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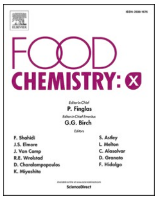

Comparison of the physicochemical properties of starches from maize reciprocal F1 hybrids and their parental lines

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ABSTRACT

Heterosis on maize yield and quality is highly variable and depends on parental selection. This study investigated and compared the starch structure and physicochemical properties among four sweet-waxy maize lines, four waxy maize lines, and their eight reciprocal F1 hybrids. Compared with the sweet-waxy maize, waxy maize and F1 hybrids had lower extent of branching of amylopectin and relative crystallinity, and larger starch granule size. Waxy maize starch had higher breakdown viscosity and retrogradation percentage, and lower setback viscosity and gelatinization enthalpy than the sweet-waxy maize starch. Meanwhile, the peak and setback viscosities, and retrogradation enthalpy of most F1 hybrid starches were higher than those of their female parent, while gelatinization enthalpy was the opposite. The F1 hybrid starches had higher onset temperature and retrogradation percentage and lower gelatinization enthalpy than their male parent in general. In conclusion, this study provides a framework for the production of new hybrids.

1. Introduction

Heterosis is of paramount agronomic importance because of the better performance of F1 hybrid plants than their corresponding homozygous parental inbred lines. Maize (*Zea mays* L.) is the global most important crop for food and feed industries and great success has been achieved in the heterosis applications of this crop. Improving grain yield and quality has become one of the important goals of modern maize breeding (Alves et al., 2019). Starch in maize grains is the main source of the starch industry and accounts for 80% of global starch production (Waterschoot et al., 2015). Maize starch is mainly composed of two main components, linear amylose and highly branched amylopectin (Singh et al., 2006). The structure and composition of starch differ among various maize types (sweet, waxy, normal, and high-amylose) and thus can be used to determine differences in starch physiochemical properties (Yu et al., 2015). In addition, the starch quality of hybrid maize is affected by both parents. Thus, the differences in the influence of male and female parents on starch quality require further exploration.

Waxy maize (*Zea mays* L. var. *certaina* Kulesh) is regulated by the *wx* gene and starch is composed by nearly 100% amylopectin in the endosperm (Dong et al., 2019). Waxy maize has high transmittance, high viscosity, low retrogradation tendency, and easy digestion in human gut and is widely used as food and industrial raw materials (Singh et al., 2006). Waxy maize starch granules are polygonal and has the highest crystallinity and largest proportion of double helix components and ordered structure in external region among various maize starches (Yu et al., 2015). Consumers prefer waxy maize with a sweet flavor, high stickiness, and soft texture (Ramekar, Sa, Park, Park, & Lee, 2020). Combinations of *wx* and other genes may alter grain quality (Wang et al., 2019). Huang et al. (2021) revealed that the synergistic regulation of *SSII-2*, *SSII-3*, and *wx* in the endosperm during amylose and amylopectin synthesis has improved rice eating and cooking quality. Moreover, the *wx-wx* and *du-wx* maize mutants maintain their respective thermal and rheological properties after β-amylolysis and isoamylase (Mendez-Montealvo et al., 2011). The starch granule size, starch microstructure, and thermal properties of *ae-wx* and *seb1a-ae-wx* maize mutants differ from those of *wx* maize (Li et al., 2007). Thus, combining *wx* with other genes can be considered in the endeavor to improve waxy maize quality.

Sweet-waxy maize is a special maize type that adds sweetness into *wx*

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background and changes the eating quality of waxy maize (Li et al., 2022). Breeders have experimented to produce high-quality maize hybrids. The F1 hybrids produced from sweet maize and field maize are characterized by high total sugar content, ear weight, and grain yield (Kumari et al., 2008). Similarly, the potential heterosis of reciprocal F1 hybrids was estimated using supersweet maize and waxy maize as parents (Dermail et al., 2020). According to an eating quality analysis of F1 hybrids, sweetness, stickiness, and tenderness have preferable genetic gain (Dermail et al., 2021). When combining different parents, the maternal effect should be considered. Altinel et al. (2019) found that the kernel size and kernel weight of F1 hybrids produced from sweet maize (maternal parent) and dent maize (male) are similar to those of the male parent, and the sugar content is similar to that of the maternal parent. However, limited studies have analyzed the starch quality of F1 hybrids using sweet-waxy maize and waxy maize inbred lines as parents.

This study aimed to examine the changes in starch granule morphology and size, amylopectin chain length, and pasting and thermal properties of reciprocal F1 hybrids using sweet-waxy maize inbred lines and waxy maize inbred lines as parents. The results provide a reference for the synergistic breeding of quality maize starch.

2. Materials and methods

2.1. Plant materials

Four sweet-waxy (SW) maize inbred lines, namely, CJ17CySW11 (SW1), 17h1sbsw9 (SW2), T42 SW03-62 (SW3), and J10BSW (SW4), and four waxy (WX) maize inbred lines, namely, W333-18 (WX1), T2 (WX2), YU45 (WX3), and W8 (WX4), were grown in the field in January 2021 at Sanya (Hainan province, China). For each inbred line, 15 plants were grown in a row of 4.2 m, with 0.5 m of space between rows. N:P_2O_5:K_2O was applied at 225:75:75 kg/ha. During plant cultivation, field management consisting of irrigation, weeding, and pest control was carried out normally.

Eight reciprocal F1 hybrids, namely, WXS1 (SW1 as maternal parent and WX1 as male parent), WXS2 (SW2 as maternal parent and WX2 as male parent), WXS3 (SW3 as maternal parent and WX3 as male parent), WXS4 (SW4 as maternal parent and WX4 as male parent), SWW1 (WX1 as maternal parent and SW1 as male parent), SWW2 (WX2 as maternal parent and SW2 as male parent), SWW3 (WX3 as maternal parent and SW3 as male parent), and SWW4 (WX4 as maternal parent and SW4 as male parent) were produced by careful manual pollination. The ears of four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight F1 hybrids were harvested at maturity, and the middle grains were subjected to starch analysis. The kernel shape is shown in Fig. S1, with shrunken kernels observed for the four sweet-waxy maize inbred lines and plump noted for the four waxy maize inbred lines and eight F1 hybrids.

