrograded starch mainly composed of amylose leached from starch granules after being hydrated. RS4 is a starch that has been chemically modified to prevent enzymatic hydrolysis. Finally, RS5 combines helical amylose and fatty acids that resist amylase digestion [8].

Several studies have assessed the genetic diversity of RS in rice. Natural genetic diversity may be accessed through breeding to improve RS content in high amylose haplotypes with minimal influence on processing quality and cooked rice texture [9]. Q. Li, et al. (2018) [10] identified the *Waxy* gene, which regulated amylose content, whereas eight soluble starch synthases coordinate amylopectin. Other studies indicate that RS was connected with starch synthase *IIa*, *Waxy*, and other starch synthesis-related genes in rice varieties with amylose levels [11, 12]. According to S. Parween, et al. (2020) [12], RS is predominantly linked to the starch synthase *IIa* gene. To find the quantitative trait locus (QTLs) and candidate genes relating to RS, seven QTLs were associated with the RS content among which the SNP 6 m1765761 was located on *Waxy*. Starch branching enzymes IIa (BEIIa), close to QTL *qRS-I4*, were detected and further identified as a specific candidate gene for RS in *Indica* [13]. These studies show that a complicated metabolite network regulates the formation of RS in rice. Even though Vietnam has an extensive collection of rice, there have not been any studies investigating the diversity of RS content within a Vietnamese rice collection.

Therefore, this study aims to determine the content of RS from common rice varieties in Vietnam. The new findings from this study contribute to the global development of the rice field, assist plant breeders and food processors in attempts to develop new rice varieties, and partly solves the diet problem for individuals with obesity and type 2 diabetes.

2. Materials and methods

2.1. Plant materials

The rice population in this study consisted of 75 characterised varieties that were grown in the Vinh Ngoc commune, Hanoi [14].

2.2. Chemicals

Kits: The RS assay kit (Rapid) (Megazyme, Ireland) was composed of amyloglucosidase (AMG) (12 ml, 3,300 U/ml on soluble starch), pancreatic α-amylase (pancreatin, 10 g, 3 ceralpha units/mg), glucose oxidase/peroxidase reagent (GOPOD) reagent buffer**,** and GOPOD reagent enzymes.

2.3. Other chemicals

Maleic acid (CAS 99 110-16-7), sodium hydroxide (CAS 1310-73-2), calcium chloride (CAS 10043-52-4), sodium azide (CAS 26628-22-8), glacial acetic acid (CAS 64-19-7), KOH (CAS 1310-58-3), and ethanol (CAS 64-17-5) were purchased from Sigma-Aldrich.

2.4. Methods

Extraction of RS: The process of RS extraction is presented in Fig. 1. At the beginning of the process, the rice flour samples were mixed with solution 2 containing pancreatic alpha-amylase and dilute AMG (300 U/ml). Then each mixture was incubated in the IKA Incubator shaker KS 4.000 at a shaking speed of 200 strokes/min at 37°C for 16 hours. During the set time, non-RS had the combined action with two enzymes (pancreatic alpha-amylase and dilute AMG (300 U/ml) to convert into D-glucose. The reaction was completed by presenting an equal volume of ethanol (96% v/v), followed by centrifugation at 3,000 rpm for 10 min, and the RS starch was formed as a pellet. The soluble starch was entirely removed from RS by washing twice with ethanol (50% v/v). The non-RS liquid was removed by decantation.

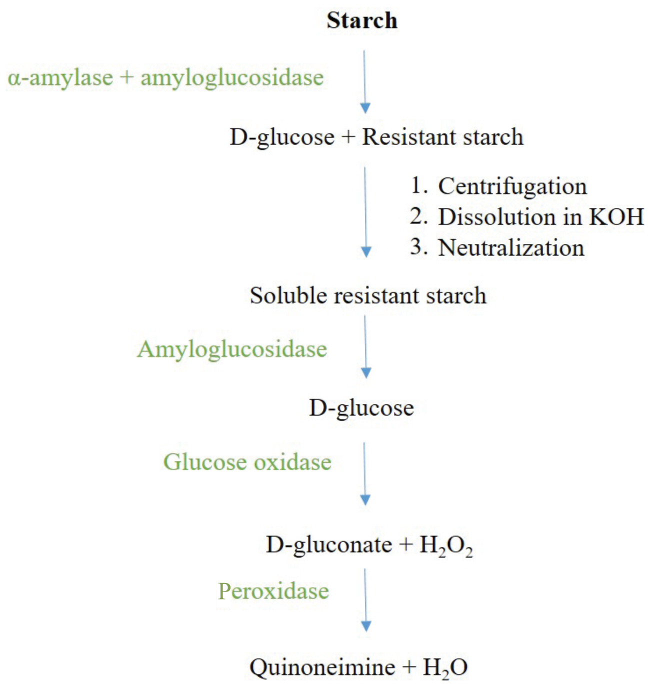

Fig. 1. RS and non-RS hydrolysis reaction (adapted from [15]).

