Diversity analysis of starch physicochemical properties in 95 proso millet (Panicum miliaceum L.) accessions

Kehu Li⁎, Tongze Zhang⁎, Shwetha Narayanamoorthy⁎, Can Jin⁎, Zhongquan Sui⁎, Zijun Li⁎, Shunguo Li⁎, Kao Wu⁎, Guoqing Liu⁎,⁎, Harold Corke⁎,⁎

⁎Department of Food Science and Technology, Shanghai Jiao Tong University, Shanghai 200240, People’s Republic of China
⁎Institute of Millet Crops, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang 050035, People’s Republic of China
⁎School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong Special Administrative Region
⁎Glyn O. Philips Hydrocolloid Research Centre at HUT, Hubei University of Technology, Wuhan 430068, People’s Republic of China

A R T I C L E  I N F O

Keywords:
Proso millet
Genetic diversity
Starch physicochemical properties

A B S T R A C T

In this study, 95 accessions of proso millet (Panicum miliaceum L.) were characterized for starch physicochemical properties, including apparent amylose content (AAC), gel textural properties, Rapid Visco Analyzer (RVA) pasting viscosity properties, thermal and retrogradation properties. Based on genotypic data, the genetic diversity and inter-relationship of these starchy traits were analyzed. Diverse starch quality was found, for example, AAC ranged from 0 to 32.3%, gelatinization temperature (GT) varied from 71.5 to 79.0 °C, and RVA profile showed distinct patterns among proso millet of different AAC types. Interestingly, high AAC proso millet usually had GT lower than that of low AAC proso millet, which is different from the findings in rice starch. Many starch traits were significantly correlated and most of the 18 tested traits could be classified as either AAC-related traits or GT-related traits. In summary, the information presented here will be useful for further development of proso millet products.

1. Introduction

Proso millet (Panicum miliaceum L.) is one of the oldest cereals of the Old World. Archaeological evidence indicates that it was domesticated in China about 10,000 years ago (Lu et al., 2009). Because of its high tolerance to poor soil, drought and high temperature, proso millet is still serving as a major food crop in arid and semi-arid areas of China, and is also being extensively cultivated in arid regions of many other countries. This cereal has diverse utilization in foods and as a forage plant (Habiyaremye et al., 2017; Sakamoto, 1987).

Starch is the major component in proso millet grain, accounting for 58.1–77.9% of the total grain weight (Yang et al., 2018). Therefore, starch physicochemical properties largely determine the eating and cooking quality, and the processing characteristics of proso millet. Fully understanding the diversity of starch characteristics of proso millet is essential for expanding its utilization in food and non-food industries. However, reports on proso millet starch quality are rather limited. Available publications only cover a small number of germplasm accessions. For example, some physicochemical properties of proso millet starch were studied in four waxy genotypes (Chao et al., 2015). Recently, the physicochemical properties and cooking quality of waxy and non-waxy proso millet were compared, using a total of ten samples (Yang et al., 2018). Previously, correlation analysis was performed in proso millet starch, aiming to elucidate the inter-relationship among starch traits (Chao et al., 2015). However, small sample size in correlation analysis could lead to unreliable conclusions (Bujang & Baharum, 2016). Other publications are mainly on starch modification using physical or chemical methods (Singh & Adeleji, 2017; Sun, Gong, Li, & Xiong, 2014a, b). In summary, previous studies have provided some fundamental information and methodologies to describe the characteristics of proso millet starch. However, the diversity and the inter-relationship among various starch traits need to be analyzed using germplasm sets of adequate size. Furthermore, characterization of starch quality for large proso millet collections is meaningful in many

Abbreviations: AAC, apparent amylose content; ADH, adhesiveness; BD, breakdown; COH, cohesion; CPV, cold paste viscosity; CS, consistency; CV, coefficient of variation; DSC, differential scanning calorimeter; GT, gelatinization temperature; HD, hardness; HPV, hot paste viscosity; Ptime, peak time; PT, pasting temperature; PV, peak viscosity; RVA, Rapid Visco Analyzer; R%, percentage of retrogradation; SB, setback; To, onset temperature; Tc, conclusion temperature; Tp, peak temperature; ΔHgr, enthalpy of gelatinization; ΔHrr, enthalpy of retrogradation

