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Variability of *in-vitro* starch digestibility, predicted glycemic index and microstructure of north Indian maize genotypes

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Abstract

Due to increasing lifestyle problems like diabetes, high blood sugar, and cholesterol, a significant refinement of research has been diverted towards high amylose and resistant starch cereal. Resistant starch (RS) is the portion of starch that escapes digestion in the small intestine and is known to have an array of health benefits. The present study aims at starch profiling, digestibility characteristics, and glycemic index prediction of maize genotypes. A range of RDS (75.4-91.1%), SDS (5.78-12.7%), and RS (0.35-10.95%) has been observed in the experimental genotypes. Moreover, results revealed that a significant positive correlation exists between amylose and resistant starch content (r= 0.96) whereas the negative correlation between amylose and SDS (r=-0.74); amylose and pGI (predicted Glycemic Index) (r= -0.98). ADHAM 15 mutant genotype was found to have maximum amylose and RS content making it a potential donor for producing low glycemic maize hybrids with pGI of 56.77. The scanning electron micrographs of three variants having the highest, intermediate, and lowest amylose content revealed the behavior of enzymatic digestion based on the difference in starch granule structure and organization pattern. SEM results depicted that high amylose maize starch granules were small, spherical, and more densely organized than low amylose maize.

Keywords: Resistant starch, slowly digestible starch (SDS), rapid digestible starch (RDS), high amylose maize, SEM

Abbreviations-

Chain-length distribution (CLD)

Glycemic index (GI)
Rapidly digestible starch (RDS)
Slowly digestible starch (SDS)
Resistant starch (RS)
Scanning electron microscope (SEM)
Predicted Glycemic index (pGI)

Introduction

Maize (*Zea mays* L.) is a globally significant and the third most important cereal crop in world with cultivation in more than 160 countries [1]. It is consumed as staple food in Africa, South America, and some parts of Asia and thus holds a significant position in human nutrition [2]. Maize is a good source of macronutrients mainly starch (70-72%), a primary dietary source of carbohydrates and energy [3]. It occurs as granules in the chloroplast of green leaves and the amyloplast of kernel [4]. Amylose and amylopectin are the two primary components of starch made up of α-glucans[5]. The former is a straight chain molecule connected by α-(1-4) linkages, whereas the latter is a branched polymer with α-(1-6) glycosidic connections acting as branching points [5]. The amylose-amylopectin ratio, molecular size, degree of branching, and chain-length distribution (CLD) of these two α—glucans all significantly impact the aggregation of starch granules' lamellar and crystalline structures [6]. In comparison to those with relatively less amylose content, high amylose content in starch granules causes significant hydrogen bonding and disturbs the crystalline lattice [7]. Because of this, food with a higher amylose content takes longer to cook and digests more slowly in the intestine, which lowers glycemic index (GI) and lessens the insulin surge [8]. Dietary starch shows great variation for the digestibility of starch molecules and its effect on the utilization of other nutrients. Based on its susceptibility to enzymatic digestion, starch can be classified into three categories: (1) rapidly digestible starch (RDS) (2) slowly digestible starch (SDS) (3) resistant starch (RS) [9]. Resistant starch is the total quantity of starch and products of starch breakdown that are not absorbed in healthy individuals' small intestines [10]. The burgeoning interest in non-digestible starch fractions can be attributed to the increasing recognition that the partial digestion and absorption of starches within the small intestine is a typical physiological process [7].

Resistant starch has health benefits, particularly for people with diabetes and obesity, due to several physiological factors. Type II diabetes is becoming more and more prevalent across the globe, with low- and middle-income groups suffering the most devastating effects [11]. In

2025, diabetes affected 589 million people worldwide, with 100 million individuals reportedly hailing from India [12]. By 2045, it is anticipated that 783 million adults will have diabetes, making diabetes management one of the largest health challenges of the twenty-first century. However, RS rich foods have potential to prevent high glycemic response due to lack of availability of digestible starch [13]. Further, resistant starch is considered as a good substrate for fermentation by colonic microflora because of high production of butyrate which is one of the principal SCFA (Short Chain Fatty Acids) produced in colon [14, 15]. In addition to supplying energy to the brain, heart, and muscle, SCFAs also boost bile salt solubility, mineral absorption, leptin production and regulation which serve as protection against obesity and metabolic disorders [16]. Moreover, SCFAs cause a concurrent decrease in luminal pH [15]. As a result, the intracolonic environment's acidity contributes to the suppression of activity of a variety of bacterial metabolites associated with colonic diseases [17]. Besides serving as prebiotics as res boosts the population of *Lactobacillus*, *Bifidobacteria*, and *Streptococci*, RS also serves as a substrate for probiotics [18]. Digestible starch leads to formation of gall stone by increasing insulin secretion which in turn stimulate cholesterol synthesis. Therefore, an elevation in the amount of RS is found to reduce the incidence of gallstones [18]. Additionally, replacing 5.4% of the meal's total dietary carbohydrates with RS could considerably enhance postprandial lipid oxidation, indicating a reduction in long-term fat accumulation [19]. Furthermore, significant research has demonstrated that resistant starch has physiological properties similar to dietary fibre [20]. The diversity of the modern food industry and the enormous variety of food products it produces also require starches that can tolerate a wide range of processing techniques and preparation conditions [21]. These requirements are satisfied through modification of native starches employing chemical, physical, and enzymatic processes resulting in the formation of indigestible residues [22, 23].

The present study, therefore, determined digestibility profile of starch in terms of RDS, SDS and RS and glycemic index was predicted based on hydrolysis index of the kinetics involved in starch hydrolysis. Further, the microstructure of maize starch granules was examined via Scanning electron microscope (SEM) to develop a better understanding of morphology and differences in the organization patterns of amylose and amylopectin in low, intermediate, and high amylose maize genotypes and to validate the results obtained from biochemical analysis of starch digestibility parameters.

