A New Strategy for Specific Qualities of Waxy Rice Breeding by Analyzing Physicochemical Properties Between Five Waxy Mutants and Corresponding Their Wild Types

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Research Article

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Abstract

Waxy rice is an essential mutant type of rice, which quality is controlled by amylopectin fine structure and apparent amylose content (AAC). The influence of amylopectin structure and AAC on quality and the waxy rice can be obtained by editing the Waxy (Wx) gene have been elucidated. However, the quality of waxy rice cannot be predicted before breeding, especially how to determine the quality of waxy (wx) mutants by wild types (WT) quality remains unclear. Herein, the quality of waxy rice has been successfully predicted through analyzing the association in physicochemical properties before and after Wx gene knockout. We demonstrated that the higher amylose WT would obtain higher amylose wx mutants, and wx mutants were endowed gelatinization temperature, amylopectin chain ratio and agronomic traits similar to WT. These data indicate that the quality of wild varieties played a decisive role in waxy rice breeding. Overall, we provide a new strategy for the specific quality breeding of waxy rice, which can get waxy rice of prescribed quality and contribute to expanding the particular type of waxy rice germplasm resources.

Summary

In this study, five new waxy rice varieties were obtained using CRISPR/Cas9 to edit the *Wx* gene. Our study also found that if the rice varieties have higher AAC, corresponding their *wx* is also higher. Moreover, *wx* and WT have similar GT type, which is controlled by the ACR value. Based on these findings, it is shown that any new waxy rice variety can be created. This research provides new insight into expanding waxy rice germplasm resources and breeding directed quality waxy rice.

1. Introduction

Waxy rice is widely used in food processing, industry, medicine, and cosmetics because of its unique functional characteristics (Bao et al. 2004, Chun et al. 2010, Lee et al. 2009, Puchongkavarin et al. 2005). It contents 80% starch of the dry weight, and almost all of the starch is amylopectin with only a small amount or no amylose (Bean et al. 1984). Some studies suggest that the different waxy rice amylopectin structures have entirely different properties and applications (Guo et al. 2019, Guo et al. 2017, Precha Atsawanan et al. 2018). Therefore, breeding waxy rice varieties with diverse amylopectin structures is an essential premise in expanding their application.

The *Waxy* gene (*Wx*) is located on rice chromosome 6 and encodes *GBSSI*, which mainly controls amylose synthesis in endosperm and directly affects rice quality (Sano 1984, Wang et al. 1995). Numerous allelic variations of *Wx* have been found, namely, Wx^{lv}, Wx^{a}, Wx^{in}, Wx^{b}, Wx^{op}, Wx^{mp}, and *wx*, and different alleles contain varying AAC in the endosperm and has affected people preferences (Chen et al. 2008, Dobo et al. 2010, Larkin and Park 2003, Mikami et al. 2008, Sano 1984, Teng et al. 2012, Zhang et al. 2019). Therefore, crop breeders can control the *Wx* expression to get a new variety by hybridization, mutagenesis, transgenic methods, etc. (Kong et al. 2015, Liu et al. 2003, Liu et al. 2005, SATOH and OMURA 1981). For instance, in recent years, the *Wx* gene-edited by the CRISPR/Cas9 system obtained a

wx mutantwith significantly reduced AAC without changing other agronomic traits (Zhang et al. 2018). However, the correlation of physicochemical properties between *wx* and WT before and after *Wx* editing remains unclear, which leads to uncertainty in waxy rice breeding.

The amylose content (AC), chain-length (CL) distribution of amylopectin and amylopectin fine structure have been proved to be vital indicators influencing waxy rice's gelatinization, retrogradation, and rheology (Huang and Lai 2014, Jane et al. 1999, Vandeputte et al. 2003a, Villareal et al. 1997). For instance, waxy rice starch with amylose as the donor has increased in the degree of crystallinity, solubility and paste clarity, gelatinization temperature and enthalpy (Guo et al. 2019). The starch retrogradation is not accessible to retrograde with the increase of amylose content. (Sasaki et al. 2000). On the other hand, the higher branch density of amylopectin increases the gelatinization enthalpy (ΔH) and solubility but decreases the viscosity of waxy rice starch (Ren et al. 2017, Sorndech et al. 2015).