2.5. Measurement of RS

The RS pellet was dissolved using KOH in an ice water bath with a magnetic stirrer within 20 min. After that, the solution was neutralised by adding a 1.2 M sodium acetate buffer (pH 3.8). Soluble RS was solubilised and hydrolysed to D-glucose by presenting AMG (3,300 U/ml), followed by incubating for 30 min in a hot water bath. Finally, the d-glucose was measured with GOPOD. D-glucose was oxidised by glucose oxidase into d-gluconate and hydrogen peroxide. The hydrogen peroxide reacted with peroxidase to create quinonimine. The absorbance was measured at 510 nm against a reagent blank using a UV-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

The content of RS was calculated by using the equation:

% RS = amount of RS in 100 mg sample (mg) = $\Delta E x F x 0.00927$

where ΔE: absorbance read against the reagent blank and F: conversion from absorbance to micrograms divided by the GOPOD absorbance for this 100 µg of d-glucose.

Statistical analysis: The correlation analysis between RS in different groups was performed using Graph Path v.8.4.3. A t-test was used to calculate the significant difference in RS content among different groups including grain size, *Indica* and *Japonica* groups, and ecosystems (irrigated, upland, RL, and mangrove). The experiment was repeated three times.

3. Results

3.1. Diversity of RS in 75 Vietnamese characterised varieties

The content of RS in 75 rice samples exhibited a continuous distribution ranging from 0.015 mg/100 mg to 0.71 mg/100 mg (Fig. 2). Some rice accessions, including G203, G161, G46, G17, and G160, had the lowest RS content. On the other hand, G98, G95, G70, G133, and G94 were the five rice samples with the highest RS content (Table 1).

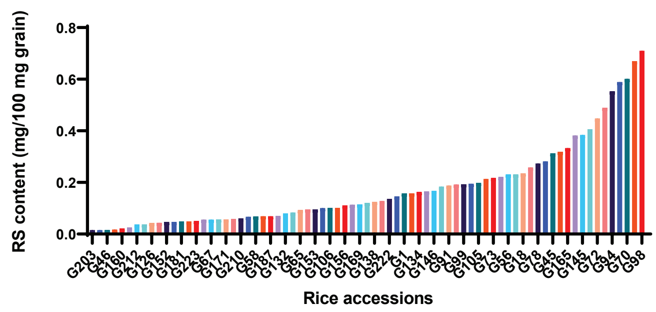

Fig. 2. Diversity of RS content in 75 Vietnamese characterised varieties.

Table 1. Accessions with the highest and lowest RS content.

ID	Name	Mean (mg/100 mg grain)	Origin	DArT P
G203	Plau Ca Banh	0.015	Dien Bien	*Japonica*
G161	Bn1	0.015	An Giang	*Indica*
G46	Nep Ba Lao	0.016	Nam Dinh	*Japonica*
G17	Nep Ga Gay Hai Duong	0.017	Hai Duong	*Indica*
G160	Jasmine	0.022	An Giang	*Indica*
G94	Lua Do	0.553	Thua Thien Hue	*Indica*
G133	A 330	0.589	Khanh Hoa	*Indica*
G70	Coc Moi Dang 2	0.601	Binh Dinh	*Indica*
G95	Lua Cham	0.67	Nam Dinh	*Indica*
G98	Ngoi Tia	0.71	Nam Dinh	*Japonica*

3.2. Frequency distribution of RS in 75 Vietnamese characterised varieties

Among the 75 samples, there were 53 samples with RS content between 0.015 and 0.2 mg/100 mg, 14 samples with RS concentration between 0.2 and 0.4 mg/100 mg, 5 samples with RS quantities in the range of 0.4-0.6 mg/100 mg, and 3 samples with RS levels in the range of 0.6-0.8 mg/100 mg (Fig. 3). The results indicate that most rice varieties had an RS level of 0-0.2 mg/100 mg, while some had lower or higher RS content than the common group.