2.2. Starch isolation

The starch isolation in this study referred to the method of Lu & Lu (2012). Firstly, 100 g grains were steeped in 500 ml of 0.1% $NaHSO_3$ for 48 h at ambient temperature. Secondly, the soaked samples were beaten into a homogenate, passed through a 100-mesh sieve, and the filtrate was collected and allowed to stand for 4 h. Thirdly, the settled starch layer was collected in 50 ml centrifuge tubes and centrifugated at 3,000*g* for 5 min. Fourthly, after the top layer impurities were removed, the white layer was resuspended with distilled water. Centrifuge the resuspension several times to remove impurities until the purity of the collected starch reaches the Chinese National Standard of edible maize starch (GB/T 8885–2017, protein content $<$ 4 mg/g, lipid content $<$ 2 mg/g, ash content $<$ 2 mg/g).

2.3. Starch granule size analysis

Starch granule size analysis was carried out using a Laser Particle Size Analyzer MS-2000 (Malvern, Worcestershire, England), which can measure the size of 0.1 to 2,000 μm. The disperse phase was absolute ethyl alcohol and stirred at 112 *g*. The obscuration in all measurements was $>$ 10%. Starch size distribution was expressed in terms of the volume of equivalent spheres. The average granule size was defined as the volume weighted mean. Experiments were repeated three times.

2.4. Scanning electron microscope observation of starch

Starch samples passed through a 100-mesh sieve were attached to an aluminum column with double-sided tape for scanning electron microscope (SEM) observation. The starch samples were plated with ion-sputtered gold using a sputter coater, and starch granules were observed using the GeminiSEM 300 (Carl Zeiss, Jena, Germany) environmental scanning electron microscope at 1,000 magnifications.

2.5. Gel permeation chromatography and amylopectin chain-length analysis

Starch samples passed through a 100-mesh sieve were deproteinized with protease and sodium bisulfite and debranched with isoamylase according to the methods of Li et al. (2011). The molecular weight distribution of debranched starch was analyzed using a PL-GPC 220 high-temperature chromatograph (Agilent Technologies UK Limited, Shropshire, UK) with three columns (PL110–6,100, 6,300, and 6,525) and a differential refractive index detector (Lin et al., 2016).

Amylopectin branch chain length distribution of starches were analyzed by using high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detector (PAD; Dionex ICS 5000 system). The technical support of gel permeation chromatography and amylopectin chain-length analysis are provided by Sanshu Biotech. Co., ltd (Shanghai, China). The experiments were carried out two times.

2.6. X-ray diffraction pattern of starch

X-ray diffraction (XRD) pattern of starch was obtained with an X-ray diffractometer D8 Advance (Bruker–AXS, Karlsruhe, Germany). The starch samples were exposed to the X-ray beam at 200 mA and 40 kV. The scanning region of the diffraction angle (2θ) ranged from 3° to 40° in step size of 0.04° and the speed was 0.6 s/step. The relative crystallinity (%) was calculated as the ratio of total crystalline peak area to the total diffraction using the MDI Jade 6 (MDI, Livermore, CA, USA) software. The experiments were performed three times.

2.7. Pasting properties

The pasting properties of starch were evaluated by a Rapid Viso Analyser RVA-TecMaster (Perten, Australia). In an aluminum crucible, 1.96 g of starch (dry weight) was mixed with 26.04 ml of distilled water form a 7% starch paste. The mixture was heated from 50 to 95 ℃ at the rate of 12 ℃/min, maintained at 95 ℃ for 2.5 min, cooled to 50 ℃ at the same rate, and finally held at 50 ℃ for 1 min. Two replicates were used for each sample.

2.8. Thermal property

The thermal characteristics were explored using differential scanning calorimetry DSC200F3 (NETZSCH, Bavaria, Germany). Five mg of starch (dry weight, through a 100-mesh sieve) was mixed with 10 μl of distilled water in an aluminum pan (25/40 μl, Diameter = 5 mm) to form a 66.7% starch paste. The aluminum pan was sealed and placed at 4 ℃ for 24 h. The sample pan was heated from 20 to 100 ℃ at the rate of 10 ℃/min, and an empty pan was used as a reference. Thermal transitions of flour were defined as onset temperature (T_o), peak gelatinization temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH_{gel}). After thermal analysis, the samples pan was stored at 4 ℃ for 7 days for retrogradation enthalpy (ΔH_{ret}) and retrogradation percentage ($\%R = 100 \times \Delta H_{ret}/\Delta H_{gel}$) analysis. The experiment was repeated three biological times.

2.9. *Statistical analysis*

The data obtained in the present study were subjected to analysis of variance (ANOVA) using SPSS statistic v.19.0 (SPSS Inc; Chicago, IL., USA). The significance levels of the data were calculated by Duncan's test at the 0.05 probability level ($P < 0.05$). The figures were plotted in GraphPad Prism software v.8.0.0 (San Diego, CA, USA).

3. Results and discussion

3.1. *Morphology structure and distribution of starch granules*

Scanning electron microscopy showed that the starch granules were small and oval in sweet-waxy maize lines but large and irregular in waxy maize lines (Fig. 1). A large number of studies also found that the endosperm of sweet maize is mainly composed of small starch granules, and that of waxy maize is mainly composed of large starch granules (Yu et al., 2015; Peng and Yao, 2018; Zhang et al., 2018). Our study found that the starch particles of sweet-waxy maize had a diameter range of 0.45–20 μm (Fig. 1) and were dominated by intermediate starch granules (5 μm < d < 15 μm) (Table S1). In waxy maize, the starch particles had a diameter range of 0.45–40 μm (Fig. 1) and were dominated by large starch granules (d > 15 μm). Meanwhile, the average starch granule size (ASGS) in waxy maize line was almost twice that in sweet-waxy maize (Fig. 2), which was similar to the results of previous studies (Yu et al., 2015; Zhang et al., 2018). Zhang et al. (2018) also found that the starch granules in waxy maize are mostly polygonal with rough surface, and those in sweet maize are mostly oval and spherical. The conversion of sugar to starch in sweet maize kernels is hindered, which in turn restricts starch deposition and development, resulting in small starch granules (Dong et al., 2019).