2. **Materials and Methods**

2.1 Collection of germplasm and preparation of samples

Genetically pure seeds of 200 maize inbreds were procured from Indian Institute of Maize Research, Ludhiana, India. Samples were collected at the stage of seed maturity, dried and crushed to form fine powder for estimation of starch via Polarimetric method and amylose through the conventional iodometric method [24]. The other fraction was treated with 0.2N NaOH over night at -4 ℃ to isolate starch. The resulting mixture was centrifuged and the supernatant containing undesired products was discarded. The wet sample containing starch was dried and further used for digestibility profiling and SEM.

2.2 Determination of Starch and amylose content

a) Starch estimation was done according to Polarimetric method with certain modifications [25]. A 2 dm observation tube was used for determination of optical rotation in a polarimeter against a blank, and the percent starch (dry basis) was calculated using the following formula:

$$\% \text{ Starch (dry basis)} = \frac{\text{Degrees angular rotation x100 x 100 x 100}}{\text{2dm x 184.5 x Sample wt. (g) x (100 } - \text{ Sample moisture \%)}}$$

Where, 184.5 = Specific rotation of maize starch

b) Amylose estimation was done [24]. The absorbance was recorded against blank at 620 nm. Standard curve for amylose was prepared at a concentration of 1µg/ml of D- Glucose.

2.3 *in vitro* starch Digestibility

A modified protocol was used to estimate *in vitro* starch digestibility [26]. Finely powdered starch sample (0.1g) was mixed with sodium acetate buffer (pH 4.5) and placed in boiling water bath for 30 minutes. After cooling the mixture to room temperature (25°C), hydrolysis was initiated by addition of α-amylase (400 units) and amyloglucosidase (30 units). 0.1 mL aliquot were withdrawn at specified time (20, 120, 240 min). The reaction was stopped by adding 95% ethanol and glucose content was analyzed by Megazyme GOPOD kit. The absorbance (A_{20}, A_{120}, A_{240}) was taken at 510 nm and RDS, SDS, and RS were calculated using following equations (Note- Dilute the test sample if necessary).

i. For RDS

$$\% \text{ RDS} = \frac{A_{20} \text{ x F x TV x DF x 162 x 100}}{\text{W x V x 180 x } 10^6}$$

ii. For SDS

$$\% \text{ SDS} = \frac{A_{120} \text{ x F x TV x DF x 162 x 100}}{}$$

$$\frac{}{W \times V \times 180 \times 10^6}$$

iii. For RS

$$\% \text{ RS} = \frac{A_{240} \times F \times TV \times DF \times 162 \times 100}{W \times V \times 180 \times 10^6}$$

Where, $F = \frac{\text{Conc.(100µg D Glucose)}}{\text{GOPOD Absorbance for 100µg of D Glucose}}$,

T V= Total Volume taken, DF = Dilution Factor of sample, W= Weight of sample taken, V = volume used for estimation

2.4 Predicted Glycemic index (pGI)

For estimation of pGI, the percentage of total starch hydrolyzed at different times (0, 30, 60, 90, 120, and 180 min) was expressed as the rate of starch digestion. Hydrolysis Index (HI) was calculated by dividing the Area Under the Hydrolysis Curve (AUHC) of the sample by the corresponding Area Under the Hydrolysis Curve of the reference food (white bread).Further, pGI was calculated from the standard formula [27].

$$pGI = 39.71 + 0.549\text{HI}$$

2.5 Microstructure determination of maize starch granules via Scanning Electron Microscope (SEM)

The surface topography of maize genotypes was observed via SEM to understand the morphology and microstructure of starch granules having different amylose content. SEM facility of Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh was utilized to serve the purpose. Dried extracted starch was mounted on an aluminum stub using double-sided tape and coated with a thin film of gold [28]. Then, the samples were examined at an accelerating voltage of 20 kV. Finally, three samples with variable amylose content (low, intermediate, and high) were analyzed at X 500, X 1500, and X 3000 magnification.

2.6 Statistical analysis

The statistical difference of measured parameters among the selected maize varieties was ascertained using one-way analysis of variance (ANOVA). Data analysis was performed using Statgraphia software. The Pearson's correlation coefficient (r) was computed to ascertain the relationships among the analyzed parameters. Further, the scatter plot and linear regression equation for amylose content v/s resistant starch, amylose content v/s SDS; and starch hydrolysis kinetics were derived using MS Excel.

3. Result and Discussion

The complete set of 200 inbreds were screened for amylose content and 21 promising lines, seven each belonging to low (<20%), intermediate (20-30%), and high amylose (>30%) content were selected and further analyzed for starch digestibility characteristics including RDS, SDS & RS. Further, Hydrolysis index of starch was determined to predict glycemic index of maize with lowest, intermediate, and highest amylose content. Further, to reveal the behavior of enzymatic digestion based on the difference in starch granule structure and organization pattern, microstructures of maize starch was determined via scanning electron micrograph (SEM).

a) Starch and Amylose content

The wide variation observed in amylose content among the selected maize genotypes reflects the substantial genetic diversity present for starch composition within the germplasm evaluated. While starch content varied within a relatively narrow range (62.35-73.33%), amylose content exhibited more than a three-fold difference, ranging from 13.57% in CML-499 to 45.33% in ADHAM-15 (Table-1). This indicates that differences among genotypes are primarily associated with starch composition rather than total starch accumulation. Similar levels of variability in amylose content have been reported in diverse maize germplasm and are known to influence starch functionality, digestibility, and nutritional quality [7, 23].