⁎ Corresponding authors.
E-mail addresses: guoqingliu@hotmail.com (G. Liu), hcorke@yahoo.com (H. Corke).

https://doi.org/10.1016/j.foodchem.2020.126863
Received 30 January 2020; Received in revised form 2 April 2020; Accepted 17 April 2020
Available online 23 April 2020
0308-8146/ © 2020 Elsevier Ltd. All rights reserved.
other aspects. For instance, individual accessions of certain merit could be identified and used in breeding programs. In rice, various germplasm collection were characterized for starch physicochemical properties (Bao, Shen, Sun, & Corke, 2006; Caffagni et al., 2013; Li, Bao, Corke, & Sun, 2017). Also, scrutinizing the starch quality of current available cultivars should provide guidance for future breeding. Recently, 34 accessions of foxtail millet were investigated for diversity in starch quality, and the results showed a low diversity in current market available cultivars (Li et al., 2019). Since description and analysis of phenotypic traits of proso millet starch is scarce, the present study is of importance in this field.

To date, the diversity and the relationship among various starch quality parameters have not yet been properly studied in proso millet. Therefore, the objective of the present study was to analyze the genetic diversity and to determine the inter-relationships among starch quality parameters, using a germplasm collection containing 95 proso millet accessions.

2. Materials and methods

2.1. Plant materials

The seeds of proso millet were provided by Institute of Millet Crops, Hebei Academy of Agriculture and Forestry Sciences (Shijiazhuang, China). These accessions were grown in uniform field plot conditions in 2017 in Hainan Island, China. As shown in Fig. 1a, 93 accessions geographically originated from 13 provinces of China, and the other two accessions were from India and Poland. Of the Chinese accessions, most of them originated in Shanxi province (23 accessions), followed by Hebei (12 accessions), Shaanxi (9 accessions) and Ningxia provinces (9 accessions).

2.2. Starch extraction

Starch was extracted using a previously described alkaline steeping method (Li et al., 2019). In general, 50 g seeds were mixed with 300 mL NaOH (0.2%) and ground in a blender (ProBlend 4, Philips, Amsterdam, The Netherlands) for 5 min at the full rotation speed. Then the mixture was kept on a rotator at room temperature for 16 h. Then, the solution was filtered through 100 mesh and 200 mesh sieves, and centrifuged at 3000 xg for 10 min, and the supernatant and top yellowish layer were discarded. This step was repeated till the yellowish layer was completely removed. Then, the starch was finally suspended in distilled water and neutralized with HCl (1 mol/L). After drying in oven at 37 °C for 24 h, the dry starch was ground to powder and passed through a 100 mesh sieve.

2.3. Apparent amylose content (AAC)

AAC was tested based on a colorimetric method described by Li et al. (2019). Briefly, 100 mg starch flour was transferred into a 100 mL measuring flask, then 1 mL of 95% ethanol was used to wet the sample. Then, 9 mL of NaOH (1 mol/L) was added and the mixture was shaken gently to avoid major lumps. The flask was boiled till the starch solution was completely clear. After cooling, the starch solution was diluted to 100 mL with distilled water. Then, 500 μL of starch solution was pipetted into a 15 mL centrifuge tube, in which the starch solution was mixed with 200 μL of I2 (0.2%) / KI (2%), 100 μL acetic acid (1 mol/L) and 9 mL distilled water. This mixture was allowed to stand at room temperature for 10 min before measuring the absorbance value in a spectrometer (UV 1800PC, Jinghua Instruments, Shanghai, China). To calculate the AAC of each sample, a standard curve was made simultaneously by using maize amylopectin (10120 Sigma-Aldrich) and potato amylose (A0512 Sigma-Aldrich) dilutions.

2.4. Rapid Visco Analyzer (RVA) pasting properties

Pasting properties were analyzed using a Rapid Visco Analyzer (RVA4500, Perten Instruments, Hägersten, Sweden). Starch sample (2 g, 12% moisture basis) was mixed with 26 mL of 0.5 mM AgNO3 solution in a RVA sample can. A heating and cooling program was used where the starch solution was held at 50 °C for 1 min, heated to 95 in 3.7 min and kept at 95 °C for 2.5 min before cooling down to 50 °C in 3.8 min and maintained at 50 °C for 2 min. The peak viscosity (PV), hot
paste viscosity (HPV), cool paste viscosity (CPV), peak time (Ptime), pasting temperature (PT) and the derivative parameters breakdown viscosity (BD = PV - HPV), setback viscosity (SB = CPV - PV) and consistency (CS = CPV - HPV) were recorded.