Similarly, the higher proportion of the long chain of amylopectin accelerates starch's retrogradation (Karim et al. 2000). Reassociating long chains during retrogradation will improve gels' rigidity (Singh et al. 2012). These studies revealed the vital role of amylopectin fine structure and AC in the physicochemical properties of waxy rice. Therefore, breeding waxy rice varieties with different amylopectin fine structures enriches waxy rice germplasm resources and expands its application prospects.

This study systematically analyzed the relationship between physicochemical properties of *wx* mutants and WT. The result shows that the quality of *wx* mutant is highly correlated with WT, which can predict *wx* mutant properties before breeding. Based on these findings, we enriched the knowledge of waxy rice breeding and contributed to the directed cultivation of special-purpose *waxy* rice.

2. Materials And Methods

2.1 Plant materials and growth conditions

We carefully selected two elite rice varieties with different genetic backgrounds (Rice Research Institute of Sichuan Agricultural University), mainly planted in eastern Asia, central China, and western China. *Japonica* waxy NY1 and *indica* waxy NY2 were used as controls. Then, we enlarged the study to five elite rice varieties to find a potential link between *wx* and WT. The CRISPR/Cas9-targeted genome editing tool, was constructed as previously described (Feng et al. 2013). The primer sequences used to construct the vector are displayed in Table S1. Unless indicated, all rice lines were grown in paddy fields in Chengdu, China, during normal rice-growing seasons.

2.2 Gene cloning

The primers used for *Wx* sequences, *Wx* allele genotype, target sequences and reporter gene detection are listed in Table S1.

2.3 Measurements

Before *Wx* rice and rice starch physicochemical property determination, the seeds of a single *Wx* mutant with stable inheritance in T5 plants were dried at 37 °C for two weeks. The dried seeds were shelled, polished, and milled by a pearling rice mill, and finally screened through a 74-micron mesh. Starch was prepared as the method of Precha Atsawanan et al. (2018).

2.3.1 Quantification of starch and amylose

Before measurement, the rice flour was balanced in a constant temperature and humidity cabinet for seven days. The starch content was determined according to the method of Smith and Zeeman (2006), and the amylose content of rice was determined using a colourimetric assay (Zhang et al. 2018). Standard curves were plotted with standard samples of rice amylose and amylopectin (China National Rice Research Institute, Zhejiang, China).

2.3.2 Quantification of gel consistency and total protein

The gel consistency (GC) of grains was evaluated as described previously (Cagampang et al. 1973). According to the Comin amylose assay procedure, the total protein content of grain was assayed by the Kjeldahl method.

2.3.3 Waxy rice powder pasting properties

The pasting properties of waxy rice, such as the peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV=PKV-HPV), setback viscosity (SBV=CPV-HPV), and pasting temperature (PT), were evaluated using a rapid viscosity analyzer (RVA, Newport Scientific, Australia) according to the method of Chung et al. (2011).

2.3.4 Waxy rice starch thermal properties

The thermal properties of waxy rice starch were measured using differential scanning calorimetry (DSC Q2000, TA Instruments Ltd. Crawley UK) (Yang et al. 2018). The onset temperature (*To*), peak temperature (*Tp*), conclusion temperature (*Tc*), and gelatinization enthalpy (ΔH) were recorded with Universal Analysis 2000 (TA Instruments Ltd. Crawley UK).

2.3.5 Amylopectin chain length distribution

The chain-length distribution of amylopectin was measured using high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAE-PAD) according to the method of Kowittaya and Lumdubwong (2014).