The categorization of genotypes into low-, intermediate-, and high-amylose groups provides a useful framework for understanding the influence of amylose concentration on starch digestibility characteristics. High-amylose genotypes, particularly ADHAM-15 and ADHAM-18, may serve as valuable genetic resources for the development of nutritionally improved maize cultivars because elevated amylose levels are often associated with increased resistant starch formation and slower enzymatic digestion [21, 23]. In contrast, low-amylose genotypes such as CML-499 and GM 146 C1 may be useful in breeding programmes targeting waxy maize development due to their comparatively lower amylose content.

The observed diversity in amylose content among the selected genotypes is expected to influence starch hydrolysis behaviour and glycemic response, as amylose-rich starches generally possess a more compact molecular arrangement and greater resistance to enzymatic attack than amylopectin-rich starches [7]. Therefore, the selected contrasting genotypes were further evaluated for starch digestibility fractions, including rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and predicted glycemic index.

b) Starch Digestibility Profile

Genotypes with a range of amylose content were studied to determine the digestibility profile of maize starch. Based on digestion rate, starches are classified as RDS, SDS, and RS. A range of RDS (82.87± 0.03 to 99.25 ± 0.04), SDS (0.78 ± 0.10 to 12.70 ± 0.1), and RS (0.35 ± 0.01 to 10.95 ± 0.12) have been observed in the experimental material (Table 1). RDS had the highest starch fraction of all the experimental samples. However, maize genotypes showed variation for SDS and RS fractions, which proves that genetic variations have a high potential to change the nutritional quality of starch materials. Interestingly, with the increasing percentage of amylose in maize starch samples, RS content also increased, whereas the SDS content decreased (Figure 1 a, b). A significant positive correlation ($r= 0.962$, $P < 0.00001$ at a significance level of 95%) between RS and amylose content (Figure 2) indicates that amylose is the major contributor to RS content. Moreover, a linearly increasing trend in resistant starch is seen from low amylose to high amylose lines (Figure 1d) indicating RS levels increase with increasing amylose content. This may be attributed to the structural characteristics of amylose molecules. Amylose consists predominantly of linear chains that tend to associate through intermolecular hydrogen bonding, resulting in the formation of compact and ordered structures that are less susceptible to enzymatic hydrolysis. Consequently, starches containing higher proportions of amylose generally exhibit greater resistance to digestion and contribute more significantly to the resistant starch fraction than amylopectin-rich starches [7,21]. Similar observations have been reported in high-amylose maize hybrids, where elevated amylose content was associated with increased resistant starch levels and reduced starch digestibility due to restricted enzyme accessibility and enhanced molecular organization [7]. The present findings are also in agreement with the observations of Obadi et al. [23], who reported that high-amylose maize starches possess unique structural features that limit enzymatic attack and promote the formation of slowly digestible and resistant starch fractions. Likewise, Ratnaningsih et al. [13] demonstrated that starches with higher resistant starch content exhibited lower digestibility and improved nutritional functionality. Therefore, the positive association between amylose and resistant starch observed in the present study highlights the potential of exploiting naturally occurring genetic variability in maize for the development of nutritionally superior cultivars with enhanced resistant starch content.Therefore, high amylose maize genotypes are a prerequisite for increasing maize RS fractions.

A strong negative correlation ($r = -0.745$, $P < 0.00001$ at a significance level of 95%) between SDS and amylose content (Figure 3) signifies that amylopectin, with its complex branched fine

structure, is probably the major starch molecule responsible for the delayed digestion of starchy foods. A possible explanation can be derived from starch debranching analysis that demonstrated parabolic relationship between SDS content and the weight ratio of amylopectin short chains (DP < 13, named SF) to long chains (DP > 13, named LF) [29]. This indicates that amylopectin with a higher proportion of either short chains or long chains can produce relatively high amounts of SDS. Previous studies have similarly reported that the digestibility behaviour of starch is governed not only by amylose content but also by the molecular architecture and chain-length distribution of amylopectin [29,30].

Resistant starch is resistant to digestion, and no glucose is available for glycemic response. Therefore, SDS and RS, individually or in combination, contribute to an improved nutritional quality of starch. In present study, highest RDS content (91.1%) was observed for GM 163 C4 with SDS (8.94%) and RS (0.46%). However, lowest RS content was observed for CML-499 (0.35%) with RDS and SDS equivalent to 89.25 and 10.3%, respectively (Table 1). However, ADHAM 15 mutant genotype was found to have maximum amylose (45.33%) and resistant starch content (10.95%) followed by ADHAM 18 and DQL 2261 with 7.34 and 7.2% RS, respectively. Among the evaluated genotypes, ADHAM-15 exhibited the highest amylose (45.33%) and resistant starch (10.95%) contents, followed by ADHAM-18 and DQL-2261, indicating their potential as donor lines for the development of high-amylose and low-glycemic maize cultivars. Conversely, CML-499 and GM 163 C4 possessed comparatively low resistant starch content and high rapidly digestible starch fractions, characteristics that may be useful in breeding programmes targeting waxy or specialty maize products. The contrasting digestibility profiles observed among these genotypes demonstrate the considerable scope for improving starch nutritional quality through germplasm selection and breeding.

c) Predicted Glycemic Index (pGI)

The kinetics of starch hydrolysis for low-, intermediate-, and high-amylose maize genotypes are presented in Figure 4. A rapid increase in starch hydrolysis was observed during the initial 30 min of enzymatic digestion, followed by a comparatively slower rate of hydrolysis up to 180 min. This behaviour indicates that a substantial proportion of starch was readily accessible to enzymatic action during the early stages of digestion, whereas the remaining starch fractions were hydrolyzed more gradually. Similar digestion patterns have been reported in cereal starches, where the initial rapid phase is attributed to the hydrolysis of amorphous and readily accessible starch regions, while the subsequent slower phase reflects the digestion of structurally ordered fractions exhibiting greater resistance to enzymatic attack [28, 30].

