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Exploring the interplay: Amylose to amylopectin ratio and formation of resistant starch

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Abstract

With the global population steadily increasing, there has been a substantial rise in demand for rice production and consumption. Rice grain primarily yields starch as its principal constituent. The quality of starch is influenced by its components, specifically amylose and amylopectin, alongside factors such as resistant starch content. Elevated health concerns linked to rice consumption stem from its high glycemic index. Consequently, the starch composition of six Pusa rice genotypes was analyzed. The starch content among these genotypes ranged from 77.62% to 83.65%. Amylose content ranged from 22.53% to 31.49%, while amylopectin content ranged from 47.07% to 59.23%. Resistant starch content varied from 1.06% to 2.37%. Furthermore, the amylose to amylopectin ratio ranged from 0.40 to 0.67. These findings indicate moderate amylose to amylopectin ratios in the studied Pusa rice genotypes. Pusa 1174 exhibited the highest amylose to amylopectin ratio, which positively correlated significantly with resistant starch content. Consumption of such genotypes may mitigate blood sugar spikes, potentially reducing risks associated with diseases like diabetes, obesity, fatigue, and CVDs. Future strategies could involve enhancing resistant starch and altering amylose to amylopectin ratios through techniques such as marker-assisted backcross breeding and genetic engineering, tailored to specific applications of rice starch.

Keywords: Rice, amylose, resistant starch, glycemic index, diabetes

1. Introduction

Rice (*Oryza sativa* L.) stands as one of the oldest cultivated cereals globally, serving as a dietary staple for over 4 billion people worldwide. Approximately 90% of the world's rice production originates from Asia. Carbohydrates constitute the majority of the grain's dry weight, with starch being the predominant storage polysaccharide in plants and a primary dietary carbohydrate. Enzymes such as amylase and amyloglucosidase break down starch into glucose, making it a crucial energy source, with dietary recommendations suggesting that 50-60% of daily caloric intake should derive from carbohydrates, particularly starch. Starch is a versatile natural polymer extensively utilized in industries like food packaging, biomedical, and pharmaceuticals due to its abundance, low cost, biodegradability, and edibility (Bashir and Aggarwal, 2019; Guo *et al*., 2020) [1, 2]. Its main components, amylose and amylopectin, exhibit distinct structures: amylopectin is highly branched with α-1, 6-glycosidic linkages, while amylose consists of linear α-1, 4-glycosidic bonds (Nakamura and Kainuma, 2022) [3]. Variability in these starch components among rice varieties significantly influences their physicochemical properties. Starch digestibility categorizes starch into rapidly digestible starches (RDS), slowly digestible starches (SDS), and resistant starches (RS), the latter remaining undigested in the upper gastrointestinal tract and fermenting in the colon. This fermentation produces short-chain fatty acids, lactic acid, and gases, influencing gut microbiota and metabolic processes (Guo *et al*., 2024) [4]. Resistant starch reduces food's energy density and enhances insulin sensitivity, potentially aiding in blood glucose regulation (Kim *et al*., 2024) [5]. Foods are categorized based on their glycemic index (GI) into low (GI ≤ 55), medium (GI 56–69), and high (GI ≥ 70) glycemic index groups. Most rice varieties exhibit high GI values (~68-70). Consumption of low or moderate GI rice could mitigate risks associated with type 2 diabetes, obesity, and cardiovascular diseases (CVDs) (Ji *et al*., 2023) [6]. Given the growing demand for low GI rice varieties, this study investigates starch composition, amylose to amylopectin ratio, and resistant starch content across six diverse Pusa rice genotypes, aiming to elucidate their impact on energy density and starch digestibility.

2. Material and Methods

Mature seed kernels from six Pusa rice genotypes were obtained from the Genetics Division of ICAR-Indian Agricultural Research Institute (IARI), New Delhi-110012. Rice kernels underwent hulling using a Satake huller followed by milling in a Satake Grain Testing Miller. Subsequently, these samples were stored in hermetic vials for subsequent analysis.

Table 1: Names of the six Pusa rice genotypes included in the current study

S. No.	Name of genotype	S. No.	Name of genotype
1	Pusa 1460	4	Pusa1342
2	Pusa 1174	5	Pusa-33
3	Pusa 1301	6	Pusa Sugandh 3

2.1 Estimation of Metabolites

2.1.1 Total starch

Total starch content (TSC) was determined following the method outlined by Krishnan *et al*., 2020 [7]. Briefly, 100 mg of samples were finely ground using a mortar and pestle. Starch was extracted twice with hot 80% ethanol. The residue obtained was then treated with 6.5 mL of 52% perchloric acid and 5 mL of distilled water. Centrifugation was conducted at 25 °C for 10 minutes, after which the supernatant was collected and adjusted to a final volume of 100 mL with distilled water. An appropriate aliquot of the solution was mixed with 5 mL of anthrone reagent (0.2%) and the absorbance was measured at 620 nm. TSC was quantified using a glucose standard curve.