2.4 Date analysis

All experiments were independently repeated three times. The results were expressed as the mean ± standard deviations. Data processing was analyzed with SPSS 25.0, and significance was defined as $P < 0.05$ (* or lowercase letters) and $P < 0.01$ (** or capital letters). Snapgene was used to compare splicing and analysis carefully.

3. Results

3.1 Molecular identification

In this study, the two cultivated rice varieties with the different major *Wx* alleles (Table S2) were selected firstly, QLD (Wx^a) and YSZ (Wx^b), which are widely grown in the rice region of Southwest China. We designed a CRISPR/Cas9 (Fig. 1a) construction accurately targeting the second exon (87-109 bp) of the *Wx* gene with the expectation to generate a null mutation (Fig. 1b). Based on this vector, the results suggest four major mutant types of QLD (Fig. 1c) and five major mutant types of YSZ (Fig. 1d) were obtained. This experiment observed high mutagenesis efficiency in 80% T0 transformants mutating in QLD plants and 82.35% YSZ (Table S2). Finally, our results again proved this method did not change the main agronomic traits (Fig. S1).

3.2 The quality of *wx*

We further identified two single-base homozygous mutations, YSZ*wx1* and QLD*wx1,* and performed quality analysis on them (Table S3). The results show no significant difference in the major grain quality of the two *wx* seeds except that the colour of endosperm changed into milky white (Fig. 1e) and the kernel weight (Fig. S2). The cooking and eating quality of YSZ*wx1* rice was soft but not sticky, while the QLD*wx1* rice was very sticky (Fig. 1f), and both their gel consistency was a significant increase (Fig. 1g).

This study observed that the AAC significantly decreased to 1.16% and 2.36% (Fig. 2a) (reduced by 91.01% and 89.80%), leading to increased gel consistency (Fig. 2b). The other qualities were also experimented. For example, the total protein was increased 26.01% and 6.37%, respectively (Fig. 2c), but the total starch showed no regularity, increasing by 8.11% and decreasing by 6.19% compared to WT (Fig. 2d). The above result suggests that the other rice quality would be significantly changed by editing the *Wx* gene, not only the AAC and *wx* mutant does not always have the same quality trend. Breeders can cultivate the different qualities of waxy rice by this method.

Differential scanning calorimetry (DSC) is widely used to study grain gelatinization temperature (GT), and the GT is usually represented by peak temperature (T_p) (Sasaki et al. 2000). The GT of rice is generally divided into three types: low (<70℃), intermediate (70-74℃) or high (>74℃) (Kongseree and Juliano 1972). In this experiment, the *wx* mutants showed a slightly higher GT range (*To*, *Tp* and *Tc*) than the WT (Fig. 2e). The GT ranges of WT starch was 66.08℃ (YSZ*wx1*) and 73.46℃ (QLD*wx1*) (onset temperature, *To*) to 76.03℃ and 82.06℃ (conclusion temperature, *Tc*) with an enthalpy (Δ*H*) of 11.6 J/g and12.55 J/g (Table S4). The results suggest that *To*, *Tp*, *Tc* and Δ*H* from *wx* mutants were increased, which lead to them being gelatinized later compared with WT, but they still belonged to the same GT types as the WT. Here, we propose a hypothesis that editing the *Wx* gene does not change the GT, and subsequent experiments were to verify this hypothesis with knocked other rice varieties' *Wx* gene.

3.3 Pasting properties

The *wx* mutants and WT pasting properties were significantly variable ($p<0.05$) (Fig. 3a). For example, the PKV, HPV, BDV, CPV, SBV, peak time and PT of QLD and QLD*wx1* ranged from 2647 to 2775 cp, from 1729 to 1362 cp, from 918 to 1413 cp, from 4080 to 1757 cp, from 1333 to -1018 cp, from 6.38 s to 4.57 s and from 80.7℃ to 81.5℃, respectively (Table S5). The results show that QLD*wx1* and YSZ*wx1* have more waxy rice pasting properties (Fig. 3b).