Distinct differences in starch hydrolysis were observed among the three amylose groups throughout the digestion period. At 180 min, starch hydrolysis reached 99.55% in the low-amylose genotype, whereas intermediate- and high-amylose genotypes exhibited hydrolysis values of 95.36% and 81.35%, respectively. The lower digestibility of high-amylose starch can be attributed to the greater proportion of linear amylose chains, which promote intermolecular associations and the formation of compact molecular structures that limit enzyme accessibility. In contrast, starches rich in amylopectin possess a more open and branched architecture, facilitating enzymatic penetration and hydrolysis. Similarly, a study reported that high-amylose maize starches exhibit lower hydrolysis rates owing to their compact granular organization and enhanced structural order [7]. More recently, Obadi et al. [23] highlighted that the unique morphology and molecular architecture of high-amylose maize starch contribute significantly to increased resistant starch formation and reduced susceptibility to enzymatic digestion.

The differences in digestion kinetics were further reflected in the predicted glycemic index values of the selected genotypes. The pGI values were 60.35 for CML-499 (low amylose), 59.61 for DQL-2173 (intermediate amylose), and 56.77 for ADHAM-15 (high amylose) (Figure 5). The gradual decline in pGI with increasing amylose content demonstrates the important role of starch composition in regulating glycemic response. Furthermore, the strong negative correlation observed between amylose content and pGI ($r = –0.98$) indicates that increased amylose concentration is associated with reduced starch digestibility and lower glycemic potential. The predicted glycemic index values obtained in the present study compare favourably with those reported previously for cereal grains differing in starch composition as Aziz et al. [31] evaluated various wheat and maize genotypes in agro-climatic region of Pakistan and reported glycemic index values for wheat varieties between 62.83 ± 2.19 and 70.70 ± 2.82 in Hashim10 and Insaf, respectively, while for maize it ranged between 56.45 ± 1.95 in Sarhad white and 70.76 ± 4.40 in CHT1W. It also aligns with the present research showcasing higher amylose and resistant starch contents exhibit lower glycemic indices than those containing greater proportions of rapidly digestible starch. Similarly, Naseer et al. [32] observed lower predicted glycemic responses in rice genotypes enriched with resistant starch fractions compared with conventional varieties. Haini et al. [33] further demonstrated that incorporation of high-amylose maize starch reduced the glycemic index from 69.18 in the control steamed bun to 39.11, highlighting the significant impact of amylose-rich starch on glycemic behaviour. Further, Lv et al. [34] demonstrated that high-amylose corn starches exhibited lower digestibility and greater resistance to enzymatic hydrolysis due to their compact granular architecture and enhanced structural stability. Compared with these reports,

the pGI value of 56.77 observed for ADHAM-15 was substantially lower than many conventional cereal products and approached the nearly low-GI category. The comparatively lower pGI of ADHAM-15 may therefore be attributed to its elevated amylose (45.33%) and resistant starch (10.95%) contents, which likely reduced the rate and extent of starch hydrolysis during digestion. The present results suggest that naturally occurring variation in amylose content can substantially influence starch digestion behaviour and glycemic potential in maize. Therefore, genotypes such as ADHAM-15, which combine high amylose content, elevated resistant starch levels, reduced starch hydrolysis, and lower predicted glycemic index, represent valuable genetic resources for the development of nutritionally enhanced maize cultivars and low-glycemic functional food products.

d) SEM

Amylose concentration appears to be an important factor in determining starch granule integrity and thus affect digestibility characteristics. Therefore, to validate the results obtained from biochemical analysis of starch digestibility profile, scanning electron micrograph (SEM) was performed using the isolated starch from the maize genotypes with lowest, intermediate, and highest amylose content. High-amylose maize starch was found to be extremely heterogeneous and comprised of three distinct kinds of starch granules, including individual, aggregated, and elongated granules (Figure 6a). In comparison to waxy or low amylose maize, the individual starch granules from high-amylose maize had smoother surfaces, were smaller, and had a more rounded form (Figure 6b). High amylose maize produced aggregate and elongated starch granules that differed significantly from the individual granules and had rough lumpy shape (Figure 6c). Further, high-amylose maize heterogeneous starch granules' lacked pores (Figure 6c). The heterogeneous granule morphology observed in the present study is consistent with previous reports on high-amylose maize starches. Zhong et al. [7] observed that high-amylose maize starches possessed smaller and more irregular granules than low-amylose counterparts, which contributed to differences in digestibility behaviour. Lv et al. [34] further demonstrated that the compact and heterogeneous granule architecture of high-amylose corn starch restricts enzymatic penetration and promotes resistant starch formation. Similarly, Cai et al. [35] reported that high-amylose maize starch contains a mixture of individual, elongated, and aggregated granules, resulting in considerable structural heterogeneity compared with normal maize starch. More recently, Obadi et al. [23] highlighted that the unique granule morphology and dense structural organization of high-amylose maize starch are major factors responsible for its lower digestibility and enhanced nutritional functionality. These findings

support the structural differences observed among the maize genotypes investigated in the present study.

Micrograph of the samples with highest amylose content (ADHAM 15 mutant) contained starch granules with more compactness which contributes (Figure 6a, c) towards decrease in the rate of starch hydrolysis due to lesser surface area available for enzymatic digestion. The micrograph of sample with lowest amylose content (CML 499) contained starch granules with less compactness, leading to increase in the extent of enzyme binding and time for digestion (Figure 6 a, c). Similar results were obtained by previous studies on microstructures of maize starch [23, 36]. Therefore, the surface characteristics of the starch granules have been observed to influence their enzymatic digestion. The lower rate of hydrolysis in high amylose maize genotypes is also a reflection of constraints imposed by crystallinity. Looking deeper into the structure morphology of starch granules of high and low amylose variants it was observed that starch with linear, longer chains has a greater tendency to form crystalline structures than starch with short, highly branched chains [7]. Thus, undoubtedly, it can be expected that portions of polysaccharide chains that are involved in forming crystallites are unlikely to bind securely to the active site [37]. Because amylose is less branched than amylopectin, high-amylose starch is typically more resistant to digest than low-amylose starch.

