The Andhra Agric. J 72 (3): 304-309, 2025
doi :10.61657/aaj.2025.213

Variability in amylopectin content among diverse panel of rice (*Oryza sativa* L.) genotypes

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ABSTRACT

Rice starch quality, as determined largely by its amylopectin composition, influences cooking, eating sensory qualities and glycemic response. In this study, we have evaluated the variability in the amylopectin content among 178 diverse rice germplasm . The amylopectin content ranged from 26.91 % to 57.40 %, with a mean of 42.73 %. The coefficient of variation (15.6 %) indicate moderate to high genetic variability, suggesting ample scope for selection and improvement. The cluster analysis classified the genotypes into three distinct non-overlapping groups based on amylopectin content, low (26.91–39.69%), medium (39.81–46.44%) and high (46.46–57.40%), reflecting clear variation among genotypes. The genotypes, Burma Black (57.4%) and Basmathi-370 (26.91%), were identified as contrasting genotypes representing the high and low amylopectin extremes which can be used as donor parents for breeding programs targeting specific amylopectin profiles. Comprehensive statistical analysis revealed excellent experimental precision with tight confidence intervals and high reliability of genotype comparisons. Overall, the study revealed substantial genetic diversity for amylopectin content within rice germplasm and provides a valuable foundation for developing rice cultivars with customized cooking qualities, improved nutritional value and low glycemic index, contributing to the design of functional and health-oriented rice varieties.

Keywords: *Amylopectin, Breeding, Cooking quality, Glycemic index, Germplasm and Rice*

Rice (*Oryza sativa* L.) is the staple food crop for more than half of the world's population, particularly in Asia, where it contributes between 50 and 80% of daily caloric intake (Fitzgerald *et al*., 2009). It is also valued for its eating and cooking quality traits, which strongly influence consumer acceptance and market price. Among the diverse grain-quality parameters, starch composition is the single most important factor determining texture, palatability and nutritional value (Juliano, 1992). Starch and protein are the primary components of the rice grain (Shi *et al*., 2020), with starch consisting of two polysaccharides: amylose and amylopectin (Zhu *et al*., 2021). Starch constitutes about 85–90% of the milled rice grain and is composed of two distinct glucose polymers: amylose, a predominantly linear á-1,4-linked glucan and amylopectin, a highly branched á-1,4/á-1,6-linked glucan. The ratio of these two components largely dictates the cooking characteristics of rice, with amylopectin conferring softness, stickiness, and glossy appearance, while amylose imparts firmness, fluffiness, and slower digestibility (Tian *et al*., 2009; Biselli *et al*., 2014).

Rice consumer preferences differ across geographies. The soft, sticky rice high in amylopectin is favoured in East and Southeast Asia, while in South Asia and many African regions, intermediate-to-high amylose rice with firmer texture predominates (Kaur *et al*. 2016). From a nutritional perspective, high-amylopectin rice tends to have higher glycemic index (GI), causing sharper postprandial glucose peaks (Hu *et al*. 2004; Frei *et al*. 2003). In contrast, varieties with lower amylopectin (i.e., higher amylose proportion) exhibit slower glucose release and are of interest for health-oriented diets and metabolic disease mitigation. Moreover, amylopectin's fine structure influences functional food behavior like retrogradation, resistant starch formation and digestibility (Zhou *et al*. 2021).

Genetically, starch biosynthesis in rice is under the control of a complex network of enzymes: the *Wx* gene (granule-bound starch synthase I, GBSSI) is the principal driver of amylose synthesis (and hence indirectly influences amylopectin proportion), while other enzymes—soluble starch synthases (SSSI, SSIIa), branching enzymes (SBEI, SBEII) and debranching enzymes (eg. isoamylase, pullulanase)—modulate chain elongation, branching, and trimming of amylopectin (Tian *et al*. 2009; Zhong *et al*. 2020). Natural allelic variation in these loci is responsible for much of the observed diversity in starch properties across *indica*, *japonica*, aromatic and specialty rice groups (Biselli *et al*. 2014; Yan *et al*. 2021). Therefore, phenotypic characterization of amylopectin variation is a valuable step toward breeding and genomic dissection.