3.4 Chain length distribution of amylopectin

The chain length distributions of amylopectin in *wx* mutants and WT were distinctly different (Fig. 4a). At chain length degree of polymerization (DP) 7-20, *wx* mutants increases compared to WT, while at DP > 20, *wx* was decreased (Fig. 4b).According to the value of ΣDP≤10/ΣDP≤24 (amylopectin chain ratio, ACR value), amylopectin structure from cultivated rice can be classified into three types: L-type (ACR≤0.200), S-type (ACR≥0.240) and M-type (ACR 0.201-0.239) (Nakamura et al. 2002). Interestingly, even although the ACR value of the *wx* mutants were significantly lower than that of WT, they still belonged to the same amylopectin structure type. The amylopectin structure QLD*wx1* and QLD belonged to the L-type, and YSZ*wx1* and YSZ belonged to the M-type (Fig. 4c).

3.5 Data Analysis

We applied the same method to three other rice varieties with the same material treatment to find a potential link between *wx* mutants and WT (Fig. S3 and Table S6). The other three rice varieties belonged to the Wx^b genotype (Table S6). The results showed that WT (Fig. 5a) with higher AAC and the AAC of corresponding its *wx* mutant (Fig. 5b) was also higher, and some *wx* mutants AAC were more than 2%, which means they may not belong to high-quality waxy rice. The same results were observed that the *Tc* and Δ*H* of *wx* mutants were increased, however, their GT types were not changed (Table 1). Similarly, a result showed the ACR value was altered but not beyond specified value such as M-type (ACR 0.201-

0.239), therefore, *wx* and WT have the same amylopectin structure type (Fig. 5b). Further data analysis showed a direct linear correlation between ACR and GT (Fig. 5c), and the ACR value depended on GT. Here, there are two new types be proposed to identify the structure of amylopectin, respectively, which were HGT-type (high gelatinization temperature type, ACR < 0.18) and LGE-type (low gelatinization temperature type, ACR > 0.18). Based on these findings, we demonstrated that GT type and ACR type would not be changed by editing the *Wx* gene and if WT with a higher AAC, its *wx* mutant would be endowed a higher AAC.

In this study, we also separately analyzed the correlation between the related physicochemical properties of WT or *wx* mutants. Pearson's correlation analysis suggested that WT and *wx* mutants had dissimilar correlations between rice quality, amylopectin structure and physicochemical properties (Fig. 6). The data of WT reflected that GT was negatively correlated with DP6-12 and ACR and positively correlated with DP13-24. The AAC was positively correlated with peak time, CPV, HPV, SBV, and was negatively correlated with BDV and GC. However, the *wx* mutantsdata reflected that GT was negatively correlated with DP6-12, DP6-24 and ACR and positively correlated with DP13-24, peak time, CL, and DP25-100. The AAC was negatively correlated with GC and positively with peak time HPV and CPV.

The above results implied that amylopectin structure and AAC in diverse rice varieties determines different physicochemical properties. So, the quality data of wild and waxy varieties added together in statistics analyzing may not be an excellent way to find the relationship between the physicochemical properties. Here, we suggest that if there are accurately diagnoses of the physicochemical properties of waxy rice, it is best to use glutinous rice data only.

3.6 Suggestion of specific quality breeding of waxy rice

It is generally believed that the AC of waxy rice should be less than 2% (Juliano 1992). We recommend that if you want to obtain high-quality waxy rice, you should first choose rice varieties with low AAC, otherwise, these *wx* varieties may not be called waxy rice. On the other hand, GT also can be selected before waxy rice breeding, breeders only have to choose a rice variety with the targeted GT. Finally, any new waxy rice variety breeders want can be obtained based on the ACR value, AAC and agronomic traits.