Only three samples showed RS levels between 0.6 and 0.8 mg/100 mg. The samples with the highest RS content were G70, G95, and G98, respectively, with 0.601, 0.67, and 0.71 mg/100 mg. These are the potential candidates for further studies to create commercial high RS rice varieties.

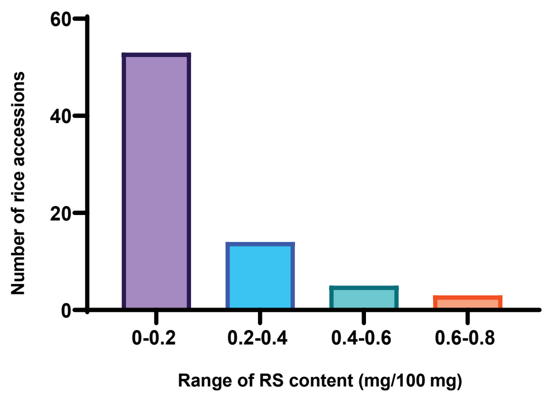

Fig. 3. Frequency distribution of RS content in the 75 characterised Vietnamese varieties.

3.3. Relationship between subgroup population and RS

Based on the structure results using the DArT markers, rice accessions can be classified into two major varieties of Asian cultivated rice: the *Indica* and *Japonica* subspecies. In our collection, there were 45 *Indica* accessions and 30 *Japonica* accessions. Fig. 4A illustrates that the RS content in *Indica* rice was 0.204±0.028 mg/100 mg, whereas it was 0.099±0.005 mg/100 mg in *Japonica* rice. As a result, the *Indica* rice had significantly greater in RS content than *Japonica* rice ($p<0.001$).

Moreover, this study also obtained a significant difference in RS content between grain sizes. RS content was higher in short (0.167±0.01 mg/100 mg) and medium grain (0.217±0.032 mg/100 mg) compared to long grain (0.107±0.004 mg/100 mg) ($p<0.05$) (Fig. 4B).

Figure 4C compared upland (UP), RL, mangrove (MG), and irrigated (IR) ecosystems in terms of the amount of RS. It was noticeable that the rice in RL could be considered to have a tremendous amount of RS (0.251±0.028 mg/100 mg) compared with the RS amount of rice from MG (0.047±0.0008 mg/100 mg) and UP (0.09±0.047 mg/100 mg) ecosystems. There were significant differences in RS content between RL or irrigated (IR) ecosystems and MG and LL ($p<0.001$). However, the RS content was not significantly different between rice is grown in IR and RL ($p>0.05$).

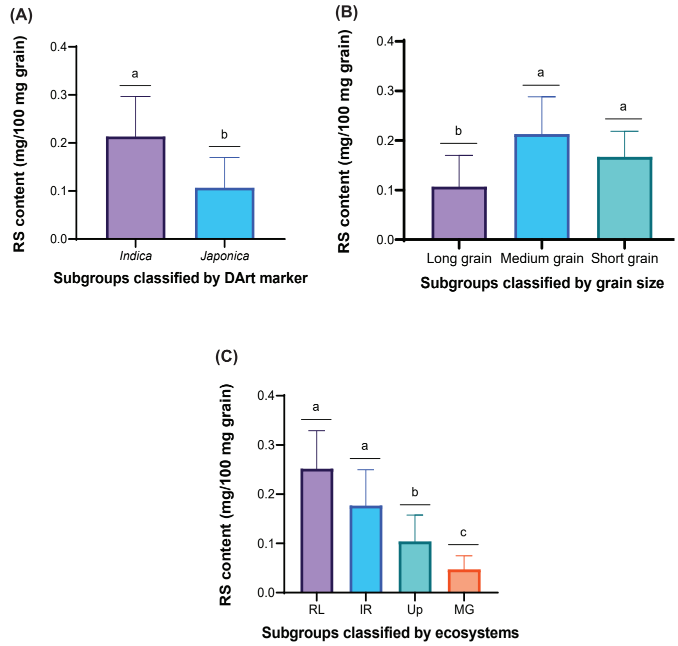

Fig. 4. The association between RS content and (A) rice varieties, (B) grain length, and (C) ecosystem. The letters above the dataset indicate the statistical difference. Abbreviations: RL (rainfed lowland), IR (irrigated), Up (upland), MG (mangrove).