4. Conclusion:

In the present study, starch profiling, *in-vitro* starch digestibility, kinetics of starch digestion and topography of starch granules were studied in different maize genotypes. A substantial difference exists in the digestibility characteristics of low, intermediate, and high amylose maize genotypes. A significant positive correlation (r= 0.96) was observed between amylose and Resistant starch content. Further, kinetics study of starch digestion revealed a significant disparity in pGI of experimental maize genotypes. It was observed that amylose content strongly and negatively affects pGI (r= -0.98). With relatively slow digestion characteristics due to maximum amylose and resistant starch content, ADHAM 15 mutant genotype was found to be a potential donor for production of low glycemic maize inbred lines. Also, it was found CML 499, GM 163 C4 and GM 146 C1 are potential genotypes for producing waxy maize inbred lines. Further, scanning electron microscope results depicted variable granule structure and pattern in low, intermediate, and high amylose maize. Heterogeneity of granules in high amylose maize starch leads to more compact matrix leading to gentle and slow enzymatic hydrolysis while large starch granules and less compactness in low amylose starch matrix

undoubtedly resulted in intense enzymatic action as revealed by SEM and digestion kinetics. The study's insights are crucial for maize breeders seeking to develop varieties with improved nutritional profiles, particularly those targeting increased RS and lower glycemic response. The results serve as a valuable foundation for future research and breeding programs focused on optimizing maize starch characteristics for both human health and industrial applications.

Ethics approval and consent to participate

The plants used in the study is owned by the breeder who generated and collected the germplasm. He is one of the authors of the manuscript. Therefore, the collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

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Author contributions: Akanksha conducted the experiment, Charanjeet Kaur conducted the experiment, analyzed the data, interpreted the results, and wrote the manuscript. Veena Devi edited the manuscript, Abhijit Kumar Das contributed experimental material, revised the manuscript. Mehak Sethi revised the manuscript. Dharam Paul Chaudhary envisaged the concept, analyzed the data, interpreted the results, and revised the manuscript. All the authors were then involved in the final review and editing. All authors have read and agreed to the published version of the manuscript.

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Table 1: Starch, Amylose, RDS, SDS and RS content for low, moderate, and high amylose maize genotypes

	S.NO.	Genotypes	Starch (%)	Amylose (%)	Rapidly Digestible Starch (RDS) (%)	Slowly Digestible Starch (SDS) (%)	Resistant Starch (RS) (%)
Low Amylose content (<20%)	1.	CML-499	64.50 ± 0.10^{ef}	13.57 ± 0.02^{k}	89.25 ± 0.06^{a}	10.3 ± 0.05^{b}	0.35 ± 0.01^{l}
	2.	GM 146 C1	72.28 ± 0.30^{ab}	13.88 ± 0.10^{k}	87.7 ± 0.01^{ab}	12.7 ± 0.12^{a}	0.49 ± 0.04^{jkl}
	3.	GM 163 C4	68.99 ± 0.08^{abcde}	14.66 ± 0.04^{jk}	91.1 ± 0.03^{a}	8.94 ± 0.08^{e}	0.46 ± 0.07^{kl}
	4.	CML 247	62.64 ± 0.20^{f}	15.54 ± 0.02^{jk}	91.08 ± 0.15^{a}	8.11 ± 0.03^{f}	0.67 ± 0.10^{jkl}
	5.	DQL -2051-1	66.90 ± 0.040^{cdef}	16.88 ± 0.05^{j}	89.16 ± 0.08^{a}	9.55 ± 0.03^{cd}	0.79 ± 0.02^{jk}
	6.	GM 146 C2	66.81 ± 0.02^{cdef}	16.93 ± 0.04^{j}	89.04 ± 0.12^{ab}	10.1 ± 0.06^{bc}	0.74 ± 0.01^{jkl}
	7.	DQL 2329	63.23 ± 0.02^{f}	20.00 ± 0.10^{i}	89.8 ± 0.08^{a}	9.01 ± 0.01^{de}	0.9 ± 0.04^{ij}
		Mean	66.47±3.14	15.89±2.02	89.59±1.11	9.81±1.36	0.62±0.18
Intermediate Amylose content (20-30) %	8.	MCFL 50	63.20 ± 0.03^{f}	21.84 ± 0.08^{hi}	89.33 ± 0.01^{a}	7.48 ± 0.06^{g}	1.3 ± 0.01^{hi}
	9.	DQL 2212	$72.19\pm0.04^{a\ b}$	22.51 ± 0.10^{gh}	87.98 ± 0.14^{ab}	6.3 ± 0.12^{klm}	1.6 ± 0.03^{h}
	10.	DQL 2097	69.40 ± 0.12^{abcde}	23.28 ± 0.07^{gh}	88.7 ± 0.05^{ab}	7.76 ± 0.01^{fg}	1.5 ± 0.02^{h}
	11.	CM 81 C2	62.35 ± 0.03^{f}	24.06 ± 0.02^{fgh}	87.11 ± 0.06^{abc}	7.19 ± 0.20^{ghi}	2.4 ± 0.05^{g}
	12.	DQL 2272	67.11 ± 0.01^{bcdef}	24.62 ± 0.10^{fg}	87.99 ± 0.10^{ab}	4.7 ± 0.08^{n}	2.43 ± 0.15^{g}
	13.	DQL 2173	63.01 ± 0.07^{f}	25.86 ± 0.03^{ef}	87.89 ± 0.03^{ab}	7.47 ± 0.10^{gh}	2.6 ± 0.02^{g}
	14.	DQL 2018	73.33 ± 0.05^{a}	27.77 ± 0.20^{e}	85.36 ± 0.01^{abc}	7.76 ± 0.03^{fg}	3.93 ± 0.05^{e}
		Mean	67.22±4.15	24.27± 1.88	87.76±1.11	6.95±1.56	2.25±0.26