2.5. Gel textural properties

After RVA analysis, the sample cans containing starch gels were sealed by Parafilm™ and kept at 4 °C for 24 h. Textural properties of starch gel were measured by a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, United Kingdom). The gel was pressed twice by a flat-ended cylindrical probe (5 mm diameter) for 10 mm with a speed at 1.0 mm/s. Hardness (HD), adhesiveness (ADH) and cohesiveness (COH) were recorded.

2.6. Thermal and retrogradation properties

Thermal and retrogradation properties were analyzed on a differential scanning calorimeter (Discovery DSC 25, TA Instruments, New Castle, DE). Starch sample (2 mg, dry basis) was mixed with 6 μl distilled water in an aluminum pan, and the pan was hermetically sealed and equilibrated at room temperature for at least 2 h. The sample was heated from 30 °C to 110 °C at a rate of 10 °C/min with an empty sealed pan used as reference. Onset (To), peak (Tp), conclusion (Tc) temperatures and enthalpy of gelatinization (ΔHg) were calculated using the Trios Program v5.0.0 (TA Instruments). The measured samples were kept at 4 °C for 7 days, before being rescanned from 25 °C to 110 °C at 10 °C/min to analyze the retrogradation properties. Enthalpy of retrograded starch (ΔHgr) was determined and the percentage of retrogradation (R%) was calculated as R% = ΔHgr/ΔHg * 100%.

2.7. Statistical analysis

All traits were measured in duplicate, and data analysis was performed using SPSS Statistics 19.0 (IBM, Armonk, NY). The Student-Newman-Keuls test at P < 0.05 was conducted for comparison of mean values of accessions from different hierarchical clusters. Pearson correlation analysis and principal component analysis were conducted to analyze the relationship among starch quality traits. The hierarchical cluster analysis on 95 proso millet accessions was computed based on Ward’s method.

3. Results

3.1. Starch physicochemical properties

3.1.1. Apparent amylose content (AAC)

AAC varied from 0 to 32.3%, with a mean value of 16.4% (Table 1). The highest AAC was found in “Hongmeizi” (Code No. 1061), and AAC was undetectable in 13 accessions (Supplementary Table 1). The mean value of AAC was 16.4%, with a CV value (79.3%) suggesting a high diversity in AAC of these 95 proso millet accessions. In this study, diverse AAC types were identified in proso millet: < 5% (33 accessions); 5-12% (8 accessions); 13-20% (6 accessions); 21-25% (6 accessions); and > 25% (42 accessions). The majority of proso millet accessions (75 out of 95) were either waxy type (AAC < 5%) or high AAC type (AAC > 25%) (Fig. 1b).

AAC, apparent amylose content; HD, hardness; ADH, adhesiveness; COH, cohesiveness; Tsd, onset temperature; Tp, peak temperature; Tc, conclusion temperature; ΔHgr, enthalpy of gelatinization; ΔHgr%, enthalpy of retrogradation; R%, percentage of retrogradation; PV, peak viscosity; HPV, hot paste viscosity; BD, breakdown; CPV, cold paste viscosity; SB, setback; CS, consistency; Ptime, peak time; PT, pasting temperature; CV, coefficient of variation.

3.1.2. Gel textural properties

Hardness (HD) varied from 1.7 g to 61.8 g, and averaged 20.3 g; Adhesiveness (ADH) varied from −94.4 to −0.8 g.s and averaged −26.2 g.s. The high SD and CV values indicated a useful range of genetic diversity in HD and ADH in proso millet (Table 1). Compared to HD and ADH, a relatively lower diversity was found for Cohesiveness (COH), as indicated by its lower CV value (36.5%) (Table 1), but still, COH varied widely (from 0.122 to 0.856), especially between waxy and non-waxy proso millet. (Supplementary Table 1).