Understanding genetic variability in amylopectin content is important for several reasons as it can offer donors for breeding programs targeting specific textural or glycemic traits, it enables association mapping or QTL discovery of starch biosynthesis genes and it provides insight into the natural allelic diversity in germplasm collections. Therefore, amylopectin content is considered as one of the major characteristics for assessing rice cooking and eating qualities. Therefore, the present study was undertaken to evaluate different rice genotypes for their amylopectin content.

MATERIAL AND METHODS

Plant Material and Experimental Design

A total of 178 rice genotypes, encompassing *indica*, *japonica*, aromatic types, local landraces and modern improved lines, were sourced from the Indian Institute of Rice Research (IIRR), Hyderabad. The field experiment was conducted during the *Kharif* 2022 season at the S.V. Agricultural College Farm, Tirupati, following standard agronomic practices. The experiment was laid out in a randomized complete block design (RCBD) with three replications to minimize environmental bias. Recommended agronomic practices and plant protection measures were followed for raising a healthy crop. At physiological maturity, panicles were harvested, and grains were processed under standardized post-harvest handling to minimize environmental noise (such as variation in drying or storage conditions). Harvested seeds were cleaned to remove impurities, dehusked and finely ground using a miller to obtain a fine powder.

Amylose content (%)

Amylose content in rice was determined by the method suggested by Juliano (1971). A standard solution of amylose was prepared by taking 100 mg amylose (A0512, Sigma) dissolved in 10 ml of 1N NaOH and made up to 100 ml with distilled water. An aliquot of 0.2 to 1.0 ml of this was taken and neutralized with 0.1N HCL using Phenalphthalein as indicator and then 1 ml iodine reagent was added. For the test samples, 100 mg rice sample was taken and 1 ml distilled ethanol and 10 ml 1N NaOH were added. Samples were incubated overnight at room temperature and then volume was made to 100 ml with distilled water. From this 2.5 ml of extract was taken, 20ml distilled water was added, neutralized with 0.1N HCL using Phenalphthalein as indicator and then 1 ml iodine reagent was added. volume made upto 50 ml with distilled water. Then absorbance was recorded at 590 nm. The amylose content of the sample was calculated by using the slope value obtained from standard curve and expressed as percentage.

Total starch content (%)

Total starch content was estimated by using Anthrone method (Hodge, J.E and Hofrieter, B.T. 1962). Rice sample of 0.5gms was taken and extracted with 10 ml of hot 80% ethanol. Centrifuged at 2,500 RPM for 5 min and retained the residue, washed the residue with hot 80% ethanol till the supernatants does not give color with anthrone reagent. The residue was dried on water bath and then 5 ml of distilled water and 6.5 ml 52% perchloric acid was added to the residue. This was maintained at 0! for 20 min and the same was repeated twice and supernatant was pooled up and made to 100 ml with distilled water. Along with test samples glucose working standard was prepared by using 10 mg glucose from stock standard. An aliquot of 0.2 to 1.0 ml of glucose working standard was taken and made to 1 ml with distilled water. 4 ml anthrone reagent was added to the samples and then heated for 8 min at 80^0 C and read the absorbance at 630 nm.

Amylopectin content

Amylopectin content was reported by substracting the measured amylose content from the total starch content.

Statistical analysis

The data on amylopectin content were subjected to statistical analysis to determine mean, median, range, standard deviation (SD), coefficient of variation (CV), skewness and kurtosis. Frequency distribution was visualized using histograms and boxplots. To classify the genotypes into groups, k-means cluster analysis (k=3) was employed on standardized amylopectin values, generating low, medium and high amylopectin classes. For each class, the number of genotypes, range, and mean values were determined. Genotype clustering through heatmap visualization. All analyses were performed using customized python scripts and statistical software.