2.1.2 Amylose content

The amylose content (AC) was determined using the colorimetric method described by Juliano (1971) [8]. Initially, 100 mg of samples were finely ground using a mortar and pestle. Subsequently, 1 mL of 95% ethanol and 9 mL of 1 N sodium hydroxide were added to the samples. The tubes were then incubated in a boiling water bath for 15 minutes, after which the volume was adjusted to 100 mL with distilled water. For analysis, a 5 mL aliquot was withdrawn and mixed with 1 mL of 1 N acetic acid and 2 mL of 0.2% iodine solution in a 100 mL volumetric flask. This mixture was left in the dark for 20 minutes to allow for color development. Absorbance was measured at 620 nm, and the AC was determined using a standard curve prepared from potato amylose (Sigma).

2.1.3 Amylopectin content

The amylopectin content (APC) was quantified using the Amylose/Amylopectin Megazyme assay kit (International Ireland, Ltd., Bray, Ireland). To remove lipids, a 20 mg powdered sample was heated with 1 mL of dimethylsulphoxide (DMSO). Starch was precipitated using 6 mL of 95% ethanol, and a 100 mM acetate buffer solution (pH 4.5) was added. Amylopectin was then precipitated by centrifugation with a 4 mL concanavalin A lectin solution. The APC was hydrolyzed into D-glucose using α-amylase and amyloglucosidase, and the resulting glucose was measured using the glucose oxidase-peroxidase (GOPOD) method at 510 nm.

2.1.4 Resistant starch content

The resistant starch (RS) content was determined using the RS assay kit from Megazyme International Ireland, Ltd., Bray, Ireland. A 100 mg powdered sample was digested with pancreatic α-amylase (10 mg/mL) containing amyloglucosidase (3 U/mL) for 16 hours at 37 °C with continuous shaking at 200 rpm. The mixture was centrifuged at 3000 rpm for 10 minutes. The RS pellet was washed twice with 50% ethanol and then suspended in 2 mL of 2 M KOH, followed by stirring in an ice-water bath for 10 minutes. Subsequently, 8 mL of sodium acetate buffer (1.2 M, pH 3.8) and 0.1 mL of amyloglucosidase (3 U/mL) were added. The tubes were vortexed and incubated at 50 °C for 30 minutes, then centrifuged at 3000 rpm for 10 minutes. A 0.1 mL aliquot of the supernatant was mixed with 3 mL of GOPOD reagent and incubated at 50 °C for 20 minutes. Absorbance was measured at 510 nm. RS content was calculated using the formula provided in the kit and expressed as a percentage.

2.2 Statistical analysis

Two biological replicates and three technical replicates were used to generate the results. The standard deviation (±) was utilized to measure the dispersion of the dataset relative to its mean.

3. Results and Discussions

3.1 Total starch contents

Almost half of the world's population relies on rice as a primary calorie source. The increasing demand and preference for high-quality rice prompted us to analyze the starch levels in various Pusa rice genotypes. In this study, six Pusa rice genotypes exhibited total starch content ranging from 77.62% to 83.65% (Fig. 1). Pusa Sugandh 3 had the highest total starch content, while Pusa 1460 had the lowest. Specifically, the total starch content was 78.56%, 78.74%, 79.52%, and 79.98% in Pusa 1174, Pusa 1342, Pusa-33, and Pusa 1301, respectively. These findings align with John *et al*. (2023) [9], who reported a total starch content of 75.2 g/100 g in rice landraces from Assam. Similarly, Deepa *et al*. (2010) [10] measured the total starch content in three rice cultivars, finding it to be in the 79% to 89% range. Results of present work indicate that Pusa rice genotypes have an average starch content of 79.68%. Regardless of total starch content, the glycemic index is influenced by the amylose-to-amylopectin ratio. Therefore, the author estimated the amylose and amylopectin content of Pusa rice genotypes to correlate with starch digestibility in the human diet.