Compared with the parents, an increased proportion of irregular starch granules was found in the eight reciprocal F1 hybrids (Fig. 1). In the endosperm cell of F1 hybrids, starch synthesis and accumulation are increased and the starch granules are closely arranged, resulting in the irregular shape of the starch granules (Yu et al., 2015). Compared with the sweet-waxy maize lines, a decrease in the proportion of small and intermediate starch granules and an increase in the proportion of large starch granules and ASGS were found in the four WXS lines (Table S1). Compared with the waxy maize lines, the proportion of small starch granules decreased in the four SWW lines, and the proportion of intermediate starch granules increased in the four WXS lines (Table S1). The ASGS of the waxy maize lines was larger than that of the WXS lines and smaller than that of the SWW lines (Fig. 2). Meanwhile, the ASGS of the eight F1 hybrids were all larger than their corresponding maternal parent, which was similar to the previous findings (Lin et al., 2016). In addition, the proportion of starch granules with 0.45 μm < d < 15 μm in WXS lines was higher than that in SWW lines, and the performance of ASGS and starch granules with d > 15 μm was the opposite (Table S1).

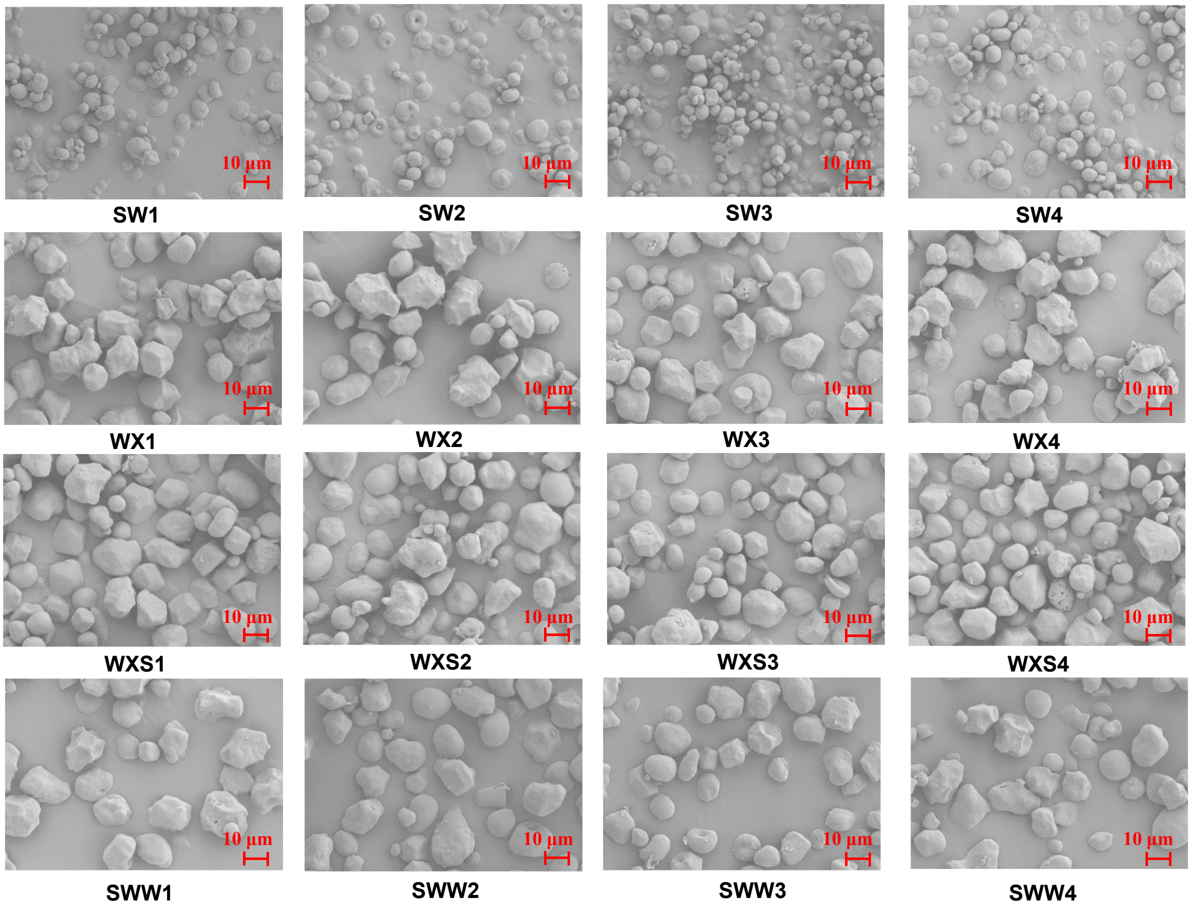


Fig. 1. Morphology of starch granules for four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight F1 hybrids. Scanning electron micrographs of starch granules were taken at 1,000 magnification, scale bar = 10 μm. SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle.