High Amylose content (>30%)	15.	CML-77	62.35±0.05^{f}	30.15±0.02^{d}	85.02±0.12^{abc}	6.89±0.15^{hij}	3.26±0.02^{f}
	16.	CM-156C4	70.08±0.03^{abcd}	30.25±0.04^{d}	85.06±0.10^{abc}	6.83±0.06^{ijk}	3.67±0.05^{ef}
	17.	DQL 2169 2	72.02±0.10^{abc}	30.87±0.02^{cd}	83.6±0.01^{abc}	6.39±0.10^{jkl}	4.5±0.02^{d}
	18.	DQL 2255	66.62±0.12^{def}	32.84±0.16^{c}	83.87±0.08^{abc}	6.22±0.01^{lm}	5.01±0.10^{c}
	19.	DQL 2261	70.05±0.04^{abcd}	36.86±0.04^{b}	80.93±0.12^{bcd}	6.380.05^{jkl}	7.2±0.04^{b}
	20.	ADHAM 18	65.67±0.05^{def}	38.53±0.01^{b}	79.37±0.03^{cd}	5.78±0.06^{m}	7.34±0.01^{b}
	21.	ADHAM 15	64.39±0.01^{ef}	45.33±0.03^{a}	75.4±0.01^{d}	5.95±0.08^{n}	10.95±0.12^{a}
		Mean	67.80±2.66	34.97±5.21	81.89±1.04	5.99±1.20	5.99±0.36

*Values are averages of experimental genotypes under study. Means that do not share a letter are significantly different

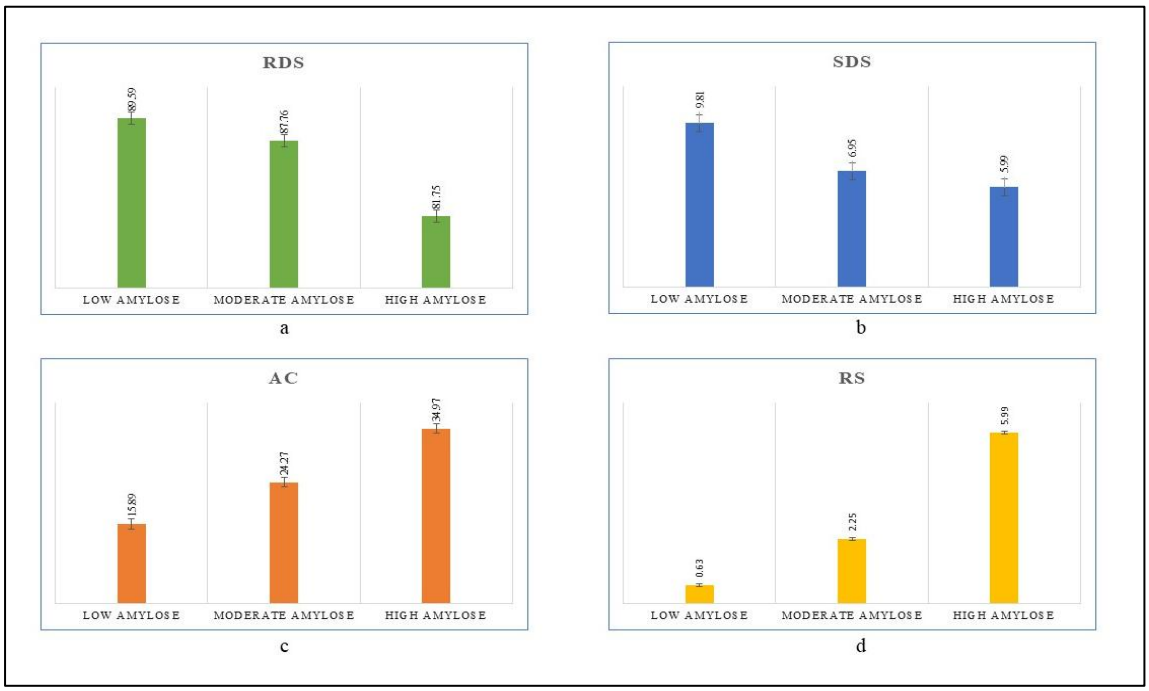


Fig.1 RDS, SDS, AC and RS in Low, Intermediate and High amylose lines * Values are averages of experimental genotypes under study