3.1.3. Rapid viscosity Analyzer (RVA) pasting properties

The RVA viscosity parameters all showed wide ranges of variation. Peak viscosity (PV), hot paste viscosity (HPV) and cool paste viscosity (CPV) ranged from 2215 to 3787 cP, 1444 to 2177 cP, and 2577 to 3373 cP, respectively. The three derivative parameters, breakdown viscosity (BD), setback viscosity (SB) and consistency (CS) ranged from 511 to 1437 cP, −1435 to 752 cP, and 238 to 2070 cP, respectively. Of these six viscosity parameters, SB showed the highest variation, as indicated by its CV value (22.9%), followed by CS (25.3%). On the other hand, peak time (Ptime) and pasting temperature (PT) showed narrower ranges of variation. The minimum Ptime was 4.04 min while the maximum was 4.45 min (Table 1). The minimum PT (78.8 °C) was found in 20 accessions, and the maximum PT (82.8 °C) was found in Xiaohongmei (Code No. 41) (Supplementary Table 1). Notably, the RVA pattern was quite different among proso millets of different AAC type, as shown in Fig. 2. high AAC proso millet usually had high CPV, hence a steeper curve from HPV to CPV.

3.1.4. Thermal and retrogradation properties

Thermal and retrogradation properties were diverse in proso millet, as shown in Supplementary Fig. 1 and Table 1. The ranges of To, Tp and Tc were 67.4–75.5 °C, 71.5–79.0 °C, and 76.5–84.0 °C, respectively (Table 1). “Baihuizi” (Code No. 1239) had the highest gelatinization temperature (GT, Tg) while “Shuzhi” (Code No. 1321) had the lowest GT (Supplementary Table 1). The majority of proso millet had a GT of 73–77 °C, while 20 accessions had a GT lower than 73 °C, and 24 accessions higher than 77 °C (Fig. 1c). ΔHg and ΔHgr varied from 11.9 to 17.6 J/g and 1.5 to 3.7 J/g, respectively. R% varied widely in proso millet, the highest R% (28.3%) was found in accession “Baiziji” (Code No. 1157), while the lowest R% (0.1%) was found in “Baishu” (Code No. 1009). R% averaged 20.4% with a 18.0% CV.

3.2. AAC-GT combination in 95 proso millets

The AAC-GT combination for each proso millet accession was visualized in Fig. 1d. Taking AAC = 20% as a threshold, the 95 proso millet accessions could be separated into two main groups: low AAC- and high AAC-GT groups. The general trend for AAC-GT combination was that proso millets with a GT lower than 77 °C usually had a high AAC, whereas proso millets with a GT higher than 77 °C usually had a low AAC, as visualized in Fig. 1d. Taking AAC = 20% as a threshold, the 95 proso millet accessions could be separated into two main groups: low AAC- and high AAC-GT groups.
Fig. 2. Representative RVA viscosity profiles for five accessions with different AAC: Heishuzi (AAC = 30.8%), Dahongshu (AAC = 23.0%), Xiaoheishu (AAC = 13.4%), Baishuzi (AAC = 0.9%), Honganchunwei (AAC = 0).

### Table 2
Comparison of means of 18 starch quality traits among three hierarchical groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC (%)</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HD (g)</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADH (g.s)</td>
<td>−2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−14.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−51.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>COH</td>
<td>0.732&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.607&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.346&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PV (cP)</td>
<td>2803&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3230&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2618&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HPV (cP)</td>
<td>1655&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1401&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1296&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CPV (cP)</td>
<td>2097&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2387&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3060&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BD (cP)</td>
<td>1328&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1829&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1323&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SB (cP)</td>
<td>−866.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−842.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>442.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CS (cP)</td>
<td>442&lt;sup&gt;a&lt;/sup&gt;</td>
<td>986&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1765&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PT (℃)</td>
<td>81.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ptime (min)</td>
<td>4.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>To (℃)</td>
<td>72.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tp (℃)</td>
<td>77.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tc (℃)</td>
<td>81.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔHg (J/g)</td>
<td>15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔHgr (J/g)</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>R (%)</td>
<td>18.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values with the same character in the same line are not significantly different at p < 0.05 using the Student-Newman-Keuls test. AAC, apparent amylose content; HD, hardness; ADH, adhesiveness; COH, cohesiveness; PV, peak viscosity; HPV, hot paste viscosity; CPV, cold paste viscosity; BD, breakdown; SB, setback; CS, consistency; PT, pasting temperature; Ptime, peak time; To, onset temperature; Tp, peak temperature; Tc, conclusion temperature; ΔHg, enthalpy of gelatinization; ΔHgr, enthalpy of retrogradation; R, percentage of retrogradation.