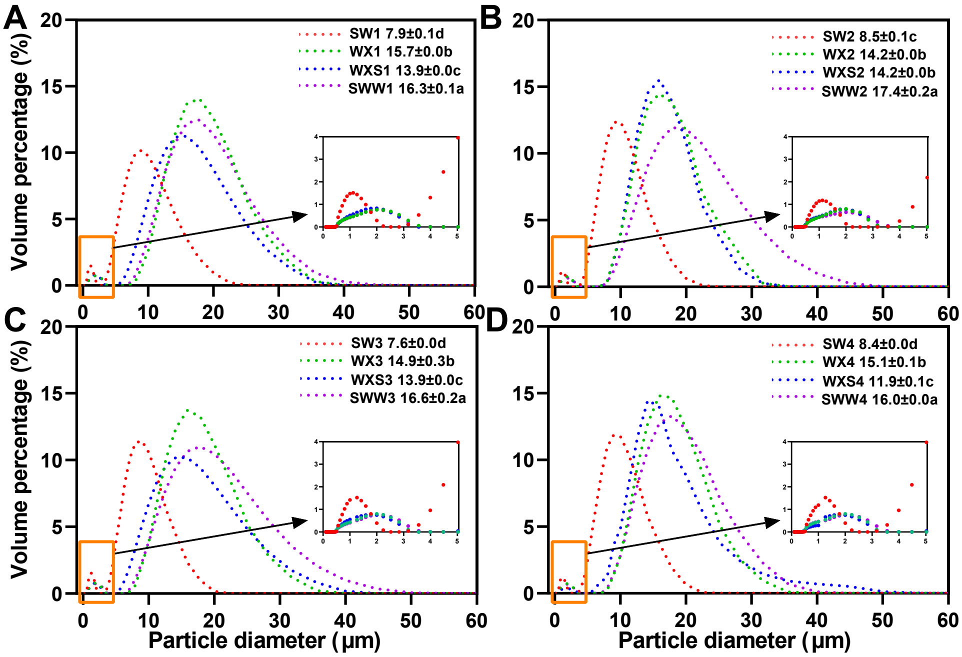


Fig. 2. Volume distributions of starch granules for four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight F1 hybrids. A, parents SW1 and WX1 and their reciprocal F1 hybrids; B, parents SW2 and WX2 and their reciprocal F1 hybrids; C, parents SW3 and WX3 and their reciprocal F1 hybrids; D, parents SW4 and WX4 and their reciprocal F1 hybrids. ASGS, average starch granule size. SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle. Mean values within each hybrid combination followed by different letters are significantly different at $P < 0.05$.

These results showed that the starch granules of F1 hybrids were influenced by maternal inheritance.

3.2. *Amylopectin chain length distribution*

The average chain-length (ACL) of waxy maize lines was longer than that of sweet-waxy maize lines (Fig. 3A-D). Our previous research found that waxy and sweet-waxy maize starches differ in amylopectin chain-length distribution (ACLD) depending on their variety (Yang et al., 2021). Furthermore, the ACL of the four WXS lines was smaller than that of the four SWW lines in the current research (Fig. 3A-D). The polyline trend of chains was consistent between maternal and male and reciprocal F1 hybrids and may be species-dependent (Hsieh et al., 2019). The chains in the four WXS lines and four waxy maize lines were smaller than those in the four SWW lines and four sweet-waxy maize lines in the range of $9 \leq$ degree of polymerization (DP) ≤ 23 (Fig. 3E-F). The proportion of longer chain-length branches (DP ≥ 24) in the four WXS lines, and four waxy maize lines were higher than those in the four SWW lines, and four sweet-waxy maize lines (Fig. 3E-F). The proportion of $9 \leq$ DP ≤ 23 in the four SWW lines were higher than those in the four waxy maize lines, and the opposite trend was observed for the proportion of DP ≥ 24 (Fig. 3E-F). Differences in ACLD between parental and F1 hybrids may be related to the expression of the genes encoding starch branching enzyme IIb (*BEIIb*), starch synthase I (*SSSI*), and soluble starch synthase IIIa (*SSSIIIa*) (Nakamura et al., 2005). Zhu et al. (2013) found that single *dull1* (encoding starch synthase III) mutant and double *dull1-wx* mutant increases the number of short chains ($6 <$ DP < 24). This result indicated that the ACLD of reciprocal F1 hybrids was influenced by the male parent.

3.3. *Molecular weight distribution of debranched starch*

The molecular weight distribution of debranched starch was determined by gel permeation chromatography (GPC). Peaks 1 and 2 consisted of short (A and short B chains) and long (long B chains) branch-chains of amylopectin, respectively (Hsieh et al., 2019). GPC results showed that the proportion of short branch-chains of amylopectin (Peak 1) in the sweet-waxy maize inbred lines starches was larger than that in the waxy maize inbred lines and reciprocal F1 hybrids; the opposite trend was observed for the proportion of long branch-chains of amylopectin (Peak 2) (Fig. 4). Peng and Yao (2018) reported that sweet maize has highly branched clusters, and waxy maize has fewer short intra-cluster chains. This extensive branching is mainly related to the deletion of the gene encoding starch debranching enzyme, *su1* (Ball et al., 1996). Therefore, the changes of sweet-waxy maize were related to the sweetness-related genes.

3.4. *X-ray diffraction and relative crystallinity*

The X-ray diffraction pattern of the starches form the four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight reciprocal F1 hybrids presented typical "A" diffraction types with main peaks at diffraction angles 2θ of 15°, 17°, 18°, and 23° as in cereal starches (Fig. S2, Cheetham and Tao 1998). The relative crystallinity (RC) of waxy maize starch was higher than that of the starch of sweet-waxy maize and eight reciprocal F1 hybrids. The RC of waxy maize starch is greater than that of sweet maize due to the greater amount of longer amylopectin chains in the former (Singh et al., 2006; Yu et al., 2015; Peng and Yao, 2018). Waxy starch with high proportions of long chains forms stable, highly crystalline structures (Chung et al., 2011). In addition, the starch RC of WXS hybrids was higher than that of SWW hybrids, and this trend was consistent with the amylopectin chain length distribution. These results showed that the male parent had a great influence on the starch RC of F1 hybrids.