4. Discussion

New waxy rice varieties have been developed in the past few decades by hybrid breeding and mutagenesis breeding. However, these methods have the disadvantages of unclear quality, time consumption and laboriousness (Deng 1992, Olsen and Purugganan 2002, Toda 1980). Therefore, a new breeding method is needed in Waxy rice breeding. In recent years, the rapid development of genome editing technology, especially the discovery and rapid development of CRISPR/Cas9 editing technology, has led to its wide use in studying plant genome functions (Belhaj et al. 2015, Bortesi and Fischer 2015, Ran et al. 2013). Since 2018, several studies have been reported using the CRISPR/Cas9 system to edit

the rice *Wx* gene (Han et al. 2018, Zeng et al. 2020, Zhang et al. 2018). Unfortunately, these studies did not address how to obtain waxy rice with directional properties. Our study found that the quality of *wx* mutantcan be predicted and has similar properties inextricably linked to WT.

Previous studies have shown that other starch-synthesizing enzymes also influence rice AAC. That different alleles have different effects, such as soluble starch synthases (*SSs*), branching enzymes (*BEs*), debranching enzymes (*DBEs*) and isoamylase 1 (*ISAI*) (Man et al. 2013, Shufen et al. 2019, Umemoto et al. 1995, Zhu et al. 2020). The AAC of rice is different, even in the same *Waxy* gene type. In this study, WT with higher AAC, its corresponding *wx* mutant AAC, will be higher than other mutants. It is reported that silencing *Wx* gene expression regulates genes related to starch synthesis, with upregulation of granule-bound starch synthase II (*GBSSII*) compensating for some amylose in the endosperm (Pérez et al. 2019). The above reasons may rationally explain the different AAC and chain length distributions of amylopectin of the *wx* mutant after editing the same *Waxy* gene type. Currently, however, the reasons for these discrepancies are undefined. This finding suggests that if you want to cultivate a lower AAC *wx* mutant variety, you should edit a lower WT, but this mechanism needs to be further studied in the future.

In rice starch, the functional properties of amylopectin have been extensively researched. Gelatinization temperature (GT) was negatively correlated with short amylopectin chains (DP 6-12) and the proportion of chains with DP ≤ 10 to those with DP ≤ 24 (the amylopectin chain ratio, ACR value) but positively correlated with amylopectin long chains (DP > 37) (Nakamura 2002, Nakamura et al. 2002, Vandeputte et al. 2003b).However, this study indicated that GT was only negatively correlated with ACR and DP6-12 when all were wild type lines, significantly negatively correlated with ACR and DP6-12 and significantly positively correlated with DP25-100 when all were waxy rice lines. Based on these findings that physicochemical properties are analyzed separately in different rice types is necessary, which can obtain accurate results.

Moreover, editing the *Waxy* gene resulted in similar ACR and GT between WT and *wx* and that ACR and GT were linearly correlated. We conclude that ACR is the main reason for the difference in GT that editing the *Wx* gene does not change the ACR type and GT type. Therefore, this makes it possible to cultivate different amylopectin fine structures and GT type waxy rice.

Abbreviations

AC Apparent amylose content

Wx *Waxy*

wx *waxy*

WT Wild type

GBSSI Granule-bound starch synthase I

HPAE-PAD High-performance anion-exchange chromatography equipped with a pulsed amperometric detector

AC Amylose content

To Onset temperature

Tp Peak temperature

Tc Conclusion temperature

Δ*H* Gelatinization enthalpy

GC Gel consistency

PT Pasting temperature

PKV Peak viscosity

HPV Hot paste viscosity

CPV Cool paste viscosity

BDV Breakdown viscosity

SBV Setback viscosity

RVA Rapid viscosity analyzer

DSC Differential scanning calorimetry

GT Gelatinization temperature

DP Degree of polymerization

ACR Amylopectin chain ratio, value

HGT-type High gelatinization temperature type

LGE-type Low gelatinization temperature type

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data supporting the conclusions of this article are provided within the article and its (Additional file 1: Figure S1- S4, Additional file 2: Table S1-7). The rice seeds of the landraces used in the studies are available at the Rice Research Institute of Sichuan Agricultural University, China.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Y.F., T.L. and J.Z. designed the strategy. Z.Z., Y.L., X.L., B.Z. and R.L. completed part of the experiments. Y.F. and J.Z. organized the figures and article modification. Y.F., T.L., and J.Z. analyzed data, and wrote the paper. All authors commented on the manuscript.