### 3.3. Correlation analysis on 18 traits of starch physicochemical properties

Various significant correlations were found among starch traits (Table 3). For example, AAC was significantly correlated to all other traits, except for BD. In detail, AAC positively correlated to HD (r = 0.900, p < 0.0001), but negatively to ADH (r = −0.926, p < 0.0001) and COH (r = −0.922, p < 0.0001). AAC had negative correlation with PV, HPV, and PT, while having positive correlation with other RVA traits, i.e., CPV, SB, CS, and Ptime. For thermal and retrogradation properties, AAC negatively correlated to To, Tp, Tc, and ΔHg, but positively correlated to ΔHgr and R%. Tp and ΔHg had significant correlation with all other traits, except for BD, Ptime, and ΔHgr. Notably, Tp had positive correlation with PT (r = 0.771, p < 0.0001).

### 3.4. Principal component analysis of 18 starch traits

Principal component analysis was conducted on 18 starch quality traits in 95 proso millet accessions. The first principal component explained 61.0% variation among the accessions, the second component explained 14.9%, and the third explained 7.4%. Totally, the first three principal components accounted for 83.3% of the variation (Supplementary Table 2). Using the first two principal components as axes 1 and 2, the distribution in space of 18 starch traits was shown in Fig. 1e. Fourteen out of 18 starch traits separated into two main groups. Group 1 included AAC, CS, CPV, HD, SB, and R%, and Group 2 consisted of To, Tp, Tc, ΔHg, PT, COH, and ADH.

### 3.5. Hierarchical cluster analysis of 95 proso millet accessions

After normalization of starch trait values using “Z Scores” method, cluster analysis of the 95 proso millet accessions was computed based on Ward’s method. Two main clusters were separated at the relative distance of 25 (Fig. 3). The first main cluster consisted of 54 accessions, and was further separated into subgroup 1 (33 accessions) and subgroup 2 (21 accessions) at a genetic distance of 6. The second main cluster consisted of 41 accessions. Proso millets of the same geographical origin did not necessarily group together.

### 3.6. Comparison of starch physicochemical properties among three hierarchical clusters

Most of the starch quality traits differed significantly (p < 0.05) among the three hierarchical groups. However, there was no significant difference in breakdown viscosity (BD) between group 1 and 3. Moreover, PT did not differ significantly between group 2 and 3; SB did not differ significantly between group 1 and 2. Generally, Group 1 had the lowest mean AAC and the highest mean GT (Tp), while group 3 had the highest mean AAC and the lowest GT (Tp), and Group 2 had the intermediate mean AAC and GT (Tp).
Correlation analysis of starch physicochemical properties of 95 proso millet accessions.

Hg ΔHgr ΔHD 0.900 **** −ADH −0.919 **** −COH 0.910 **** 0.912 **** −HPV 0.651 **** −CPV 0.924 **** 0.867 **** 0.851 **** 0.401 **** −BD 0.049 0.264 ** −PT 0.451 **** 0.436 **** 0.447 **** 0.461 **** 0.153 0.488 **** 0.375 *** 0.348 *** −Δ 0.374 *** 0.366 *** 0.287 ** −R (%) 0.702 **** 0.763 **** 0.695 **** 0.435 **** 0.354 *** 0.693 **** 0.216* 0.719 **** 0.681 **** 0.145 −ΔHgr, enthalpy of retrogradation; R(%), percentage of retrogradation.

5

(c) 2017 Elsevier B.V. All rights reserved.