3.5. *Pasting property*

Changes in the fine structure of starch, such as molecular size, degree of branching, chain-length distribution, crystalline structure of granules, will directly affect the functional properties of starch (Lin et al., 2016). The pasting parameters of four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight reciprocal F1 hybrids were summarized in Table 1. The starches of WX2, WX3, and WX4 inbred lines had higher peak (PV) and breakdown viscosities (BD) and lower setback viscosity (SB) than the starches of SW2, SW3, and SW4. In general, high PV is associated with large starch granules (Liu et al., 2017) and high RC (Hung et al., 2007); the similar results were found in this study (Fig. 2 and Fig. S2). Waxy starches have great PV, followed by a pronounced BD and low SB during pasting (Hung et al., 2007; Yu et al., 2015). The differences between sweet-waxy and waxy maize lines may be due to the

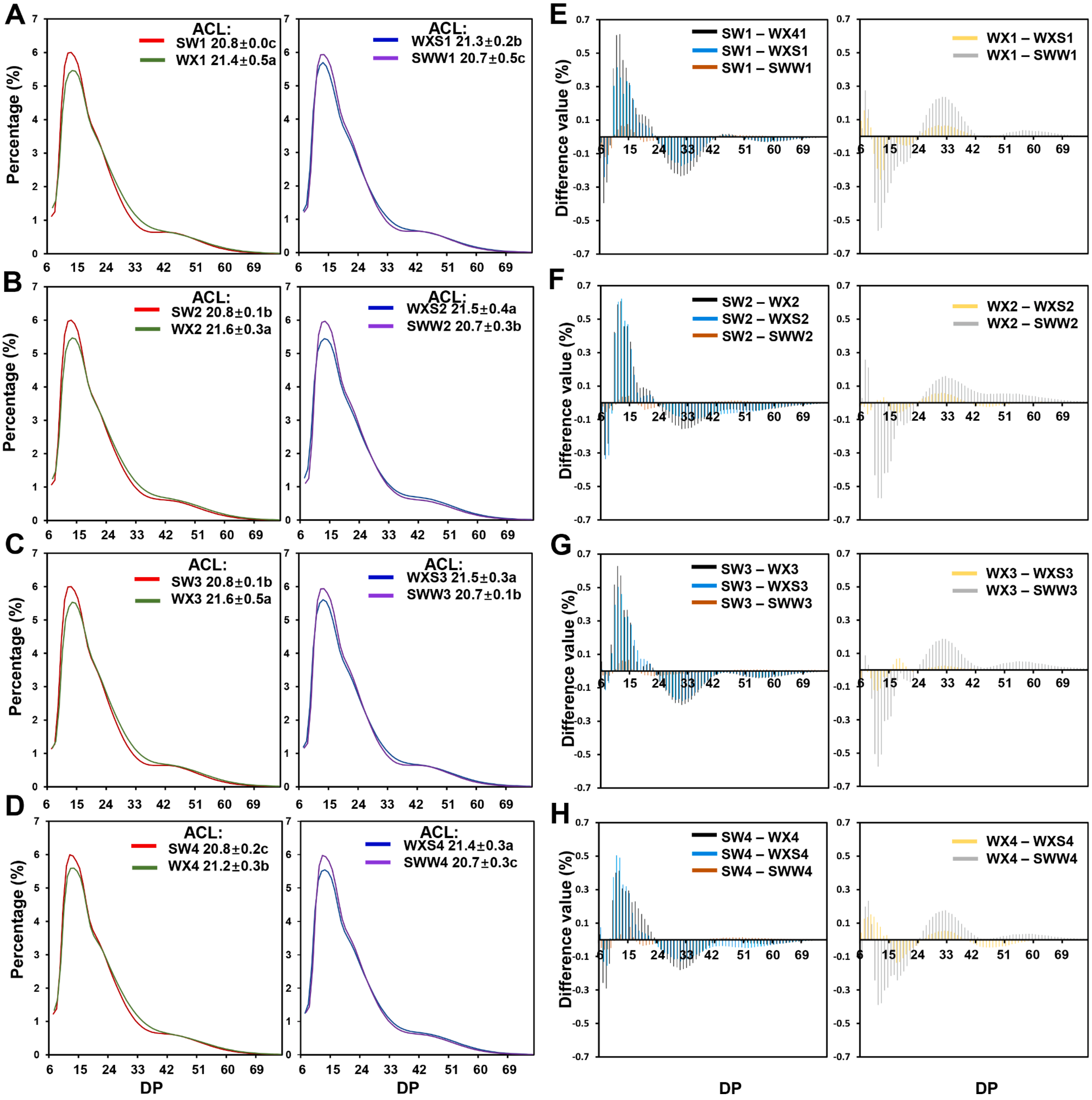
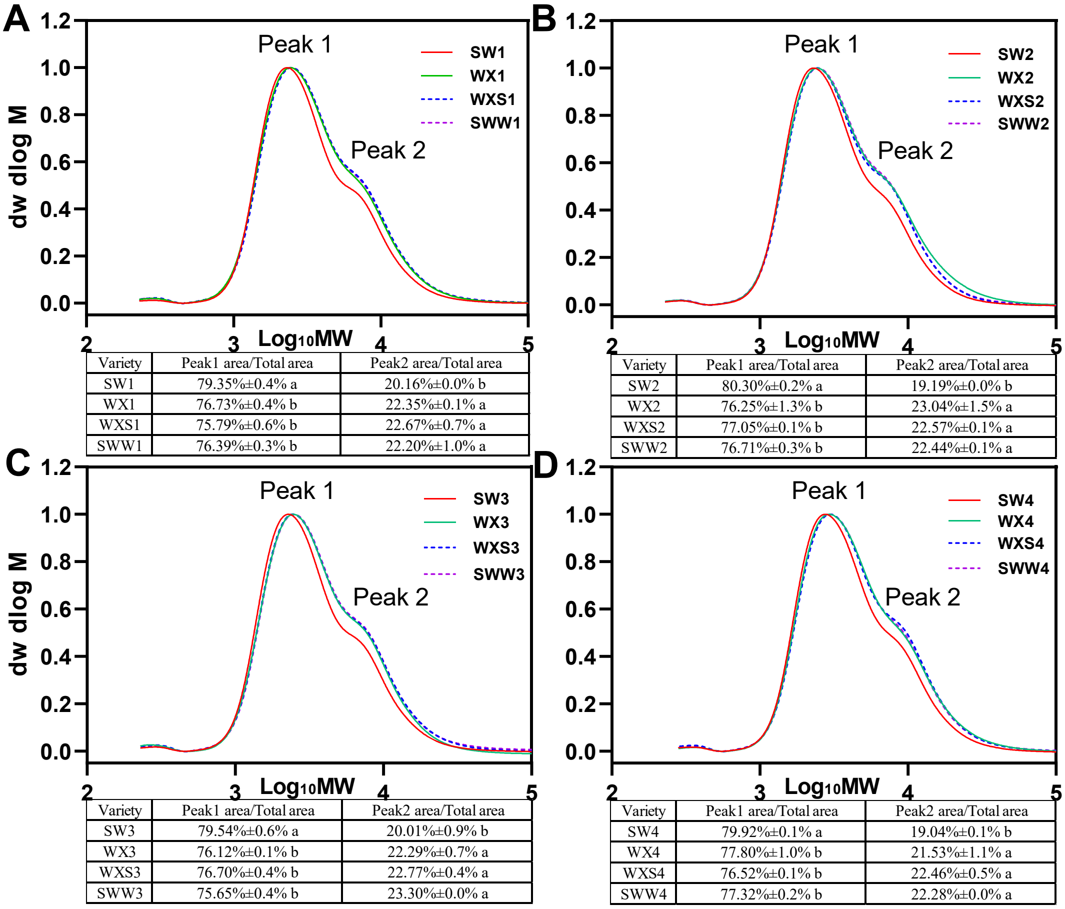