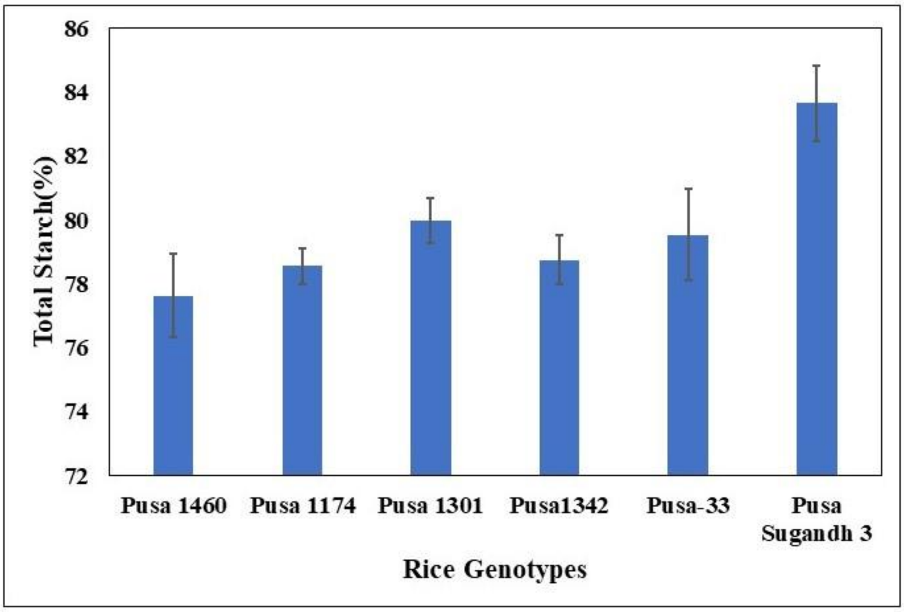

Fig 1: Total starch content in six diverse Pusa rice genotypes, depicted with results from two biological and three technical replicates, ± standard deviation.

3.2 Amylose content

Amylose is highly effective as a thickening agent, water binder, emulsion stabilizer, and gelling agent in both industrial and nutritional contexts. In the current investigation, the Author hypothesized a relationship between blood sugar levels and the amylose content in various Pusa rice genotypes. The relative amylose content of six Pusa rice genotypes ranged from 22.53% to 31.49% (Fig. 2), with Pusa 1174 having the highest amylose content and Pusa-33 the lowest. Specifically, the amylose content was found to be 24.42%, 27.00%, 27.22%, and 27.97% in Pusa Sugandh 3, Pusa 1460, Pusa 1301, and Pusa 1342, respectively. These results align with those of Naseer *et al.* (2021) [11], who reported that the apparent amylose content (AAC) among various rice genotypes varied significantly from 15.40% to 28.31%. Similarly, Govindaraju *et al.* (2022) [12] studied ten rice genotypes and found amylose content ranging from 7.50% to 28.58%. Hebishy *et al.*, 2024 [13] studied the physiochemical properties of three rice genotypes with respect to their amylose content and reported that the amylose content of rice genotypes ranged from 16.67-21.61%. The author's findings, along with current literature, suggest that Pusa rice genotypes have moderate amylose contents. Therefore, consuming such rice varieties is unlikely to cause a sharp increase in blood sugar levels, potentially reducing health issues associated with rice consumption.

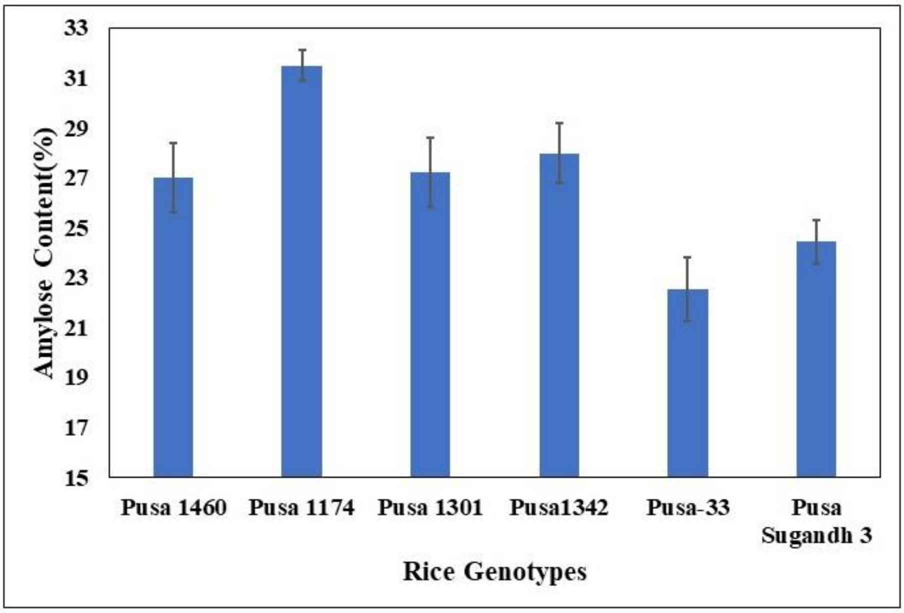

Fig 2: Amylose content measured in six diverse Pusa rice genotypes, based on results from two biological and three technical replicates, with ± standard deviation indicated.