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Supplementary Information

Fig. S1: Plant height, panicle number per plant, grain number per panicle and seed-sitting rate in YSZ*wx1,* QLD*wx1* and their corresponding WT plants

Fig. S2: Grain width, grain length and 1000 grains weight in YSZ*wx1*, QLD*wx1* and their corresponding WT plants

Fig. S3: Grain phenotypes, gel consistency, rapid viscosity analyzer profiles and gelatinization properties in SH789*wx,* HZ*wx,* TJG*wx* and their corresponding WT plants

Fig. S4: Differences in amylopectin structure between SH789*wx,* HZ*wx,* TJG*wx* and their corresponding WT starch

Table S1: Primers used in this study

Table S2: Percentage of T0 plants with mutations in the target locus

Table S3: Waxy alleles and mature seed quality of *wx* mutants and corresponding WT lines

Table S4: Differential scanning calorimetry (DSC) of *wx* mutants and corresponding WT lines

Table S5: RVA profile characteristics

Table S6: Agronomic and yield traits in additional three WT plants and their corresponding *wx* mutants

Table S7: The AAC, GC and ACR of *wx* mutants and corresponding WT lines

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Tables

Table 1. Gelatinization temperatures and enthalpy values of WT and *wx* starch.

Cultivar	To (℃)	Tp (°C)	Tc (°C)	ΔH (J/g)	Tc-To (°C)	GT type
YSZ	66.0±0.1	70.3±0.2	76.2±0.2	11.9±0.2	10.2±0.2	I
YSZ*wx1*	66.4±0.1**	71.8±0.2**	78.6±0.4**	13.3±0.3**	12.2±0.2**	
QLD	73.5±0.5	77.3±0.3	81.9±0.3	12.8±0.2	8.4±0.3	H
QLD*wx1*	74.8±0.5*	79.6±0.4**	86.0±0.3**	15.2±0.4**	11.2±0.8**	
SH789	65.9±0.1	70.1 ±0.7	74.6±0.2	10.8±0.2	8.8±0.2	I
SH789*wx*	65.5±0.2*	71.4±0.2*	78.2±0.2**	12.7±0.3**	12.7±0.1**	
TJG	66.3±0.1	71.1±0.2	77.6±0.3	10.3±0.2	11.3±0.3	H
TJG*wx*	66.6±0.1*	72.3±0.2**	80.5±0.2**	14.1±0.2**	14.0±0.3**	
HZ	76.3±0.2	79.3±0.3	83.6±0.3	14.0±0.2	7.2±0.2	H
HZ*wx*	74.9±0.2**	79.6±0.4	88.5±0.5**	15.4±0.2**	13.6±0.3	

To, Tp, Tc, ΔH (J/g), I and H = onset, peak, and final gelatinization temperature, gelatinization enthalpy, intermediate gelatinization temperature type, and high gelatinization temperature type.

Data are presented as means ± sd. *P <0.05, **P < 0.01.