Fig. 3. Starch amylopectin chain-length distribution of four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight F1 hybrids. A and E, parents SW1 and WX1 and their reciprocal F1 hybrids; B and F, parents SW2 and WX2 and their reciprocal F1 hybrids; C and G, parents SW3 and WX3 and their reciprocal F1 hybrids; D and H, parents SW4 and WX4 and their reciprocal F1 hybrids. A-D, amylopectin chain-length distribution of each line; E-H, the differences in the amylopectin chain-length distribution of the different lines. ACL, average chain-length. SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle. Mean values within each hybrid combination followed by different letters are significantly different at $P < 0.05$.

starch granules size (Perera et al., 2001). In addition, the high PV and BD in waxy maize starches may be due to the high proportion of short chains in the starch, the weaker starch binding force, and the easier swelling (Han and Hamaker 2001).

Compared with SW1, WXS1 had decreased PV and BD, and increased other pasting parameters; and SWW1 had increased BD and SB and decreased trough viscosity (TV), final viscosity (FV), and pasting temperature (P_{temp}) (Table 1). Compared with WX1, SWW1 and WXS1 had increased PV, TV, FV, and SB. An increase in PV and BD were found in WXS2 and SWW2 compared to SW2 and WX2, respectively (Table 1). An increase in PV and BD and a decrease in FV and SB were found in SWW2, and a decrease in BD and SB and an increase in P_{temp} in WXS2, compared with their corresponding male parents SW2 and WX2. Compared with SW3, PV and BD of WXS3 increased, FV and SB decreased; and the FV, SB, and P_{temp} of SWW3 increased (Table 1). Compared with WX3, the PV and TV of SWW3 reduced, while FV, SB, and P_{temp} increased; and FV and P_{temp} of WXS3 decreased. The pasting parameters of WXS4 and SWW4 were higher than those of SW4 and WX4, respectively (Table 1). Compared with SW4, SWW4 had increased PV and BD and decreased SB. Except for PV and BD, the pasting parameters of WXS4 were increased compared with those of WX4. Furthermore, the SB and P_{temp} of WXS1, WXS2, and WXS4 starches were higher than those of SWW1, SWW2, and SWW4 starches, and the opposite trend was observed for BD. These results showed that the pasting property varies among the eight F1 hybrids



Variety	Peak1 area/Total area	Peak2 area/Total area
SW1	79.35%±0.4% a	20.16%±0.0% b
WX1	76.73%±0.4% b	22.35%±0.1% a
WXS1	75.79%±0.6% b	22.67%±0.7% a
SWW1	76.39%±0.3% b	22.20%±1.0% a

Variety	Peak1 area/Total area	Peak2 area/Total area
SW2	80.30%±0.2% a	19.19%±0.0% b
WX2	76.25%±1.3% b	23.04%±1.5% a
WXS2	77.05%±0.1% b	22.57%±0.1% a
SWW2	76.71%±0.3% b	22.44%±0.1% a

Variety	Peak1 area/Total area	Peak2 area/Total area
SW3	79.54%±0.6% a	20.01%±0.9% b
WX3	76.12%±0.1% b	22.29%±0.7% a
WXS3	76.70%±0.4% b	22.77%±0.4% a
SWW3	75.65%±0.4% b	23.30%±0.0% a

Variety	Peak1 area/Total area	Peak2 area/Total area
SW4	79.92%±0.1% a	19.04%±0.1% b
WX4	77.80%±1.0% b	21.53%±1.1% a
WXS4	76.52%±0.1% b	22.46%±0.5% a
SWW4	77.32%±0.2% b	22.28%±0.0% a

Fig. 4. Starch molecular weight distribution of four sweet-waxy maize inbred lines, four waxy maize inbred lines, and eight F1 hybrids. A, parents SW1 and WX1 and their reciprocal F1 hybrids; B, parents SW2 and WX2 and their reciprocal F1 hybrids; C, parents SW3 and WX3 and their reciprocal F1 hybrids; D, parents SW4 and WX4 and their reciprocal F1 hybrids. SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle. Mean values within each hybrid combination followed by different letters are significantly different at $P < 0.05$.

possibly due to their different parental sources or differences in their starch structures (Hsieh et al., 2019). The mutation types of genes related to starch biosynthesis in SW and WX inbred lines are different, which leads to differences in the molecular structures and physical characteristics of starch, thus affecting the starch characteristics of F1 hybrids (Perera et al., 2001; Altinel et al., 2019; Revilla et al., 2021).