4. Discussion

Our study is the first study investigating the diversity of RS in Vietnamese rice among a large rice collection using the RS assay kit (Rapid) from Megazyme. The results showed a wide range of RS among the 75 investigated rice accessions, ranging from 0.015 to 0.71%. Our study's range of RS content was low compared to the RS content of 10.1 to 18% in others study, which also used the same quantification method [16]. In another study, the RS content of rice ranges from 0.1 to 25.4% [17]. Different methods for RS quantification have been proposed using enzymes [3, 17]. RS values may differ in different studies due to rice varieties, ecosystems, and the quantification methods.

Among the 75 samples analysed, approximately 70.67% of samples had RS content of between 0.015 and 0.2 mg/100 mg, 18.67% of samples had RS concentrations between 0.2 and 0.4 mg/100 mg, 6.67% of samples had RS quantities in the range of 0.4-0.6 mg/100 mg, and only 4% samples had RS levels in the range of 0.6-0.8 mg/100 mg.

Our study also indicated more amylose in *Indica* rice grains than in *Japonica* rice grains. Generally, the amount of amylose in rice grain is positively associated with the amount of RS [18]. In contrast, amylopectin, which accounts for 80-85% of the starch humans can readily digest, is negatively correlated with the amount of RS [19]. That means more amylose content in the diet will increase the RS level while more amylopectin content in the diet will decrease the RS level. If there is more amylose, rice is digested more slowly and has a lower glycaemic index [1]. This result implies that *Indica* rice is digested slower than *Japonica* rice because it has a higher proportion of RS. Thus, *Indica* rice is a primary source of RS, and it may be used as a therapeutic agent in the diets of people with metabolic problems [20]. The waxy rice, such as *Japonica* and low amylose rice, has faster and more complete starch hydrolysis than intermediate and high amylose rice [18]. The fact is that higher amounts of amylose or RS make it more difficult to cook rice. These types of rice also have a firm texture because RS raises the gelatinization temperature and lowers the peak viscosity values of rice [20]. Moreover, crystallisation also affects RS content. High-amylose starch, which displays a C-type crystalline structure, contains a lower content of A-type crystalline structure than waxy and normal starches in rice [21]. When side chains of clustered amylopectin and amylose form crystalline structures, it is demonstrated that a higher proportion of crystallinity correlates with more rapidly digestible starch [22]. These results indicate that more crystalline structures will decrease the RS content.

Our study found that short and medium grains had higher RS content than long grains in terms of grain size. W. Zhang, et al. (2022) [23] also pointed out that smaller starch granules had higher RS content than bigger ones. X. Shu, et al. (2014) [24] observed that the starch granule diameter is negatively correlated with starch digestibility. The larger surface area in long grains increase enzyme binding rates and consequently ease digestion [25].

Regarding ecosystems affecting RS content, our study showed that the rice from rainfed lowland ecosystems could be considered to have the most significant amount of RS compared to the RS amount of rice from the MG and UP ecosystems. There has not been any study examining the RS content in different rice varieties grown in diverse ecosystems. In some other studies, scientists compared the effects of environmental factors including light, air temperature, water stress, and soil quality in terms of producing amylose and amylopectin, which are also related to RS content [26]. Due to the impact of water supplementation, J.A. Patindol, et al. (2015) [27] reported that during the reproductive stage of growth, rice was sensitive to water deficit and rainy spells. If drought occurs during the ripening stage of rainfed lowland rice, the result is decreased amylose content, which may reduce RS content in grains after that [28]. Therefore, our research results can be considered relevant to other studies.

5. Conclusions

In summary, our research focuses on correlating RS content in rice with different rice types and ecosystems. Regarding the RS amount in different types of rice, the *Indica* rice subgroup had a higher value (0.204±0.028 mg/100 mg) than the *Japonica* rice subgroup (0.099±0.005 mg/100 mg). Short and medium grains had higher RS content than long grains. Regarding ecosystems, the RL showed the greatest amount of RS (0.251±0.028 mg/100 mg), followed by MG (0.047±0.0008 mg/100 mg), and UP (0.09±0.047 mg/100 mg). To have firm conclusions, some other results should be further evaluated in larger amounts of the sample. In the realm of food and nutrition, our results contribute to helping nutritionists and doctors develop a suitable rice-based diet for patients with diabetes or obesity.

6. Recommendations

The results from our study can provide insight into the RS in rice for a future genome-wide association study of RS content to identify the genes or QTLs relating to high RS content in rice.

CRediT author statement

Thi Mai Huong To: Conceptualisation, Methodology and Reviewing; Thi Linh Nguyen: Investigation, Data curation; Thi Ngoc An Do: Investigation; Lan Phuong Nguyen: Investigation; Nga T.P. Mai: Conceptualisation, Methodology, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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