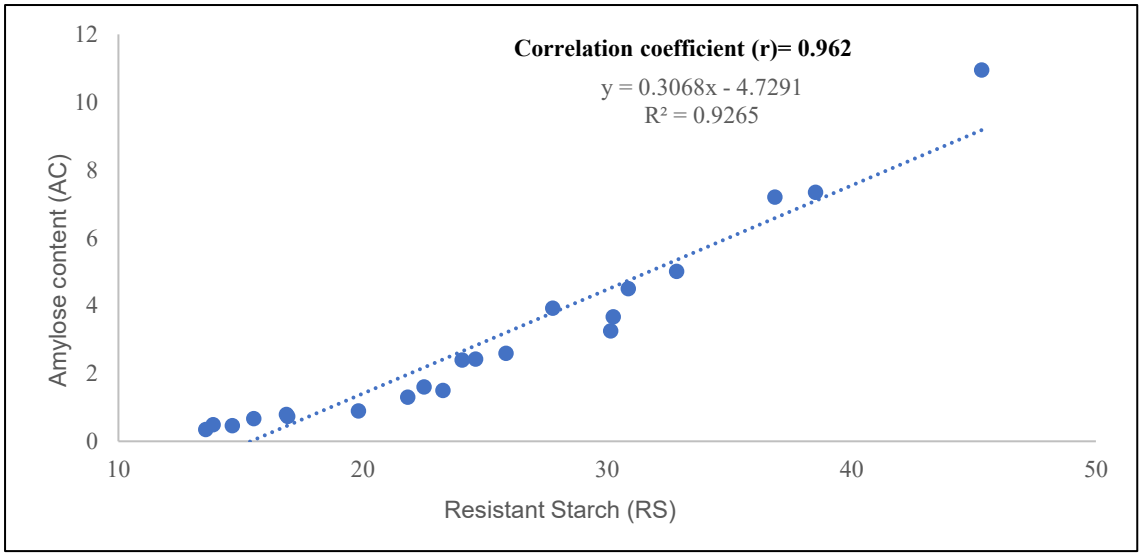


Fig. 2 Correlation between amylose content and resistant starch

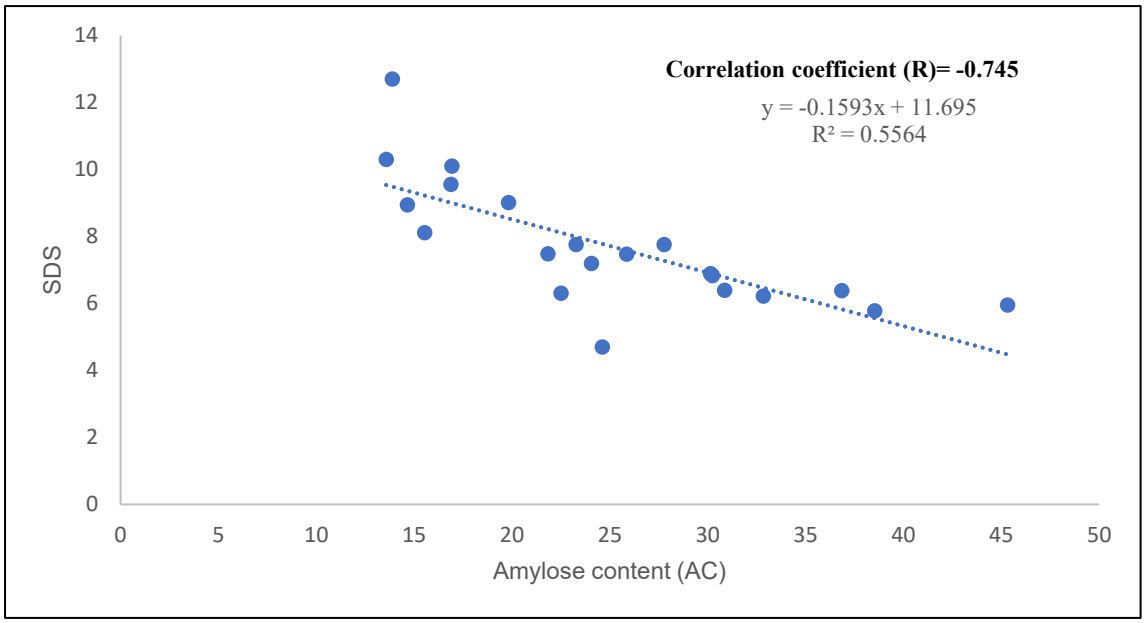


Fig. 3 Scatter Plot for SDS and Amylose content

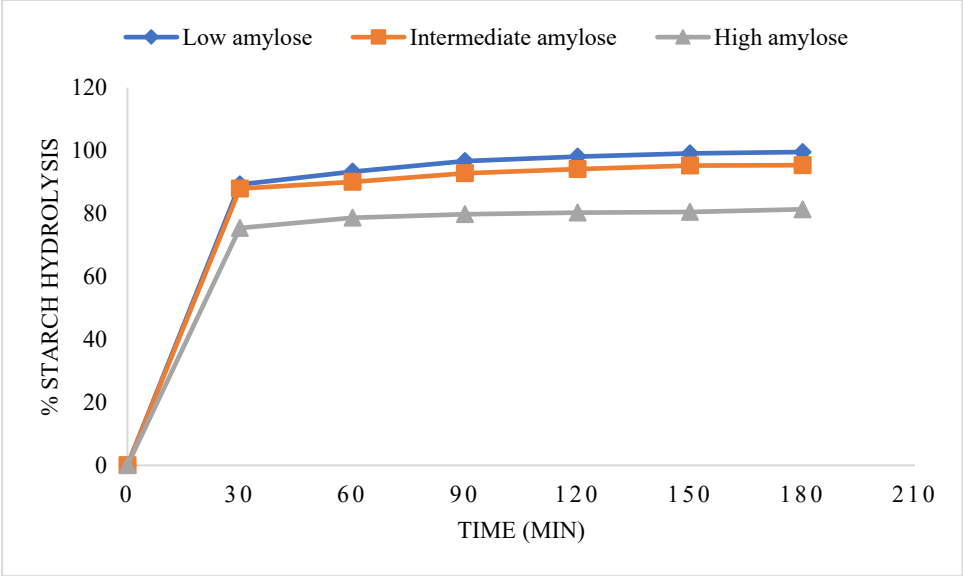


Fig. 4 Kinetics of Starch hydrolysis for Low, Intermediate, and High amylose maize