4. Discussion

4.1. Genetic diversity of starch physicochemical properties in proso millet

The results of this study show a high diversity of starch physico-
chemical properties in proso millet. By including many more ac-
cessions, more diverse starch physicochemical properties were found than in any previous report in this field (Chao et al., 2015; Wen, Liu, Meng, Zhang, & Zhao, 2014; Yang et al., 2018). Previously, AAC was found ranging from 18.5% to 38.7% in five non-waxy proso millet, and from 2.2% to 3.5% in five waxy proso millet (Yang et al., 2018). In another study, AAC ranged from 0.12% to 0.25% in four waxy proso millets. The current study found AAC varied from 0 to 4.9% in 33 waxy proso millet and from 5.2 to 32.3% in 62 non-waxy proso millets. Notably, the maximum AAC in non-waxy proso millet and the minimum AAC in waxy proso millet in this study were all lower than that of previous reports. This is likely due to the different standard starch used in AAC standard curve development. Generally, the range of AAC is very similar to that reported in rice (Bao et al., 2006; Li et al., 2017; Wu et al., 2017).

Gelatinization temperature (GT) is much more diverse than previous reports suggested (Chao et al., 2015; Wen et al., 2014). There was a continuous variation of GT from 71.5 to 79.0 °C in these 95 proso millet accessions. The phenotypic GT data provided by the current study, should be useful for future studies aiming to explore the genetic basis for GT via molecular approaches, such like association analysis, and gene cloning. In rice, SSIIa gene has been identified as the major gene controlling GT, and markers targeting sequence variations of SSIIa gene have been developed and applied in marker assisted breeding (Ayers et al., 1997; Bao, Corke, & Sun, 2006; Li, Bao, Corke, & Sun, 2017). The GT variation observed in 95 proso millet accessions implied extensive sequential variations of the underlying gene, hence a gene-trait association study for GT should be achievable in proso millet.

Proso millet with different AAC types showed distinct RVA patterns. Generally, a higher CPV usually accompanied a higher AAC, hence a steeper climb from HPV to CPV, as shown in Fig. 2. Similar findings were reported in rice starchy (Bao et al., 2006; Yu, Ma, Menager, & Sun, 2012). This implied that RVA testing could serve as an expeditious way to separate proso millet cultivars into high-, intermediate- and low-AAC groups. Other starch quality parameters all showed wide variation, as listed in Supplementary Table 1. The information provided in this paper should be valuable in guiding selection of suitable materials for later studies, such like breeding, food development, and starch synthesis gene mining.

4.2. Inter-relationship among 18 starch traits in proso millet

Correlation analysis and principal component analysis were both conducted to reveal the inter-relationship among 18 starch quality traits. Since the correlation analysis was only performed in four accessions in a previous study, the current report should present a more reliable result. As Table 3 shows, various significant correlations were found. Both AAC and Tp were significantly correlated to many traits of RVA, gel textural and thermal properties. In line with many previous studies conducted in starches of various sources (Ahmed et al., 2018; Bao et al., 2006; Li & Corke, 1999), AAC had significant positive correlation with HD, CPV and SB. The significant AAC-HD correlation (r = 0.900, p < 0.0001) was expected, since non-waxy starch usually produce a harder gel than waxy starch. The significant positive correlations of AAC-CPV (r = 0.924, p < 0.0001) and AAC-SB (r = 0.855, p < 0.0001) were consistent with the RVA pattern differentiation in proso millet, mentioned in 4.1. ΔHg negatively related to AAC (r = -0.790, p < 0.0001), agreeing with some previous reports (Czuchajowska, Otto, Paszczynska, & Baik, 1998; Li et al., 2019). Tp-PT correlation (r = 0.924, p < 0.0001) detected in this study was also well supported by previous studies (Abegunde, Mu, Chen, & Deng, 1999).
However, it should be pointed out, inter-relationship among some starch traits were not always consistent in literature, and this should be due to differences in sample selection.

The inter-relationship among starch quality traits was also visualized by PCA plotting. As Fig. 1e shows, PCA grouped 14 traits into two main groups. BD and Ptime were in neither of the two groups. Since AAC and GT are the two most important parameters in determining starch eating and cooking quality, the two PCA groups could be regarded as AAC related traits consisting of AAC, CS, CPV, HD, SB and R; and GT related traits consisted of Tp, Tc, PT, HPV, ΔHg, COH and ADH. A similar grouping was reported for rice and foxtail millet (Li et al., 2019; Wang et al., 2007; Yang et al., 2014).