RESULTS AND DISCUSSION

Genetic variability in Amylopectin Content

The analysis of 178 rice genotypes revealed substantial genetic variability for amylopectin content, ranging from 26.91 % to 57.40 %, with a mean of 42.73 % and standard deviation of 6.67 % (Table.1). This clearly indicated that most genotypes fell within a moderate amylopectin range, which agrees with the general starch composition of rice, where amylopectin constitutes 65–80 % of total starch (Juliano, 1992). The observed coefficient of variation (CV) of 15.6 % indicate moderate to high genetic diversity among the genotypes. According to Snedecor and Cochran (1989), CV values between 10–20 % represent moderate variability, while values above 20 % indicate high variability. Hence, the recorded CV value confirmed the existence of substantial genetic diversity, suitable for use in breeding programs and genetic studies targeting grain quality traits.

The wide range (26.91- 57.4) demonstrated broad genetic variation in amylopectin content among the rice genotypes, comparable to the 33-63% reported in Chinese *japonica* germplasm (Wang *et al.,* 2017) and 30-65% observed in Indian aromatic varieties (Kaur *et al.,* 2016). The large sample size (n = 180) provides robust statistical power for variability assessment (Cochran, 1977). The highly significant differences between genotypes (F = 242.43, p < 0.001) confirm the existence of substantial genetic diversity for amylopectin content. The clear separation among clusters confirms statistically distinct groupings, while the moderate to high CV (15.6%) and broad range (30.49%) further validate the existence of significant genetic diversity for amylopectin content.

Table 1. Descriptive statistics for amylopectin content

Parameter	Value
Mean (%)	42.73
Median (%)	43.04
Standard Deviation	6.67
Coefficient of Variation (%)	15.6
Minimum (%)	26.91
Maximum (%)	57.4
Skewness	-0.177
Kurtosis	-0.554

Distribution Pattern and Comparative Variability

The skewness (–0.177) indicate a slightly left-tailed distribution, suggesting fewer genotypes with extremely high amylopectin content. This pattern reflects the natural distribution of starch biosynthetic efficiency across rice germplasm. The kurtosis value (–0.554) showed a platykurtic distribution (flatter than normal), indicating a wider spread of amylopectin content across the genotypes. Such distribution is advantageous for selection, as it ensures good representation across low, medium and high amylopectin levels (Figure 1).The approximately normal distribution indicated that the trait is governed by multiple genes, suggesting polygenic inheritance, which aligns with previous findings that starch composition in rice is controlled by multiple genes with additive effects (Tian *et al*. 2009; Biselli *et al*. 2014).

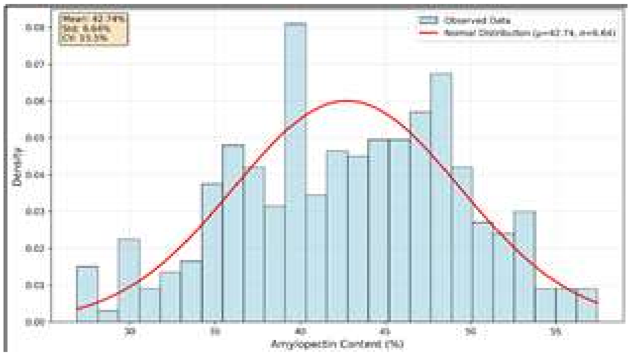

Figure. 1. Frequency distribution curve of amylopectin content among 178 rice genotypes. Blue bars in the graph indicate observed data across genotypes whereas red curve indicates the frequency distribution pattern.

Range of **30.49 %** (26.91–57.40 %) recorded in this study demonstrated broad genetic variation in amylopectin content among the rice genotypes. This range is comparable to the **33–63 %** reported in Chinese *japonica* germplasm *Wang et al*., 2017 and **30–65 %** observed in Indian aromatic varieties Kaur *et al*., 2016. The mean amylopectin content (42.73 %) also aligns with earlier reports for Indian rice as reported by Rani and Bhattacharya, 1989. The comparative analysis thus confirms the wide genetic base of 178 rice germplasm lines for starch quality traits.