3.6. *Thermal property*

The thermal parameters of sweet-waxy maize, waxy maize, and their filial generation F1 are presented in Table 2. The thermal properties of starch are affected by the morphology of starch granules, amylopectin chain length distribution, and crystalline structure (Wang et al., 2018; Peng et al., 2021). The starch of four sweet-waxy maize inbred lines exhibited higher ΔH_{gel} and lower $\%R$ than the starch of the four waxy maize inbred lines. However, the ΔH_{gel} of waxy maize is greater than that of sweet maize (Singh et al., 2006; Yu et al., 2015; Perera et al., 2001), indicating that the starch of sweet-waxy maize differs from that of sweet maize. The ΔH_{gel} of starch is mainly affected by the disruption of starch double helices, that is, a high RC is correlated with a great ΔH_{gel} (Yu et al., 2015). The high content of amylopectin in waxy maize leads to the formation of many crystalline structures within granules, so its starch requires a large amount of energy to unravel the double-helix structure during gelatinization (Singh et al., 2010). The low $\%R$ of sweet-waxy maize is mainly because of its small granules and highly branched clusters (Singh and Kaur, 2004).

Compared with the parents SW1 and WX1, WXS1 and SWW1 had decreased ΔH_{gel} and T_c and increased T_o, T_p, lower ΔH_{ret} and $\%R$ (Table 2). Compared with SW2, only ΔH_{gel} decreased in WXS2 and SWW2, while the other thermal parameters increased. The ΔH_{gel} of WXS2 and SWW2 and ΔH_{ret} and $\%R$ of SWW2 decreased and T_o, T_p, and $\%R$ of WXS2 increased in comparison with WX2. A decrease in ΔH_{gel} and an increase in $\%R$ were found in WXS3 and SWW3, compared with the SW3. Compared with WX3, WXS3 and SWW3 had increased ΔH_{gel} and ΔH_{ret} and decreased T_c. The ΔH_{gel} and T_c of WXS4 and SWW4 decreased, and the T_o, T_p, ΔH_{ret}, and $\%R$ increased, when compared with the SW4. Compared with WX4, WXS4 and SWW4 had improved T_o, T_p, and ΔH_{ret}. The differences of T_o, T_p, and T_c of starch in different hybrids may be related to the stability and perfection of starch structure (Wang et al., 2018). In addition, the ΔH_{ret} and $\%R$ of WXS2, WXS3, and WXS4 starches were higher than those of SWW2, SWW3, and SWW4 starched, respectively. The differences in swelling power, water solubility, and molecular structure of starches may also be the key factors leading to the different thermal properties between WXS and SWW lines (Lin et al., 2016).

4. Conclusion

Compared with waxy maize, sweet-waxy maize had smaller starch granules, lower proportion of long branch-chains of amylopectin and relative crystallinity, and higher proportion of short branch-chains of amylopectin; these features are responsible for the lower breakdown viscosity and retrogradation percentage, and higher setback viscosity and gelatinization enthalpy of sweet-waxy maize. Compared with the

Table 1
Starch pasting profiles of four sweet-waxy maize inbred lines and four waxy maize inbred lines and eight F1 hybrids.

Variety	PV(cP)	TV(cP)	BD(cP)	FV(cP)	SB(cP)	P_{temp} (℃)
SW1	1740.5 ± 113.5 f	1122.0 ± 54.0c	618.5 ± 59.5 gh	1180.0 ± 55.0 e	58.0 ± 1.0 g	75.2 ± 0.3 cd
WX1	1357.0 ± 152.5 h	712.7 ± 84.1f	644.3 ± 66.6 fg	744.0 ± 67.3 g	56.0 ± 11.1 g	74.3 ± 0.0 e
WXS1	1574.7 ± 87.5 g	1225.3 ± 37.3 ab	349.3 ± 55.4 i	1408.3 ± 12.9 bc	183.0 ± 24.4b	78.1 ± 0.6 a
SWW1	1686.0 ± 74.0 fg	926.7 ± 70.5 e	741.0 ± 33.0 ef	1046.0 ± 122.2f	166.7 ± 13.6c	73.5 ± 0.1 fg
SW2	1655.7 ± 53.5 fg	1255.7 ± 87.4 ab	379.0 ± 20.0 i	1399.3 ± 40.4 bc	120.3 ± 9.1 de	74.0 ± 0.5 ef
WX2	2310.7 ± 142.9b	1221.3 ± 68.1 ab	1045.7 ± 77.8 c	1343.7 ± 25.9 cd	40.3 ± 4.0 ghi	73.5 ± 0.0 fg
WXS2	2274.0 ± 138.6 bc	1303.3 ± 41.8 a	904.0 ± 12.2 d	1346.3 ± 22.2 cd	114.0 ± 3.0 e	75.1 ± 0.1 cd
SWW2	2573.3 ± 22.3 a	1243.0 ± 25.5 ab	1330.3 ± 3.2 a	1300.3 ± 16.7 d	57.3 ± 9.5 g	73.5 ± 0.1 fg
SW3	1737.7 ± 33.5 f	1190.0 ± 18.0 bc	590.0 ± 11.5 gh	1308.7 ± 14.6 d	132.7 ± 3.8 d	72.9 ± 0.5 gh
WX3	2241.7 ± 35.6 bcd	1181.7 ± 82.3 bc	1060.0 ± 109.1c	1325.0 ± 6.1 cd	47.0 ± 1.0 gh	74.5 ± 0.5 de
WXS3	2128.3 ± 80.9 cde	1102.0 ± 4.0 cd	1007.0 ± 8.2c	1143.3 ± 11.0 e	36.3 ± 2.1 hi	73.2 ± 0.2 g
SWW3	1801.0 ± 49.6 f	1273.0 ± 7.0 ab	525.0 ± 48.7 h	1462.3 ± 14.6 ab	190.0 ± 12.5b	75.5 ± 1.0c
SW4	1686.3 ± 25.5 fg	1104.3 ± 47.5 cd	582.0 ± 22.0 gh	1185.3 ± 50.5 e	81.0 ± 3.0 f	71.8 ± 0.0 i
WX4	2109.7 ± 55.5 de	1027.7 ± 7.6 d	1085.3 ± 47.0 c	1047.3 ± 14.2 f	24.3 ± 3.5 i	70.8 ± 0.6 j
WXS4	2059.0 ± 36.9 e	1262.0 ± 29.2 ab	751.0 ± 10.0 e	1509.0 ± 52.4 a	252.0 ± 11.1 a	77.2 ± 0.6 b
SWW4	2379.0 ± 16.5 b	1183.3 ± 18.6 bc	1195.7 ± 5.7 b	1219.0 ± 19.5 e	48.0 ± 2.6 gh	72.4 ± 0.5 hi

SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle. PV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SB, setback viscosity; P_{temp}, pasting temperature; cP, centipoise. Mean values in the same experiment in the same column followed by different letters are significantly different ($P < 0.05$).

maternal parent, the F1 hybrids had increased average starch granule size, decreased relative crystallinity, and altered average chain-length and molecular weight distribution of debranched starch, all of which are potential drivers for improved starch quality. The changes in the morphological, molecular, crystal, and helical structures of starch in F1 hybrids modified its thermal and pasting characteristics. Meanwhile, the maternal parent played a significant role in determining the content of grain components and starch granule size of F1 hybrids. The male parent played an important role in determining the amylopectin chain length distribution and relative crystallinity, and the degree of variation in functionality of hybrids starch were jointly affected by the parents. Overall, the use of sweet-waxy maize and waxy maize as parents shows promise in producing reciprocal hybrids with improved quality.

CRediT authorship contribution statement

Table 2
Starch thermal characteristics of four sweet-waxy maize inbred lines and four waxy maize inbred lines and eight F1 hybrids.

Variety	ΔH_{gel} (J/g)	T_p (℃)	T_o (℃)	T_c (℃)	ΔH_{ret} (J/g)	%R (%)
SW1	15.2 ± 0.6 bc	65.7 ± 0.4 b	70.5 ± 0.1 d	78.4 ± 0.5 cdef	4.6 ± 0.1 hi	30.1 ± 0.8 g
WX1	13.8 ± 0.3 de	63.3 ± 0.4 f	68.3 ± 0.3 g	77.0 ± 1.2 efg	5.0 ± 0.2 fgh	36.4 ± 1.7 f
WXS1	10.6 ± 0.1 g	66.6 ± 0.8 a	79.8 ± 0.3 c	72.4 ± 0.1 hi	5.8 ± 0.3 cd	54.9 ± 2.0 c
SWW1	10.1 ± 0.7 g	65.2 ± 0.2 bc	81.0 ± 0.5 b	70.9 ± 0.3 i	6.5 ± 0.3 ab	64.8 ± 4.2 a
SW2	15.5 ± 1.3 b	61.5 ± 0.2 g	66.1 ± 0.2 i	73.2 ± 1.2 h	4.4 ± 0.6 i	28.1 ± 1.9 gh
WX2	12.8 ± 0.1 ef	63.7 ± 0.8 ef	69.1 ± 0.4 f	79.7 ± 1.0 bcd	6.9 ± 0.1 a	53.9 ± 0.4 c
WXS2	10.9 ± 0.3 g	65.5 ± 0.2 bc	70.6 ± 0.1 d	80.4 ± 0.9 bc	6.5 ± 0.2 ab	59.8 ± 2.7 b
SWW2	10.6 ± 0.4 g	64.3 ± 0.4 de	69.3 ± 0.1 ef	79.1 ± 2.1 cde	3.7 ± 0.0 j	34.9 ± 1.4 f
SW3	17.4 ± 0.6 a	64.7 ± 0.2 cd	69.5 ± 0.2 ef	76.7 ± 2.0 fg	5.6 ± 0.2 cdef	30.2 ± 0.3 g
WX3	12.0 ± 0.7 f	65.3 ± 0.1 bc	70.7 ± 0.1 d	81.4 ± 0.3 b	5.2 ± 0.1 efg	43.1 ± 2.0 de
WXS3	15.3 ± 0.8 bc	64.3 ± 0.2 de	69.7 ± 0.1 e	78.3 ± 0.8 def	6.5 ± 0.3 ab	42.9 ± 0.5 de
SWW3	13.6 ± 0.3 de	65.5 ± 0.2 bc	70.9 ± 0.1 d	79.1 ± 0.4 cde	5.7 ± 0.1 cde	41.7 ± 1.9 e
SW4	17.6 ± 1.3 a	60.3 ± 1.0 h	65.8 ± 0.6 i	92.9 ± 1.9 a	4.5 ± 0.5 hi	25.6 ± 1.2 h
WX4	13.3 ± 0.2 de	60.5 ± 0.4 h	65.6 ± 0.3 i	75.4 ± 0.9 g	4.8 ± 0.3 ghi	35.9 ± 1.5 f
WXS4	13.1 ± 0.9 def	65.1 ± 0.1 bc	81.6 ± 0.8 a	70.5 ± 0.3 i	6.0 ± 0.8 bc	46.0 ± 2.5 d
SWW4	14.2 ± 0.1 cd	63.9 ± 0.2 def	67.6 ± 0.1 h	78.2 ± 1.4 def	5.3 ± 0.1 defg	37.2 ± 0.5f

SW, sweet-waxy maize. WX, waxy maize. WXS1, SW1 as maternal and WX1 as male; WXS2, WXS3, and WXS4 follow this principle. SWW1, WX1 as maternal and SW1 as male; SWW2, SWW3, and SWW4 follow this principle. ΔH_{gel}, gelatinization enthalpy; T_o, onset temperature; T_p, peak gelatinization temperature; T_c, conclusion temperature; ΔH_{ret}, retrogradation enthalpy; %R, retrogradation percentage. Mean values in the same column followed by different letters are significantly different ($P < 0.05$).

Jian Guo: Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Zitao Wang:** Investigation, Formal analysis, Software. **Lingling Qu:** Investigation, Formal analysis, Software. **Derong Hao:** Data curation. **Dalei Lu:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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