Figures

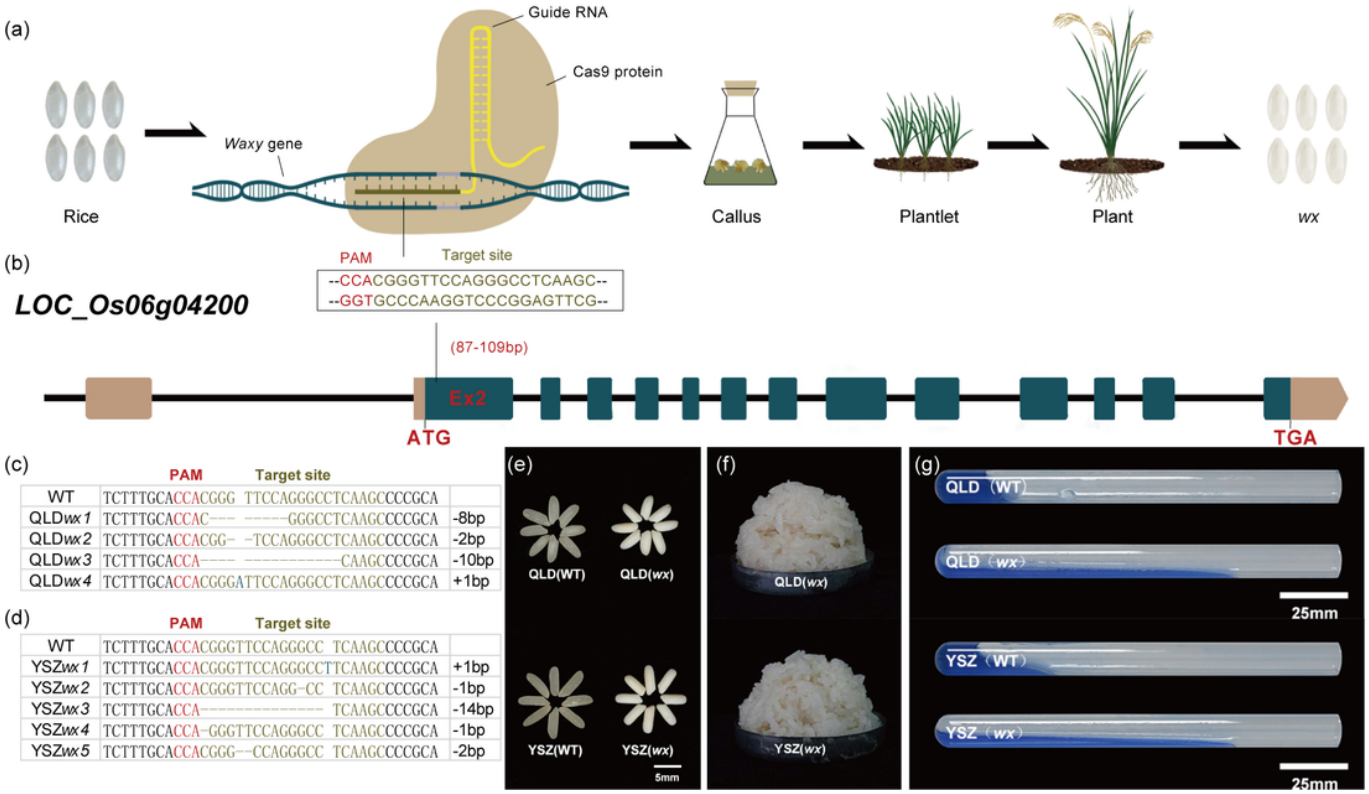

Figure 1

Identification of wx mutants lines (a) The schematic experimental design. (b) Schematic diagram of the targeted site in the Waxy gene. (c-d) Nucleotide variations at the targets (protospacer adjacent motif in blank) of homozygous mutant lines from YSZwx1 and QLDwx1. '-', base deletion, '+', base insertion. (e) Grain phenotypes of wx mutant and corresponding their WT. (f-g) Comparison of quality between wx mutant and corresponding their WT: appearance of cooked rice (f), gel consistency (g). Error bars are mean ± SD (n = 3). Significant differences were determined by Student's t-test (*P < 0.05, **P < 0.01).