Cluster Analysis and Genetic Grouping

Cluster analysis based on amylopectin content classified 178 genotypes into **three distinct groups**, representing clear genetic differentiation (Table 2, Figure 2).

Table 2. Cluster analysis results for amylopectin content

Cluster	No of genotypes	Range (%)	Mean (%)	SD (%)
Low	60	26.91–39.69	35.23	3.46
Medium	60	39.81–46.44	42.94	2.12
High	58	46.46–57.40	50.01	2.7

The three clusters represent distinct genetic groups with specific breeding applications:- Low amylopectin group (26.91-39.69%): Suitable for developing low-glycemic index rice varieties - Medium amylopectin group (39.81-46.44%): Representative of conventional commercial cultivars - High amylopectin group (46.46-57.40%): Ideal for developing soft, sticky rice varieties

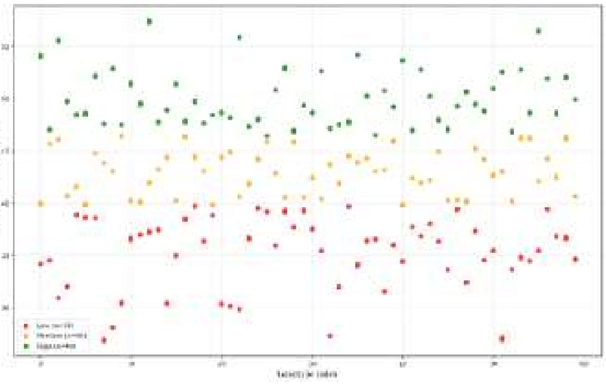

Figure 2. Scatter plot illustrating amylopectin content of 178 rice genotypes classified into low, medium and high groups

The clear separation among clusters indicated the presence of distinct genetic groups for amylopectin content, which can be exploited in targeted breeding programmes (Figure 3). Similar clustering patterns have been reported in rice starch quality studies, Tian *et al*., 2009; Yan *et al*., 2021.

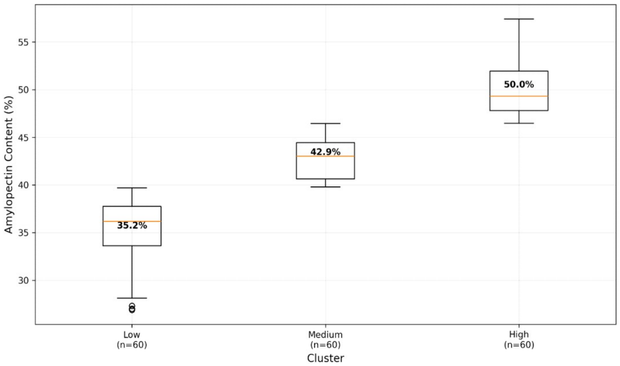

Figure 3. Boxplot exhibiting amylopectin content among three clusters of rice genotypes

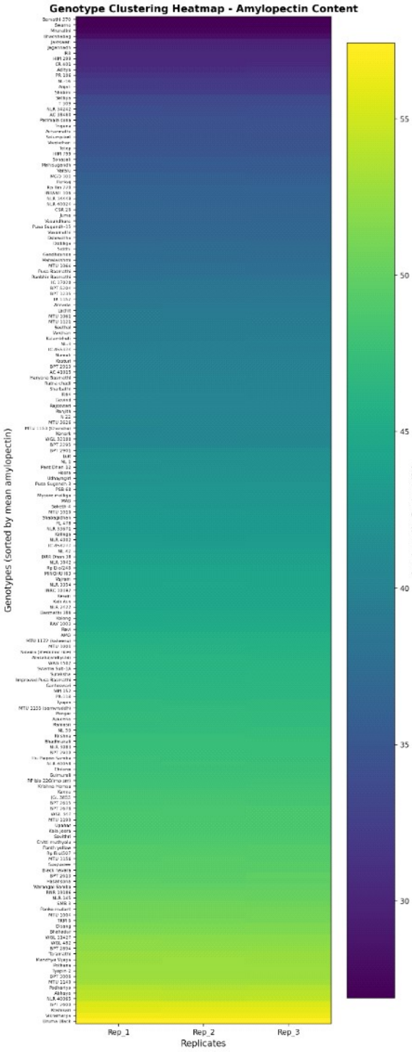

Figure 4. Heatmap representing mean amylopectin content of 178 rice genotypes.