* Values are averages of three replications

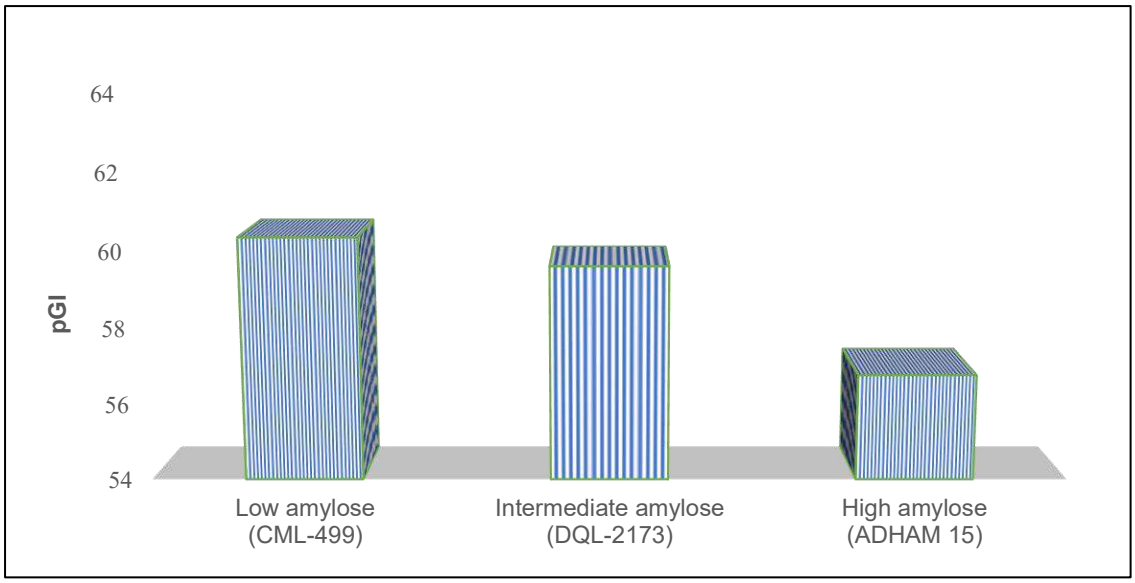


Fig. 5 Predicted glycemic index (pGI) of Low, Intermediate, and High amylose maize

* Values are averages of experimental genotypes under study

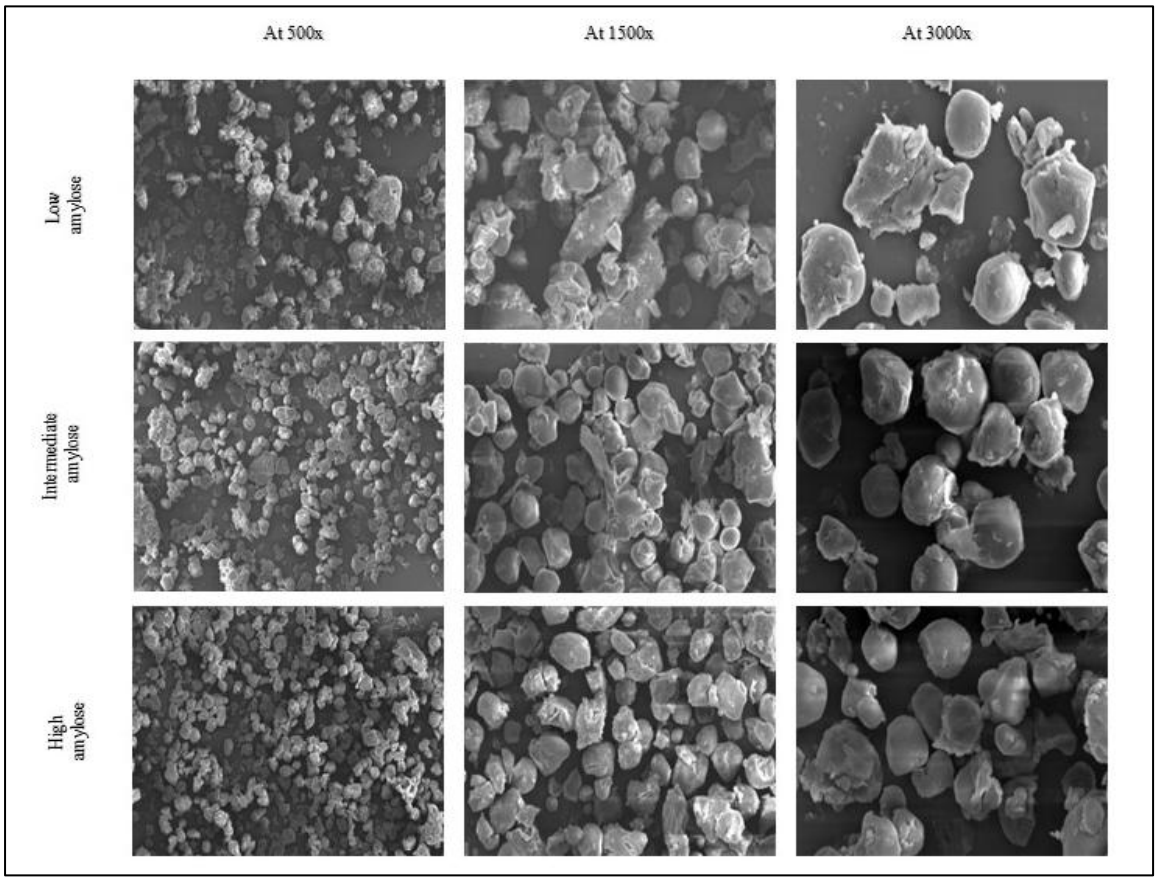


Fig. 6 Scanning Electron Microscope images of low, intermediate, and high amylose maize