3.3 Amylopectin content

Amylopectin, a highly branched starch molecule, contributes to the gelatinous and sticky texture of rice. The amylopectin content in six Pusa rice genotypes ranged from 47.07% to 59.23% (Fig. 3), with Pusa Sugandh 3 having the highest and Pusa 1174 the lowest amylopectin content.

Specifically, amylopectin content was 50.32%, 50.77%, 52.76%, and 56.99% in Pusa 1460, Pusa 1342, Pusa 1301, and Pusa-33, respectively. Chakraborty *et al*. (2009) [14] found that amylopectin content in rice genotypes varied from 74.44% to 84.75%. This higher percentage resulted from calculating amylopectin indirectly by subtracting the amylose content from the total starch content, whereas the author measured amylopectin directly using the Megazyme kit. Consequently, this study concluded that Pusa Sugandh 3, with its high amylopectin content, may produce sticky rice after cooking and be digested more quickly than genotypes with lower amylopectin levels. However, linking the digestibility and subsequent rise in blood sugar directly to amylopectin or amylose content is complex. Therefore, the author further evaluated the ratio of starch components to achieve a more precise understanding.

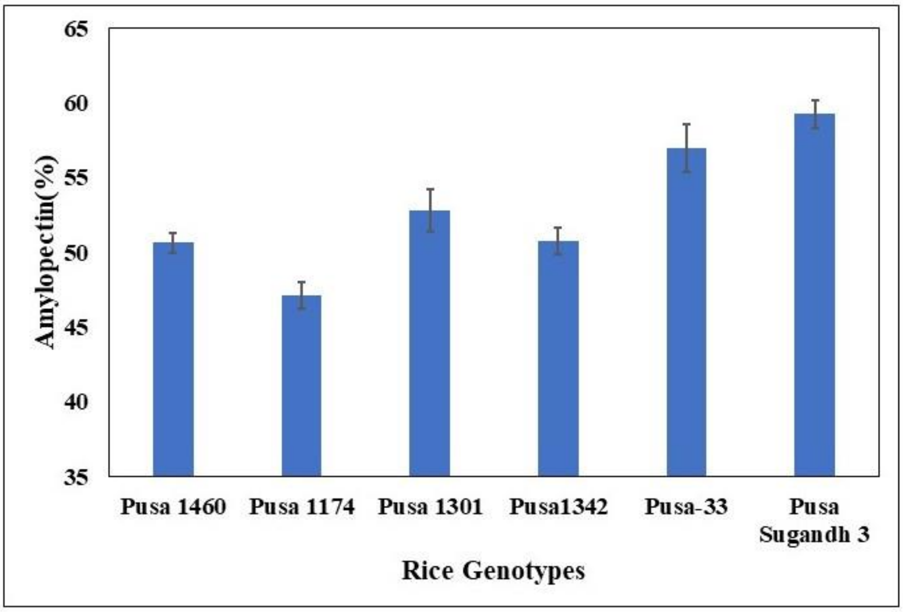

Fig 3: Amylopectin content measured in six diverse Pusa rice genotypes, based on results from two biological and three technical replicates, with ± standard deviation shown.

3.4 Ratio of amylose to amylopectin

Various types of starches and cultivars of the same crop have different amylose-to-amylopectin ratios. In this study, the amylose-to-amylopectin ratio ranged from 0.40 to 0.67 (Fig. 4), with Pusa 1174 having the highest ratio and Pusa-33 the lowest. Specifically, the ratios were 0.41, 0.52, 0.53, and 0.55 in Pusa Sugandh 3, Pusa 1301, Pusa 1460, and Pusa 1342, respectively. Kale *et al*. (2015) [15] reported an amylose-to-amylopectin ratio of 0.59 in a rice genotype, which aligns with the present findings. The amylose-to-amylopectin ratio is crucial because rice genotypes with similar amylose content can have different physiochemical properties, affecting digestibility and blood glucose response. Therefore, this study suggests that the amylose-to-amylopectin ratio may determine the digestibility quality of Pusa rice genotypes. Based on present findings, the best genotypes for eating and cooking are Pusa 1174, followed by Pusa 1342 and Pusa 1460.