The genotype clustering heatmap Figure 4 clearly demonstrated the three distinct groups based on amylopectin content, with no overlapping ranges between clusters. The heatmap visualization provides a comprehensive overview of genetic diversity and clustering patterns, facilitating the identification of potential breeding parents and the understanding of genetic relationships among genotypes.

Confidence Intervals and Statistical Precision

Detailed statistical analysis revealed high precision in amylopectin content estimation, with mean confidence interval of 0.187% (range: 0.017% - 0.392%). The standard error of mean (SEM) values ranged from 0.002% to 0.045%, which indicated high experimental precision and reliability of genotype comparisons. These tight confidence intervals validate the robustness of the experimental data and support the reliability of genotype performance evaluations.

Identification of Extreme Genotypes

Within the high-amylopectin group, genotypes such as Bruma Black (57.40 %), Vikramarya (56.49 %) and Koshikari (55.90 %) exhibited the highest amylopectin contents (Table 3). These genotypes are valuable donors for developing soft, sticky rice varieties preferred in East and Southeast Asian markets and for specialty rice products requiring high gelatinization and cohesive texture.

Table 3. List of top 10 rice genotypes based on high amylopectin content

Rank	Genotype	Amylopectin (%)
1	Bruma Black	57.4
2	Vikramarya	56.49
3	Koshikari	55.9
4	BPT 2600	55.57
5	NLR 40065	54.2
6	Abhaya	54.07
7	Podhariyo	53.62
8	MTU 1140	52.93
9	BPT 3006	52.88
10	Tyapin-2	52.76

Conversely, Basmathi-370 (26.91 %), Swarna (27.02 %) and Mrunalini (27.32 %) were identified as low-amylopectin genotypes (Table 4) representing potential donors for breeding low-glycemic index and diabetic-friendly rice. Such lines are ideal for health-oriented markets and functional food development. The contrasting extremes provide a foundation for designing crosses to develop segregating populations covering the entire amylopectin spectrum.

Table 4. List of top 10 rice genotypes based on low amylopectin content

Rank	Genotype	Amylopectin (%)
1	Aditya	30.93
2	CR-401	30.46
3	HIM 299	30.41
4	IR8	30.37
5	Jagannadh	30.15
6	Jaleswari	29.87
7	Bhadshabag	28.12
8	Mrunalini	27.32
9	Swarna	27.02
10	Basmathi-370	26.91

Thus, selection and utilization of genotypes from extreme groups can support both **quality enhancement** and **nutritional diversification** of rice.

Future Research Directions

The present findings lay the groundwork for advanced studies including, Molecular characterization of starch biosynthetic genes (eg., *Wx*, *SSII*, *SBE*) in extreme genotypes. Multi-location evaluations to assess genotype × environment stability of amylopectin content. Mapping population development to identify quantitative trait loci (QTLs) governing amylopectin variability. Comprehensive quality evaluation, including cooking, pasting, and sensory profiling of selected lines.

CONCLUSION

The study revealed substantial genetic variability (CV = 15.6 %) for amylopectin content among 178 rice genotypes, with clear genetic clustering and identification of superior and inferior donors. The presence of wide variation and well-defined genetic groups provides a strong foundation for quality-oriented breeding programmes aimed at developing rice varieties with desired amylopectin content for both traditional and health-focused markets. The comprehensive statistical analysis, including confidence intervals, normality tests, and cluster analysis, provides a robust foundation for understanding the genetic diversity of amylopectin content in Indian rice germplasm. The clear genetic differentiation and identification of extreme genotypes offer valuable resources for rice breeding programs targeting specific amylopectin profiles for diverse market requirements.

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Received on 03.07.2025 and Accepted on 08.08.2025