The hierarchical clustering of 95 accessions based on 18 starch quality parameters resulted in three groups (Fig. 3). Comparison of means showed significant differences in many quality parameters among the three germplasm groups (Table 2). Proso millets originating from the same geographical regions did not necessarily group together, indicating a wide genetic diversity in proso millet starch quality within these regions, such as Shanxi, Shaanxi, Hebei and Heilongjiang provinces. Traditionally, these regions harbor more proso millet accessions than other regions (Wang et al., 2016). There are abundant proso millet germplasm resources that could be used for research with various aims (Upadhyaya, Vetriventhan, Dwivedi, Pattanashetti, & Singh, 2015), however, a fundamental description of genotypic traits in big germplasm collections is rarely reported. The current study characterized starch physicochemical properties for 95 proso millet accessions, and the results have many implications for later studies. First, starch products for different end-uses requires native starches with different physicochemical properties. With the information provided by this study, individual germplasm of desired starch quality could be selected for grain production, breeding, as well as for food and non-food products development. Secondly, the phenotypic data offered by this study, could be incorporated with genotypic data into association analysis to develop trait specific markers, which could be used in marker assisted breeding to improve breeding efficiency.

4.3. AAC-GT combinations in proso millet

AAC and GT are viewed as the two major determinants of starch eating and cooking quality (Wang et al., 2007; Yang et al., 2014). Therefore, the AAC-GT combination in each accession were visualized in plot figure (Fig. 1d). There were two types of AAC-GT combinations in proso millet, and GT of high AAC proso millet is lower than GT of low AAC proso millet. This is very different from the findings in rice starch. In rice starch, four types of AAC-GT were found: low AAC-high GT, low AAC-low GT, high AAC-high GT and high AAC-low GT. The GT of low AAC-high GT group is higher than that of high AAC-high GT group, the GT of low AAC-low GT group is higher than that of high AAC-low GT group (Li et al., 2017; Yang et al., 2014). The reason for fewer groups of AAC-GT combinations in proso millet could be fewer samples being studied, which means that scrutinizing more samples may lead to a discovery of new AAC-GT combinations. The reason also could also be rooted in the genetic basis of AAC and GT in proso millet, meaning there are just not sufficiently varied allele combinations of underlying genes to produce more AAC-GT combinations. The reason needs to be explored by later studies.

4.4. Insights for later studies in proso millet

Proso millet is an important minor crop with a long cultivation history, but currently under-researched and underutilized. Recent studies suggest it has potential for healthy foods with better nutrient composition than many major cereals (Habiyaremye et al., 2017). Also, proso millet has a good environmental adaptability and a short growth cycle, hence serving as a major cereal plant in arid and semi-arid areas of many countries (Wang et al., 2016). There are abundant proso millet germplasm resources that could be used for research with various aims (Upadhyaya, Vetriventhan, Dwivedi, Pattanashetti, & Singh, 2015), however, a fundamental description of genotypic traits in big germplasm collections is rarely reported. The current study characterized starch physicochemical properties for 95 proso millet accessions, and the results have many implications for later studies. First, starch products for different end-uses requires native starches with different physicochemical properties. With the information provided by this study, individual germplasm of desired starch quality could be selected for grain production, breeding, as well as for food and non-food products development. Secondly, the phenotypic data offered by this study, could be incorporated with genotypic data into association analysis to develop trait specific markers, which could be used in marker assisted breeding to improve breeding efficiency.
5. Conclusions

Wide variation in starch physicochemical properties were observed in 95 proso millet accessions, and the inter-relationship among 18 starch quality parameters was analyzed. The description of starch traits in 95 proso millet accessions is useful in guiding selection of suitable germplasm for breeding programs and food or non-food product development. The diverse starch physicochemical properties revealed in this study should facilitate later studies aiming at exploring the genetic basis for genotypic variations in starch quality traits. The current study is the first one reporting proso millet starch quality in a large set of accessions, but some findings need to be confirmed and explained by later studies, for example, the less diverse AAC-GT combination in proso millet, compared to other cereal starch.

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Dr. Pinhe Liu at Sun Yat-Sen University (Guangzhou, P. R. China) for her assistance in data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126863.

References