Fig 4: The sunburst chart illustrates the amylose-to-amylopectin ratio in six diverse Pusa rice genotypes.

3.5 Resistant starch content

Resistant starch is a type of carbohydrate that remains undigested in the small intestine. Instead, it ferments in the large intestine, nourishing the beneficial gut microbiota. The resistant starch content of six Pusa rice genotypes ranged from 1.06% to 2.37% (Fig. 2), with Pusa 1174 having the highest resistant starch content and Pusa-33 the lowest. Specifically, resistant starch contents were 1.26%, 1.41%, 1.45%, and 1.53% in Pusa Sugandh 3, Pusa 1460, Pusa 1301, and Pusa 1342, respectively. This study indicates that amount of resistant starch content having a significant positive correlation with that of amylose content and the ratio of amylose to amylopectin. Kumar *et al.* (2018) [16] reported that resistant starch could play a crucial role in determining the glycemic index of rice cultivars. Therefore, the amount of resistant starch can serve as a marker to identify crops with low glycemic index, thereby improving the starch quality of rice, particularly beneficial for diabetic and obese patients. Studies indicate that consuming resistant starch (RS) can effectively improve insulin resistance in individuals with diabetes and obesity (Xie *et al*., 2019) [17].

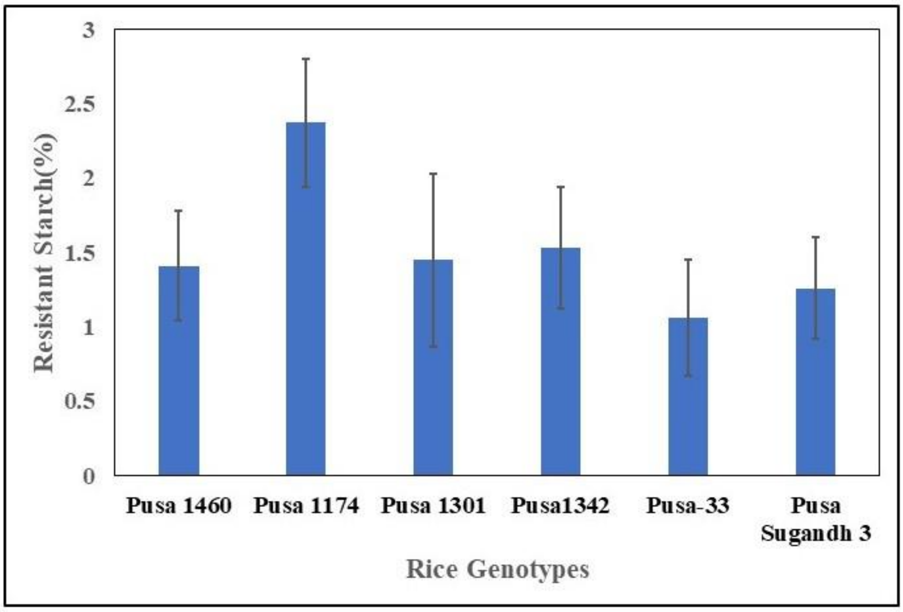

Fig 5: Resistant starch content in six diverse Pusa rice genotypes, depicted with results from two biological and three technical replicates, ± standard deviation shown.

4. Conclusion

The demand, production, supply, and consumption of rice have increased globally, with Pusa rice genotypes primarily produced in India. People derive a significant portion of their caloric intake from rice starch, which is used in various food and non-food applications. Therefore, the author estimated the starch content in different Pusa rice genotypes. The quality of starch is influenced by the amylose-to-amylopectin ratio, resistant starch content, and other food matrix components. The level of amylose, and particularly the ratio of starch components, can affect the glycemic index and thus blood sugar levels. Resistant starch showed a significant positive correlation with the high amylose-to-amylopectin genotype (Pusa 1174). Among the six Pusa rice genotypes studied, Pusa Sugandh 3 had the highest total starch content, while Pusa 1174 had the highest amylose content and Pusa Sugandh 3 had the highest amylopectin content. The amylose-to-amylopectin ratio was highest in Pusa 1174, which also had the highest resistant starch content. This suggests that genotypes with high amylose content might form complexes with other components, such as fatty acids, resulting in incomplete digestion in the small intestine. Additionally, high amylose levels may induce structural changes in the starch granule, contributing to increased resistant starch content. Therefore, consuming such rice may reduce the risk of diabetes, obesity, fatigue, cardiovascular diseases (CVDs), and other health issues. Breeders can use these genotypes to improve rice starch quality through hybridization or genetic engineering approaches.

5. Conflict of interest

Author confirms that there are no known conflicts of interest associated with this work.

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