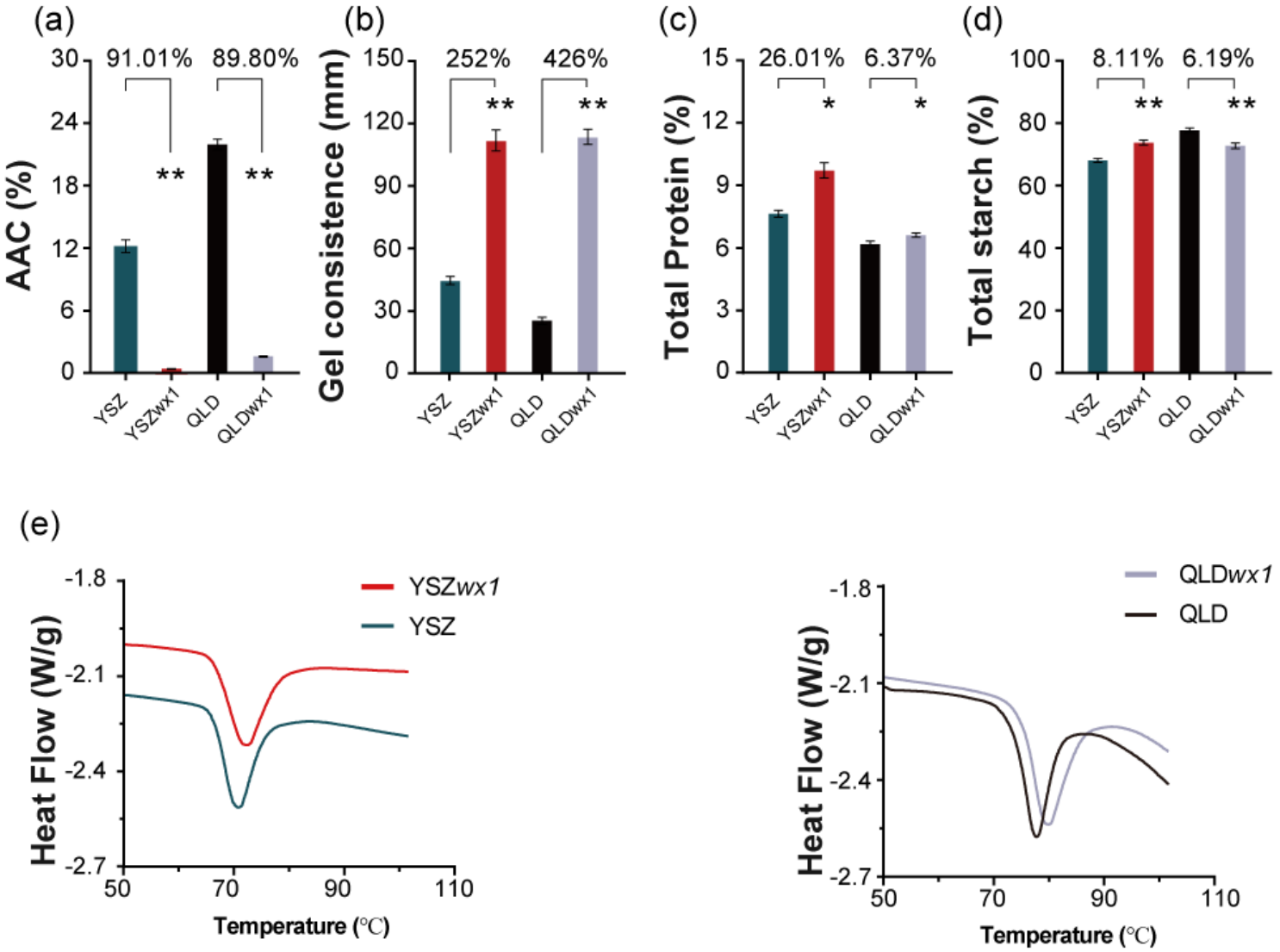

Figure 2

Quality of WT and their wx mutant lines (a) Apparent amylose content of wx mutant and corresponding their WT. (b) Gel consistence of wx mutant and corresponding their WT. (c) Total protein of wx mutant and corresponding their WT. (d) Total starch of wx mutant and corresponding their WT. (e) Gelatinization properties curve of wx mutant and corresponding their WT. Error bars are mean ± SD (n = 3). Significant differences were determined by Student's t-test (*P < 0.05, **P < 0.01).

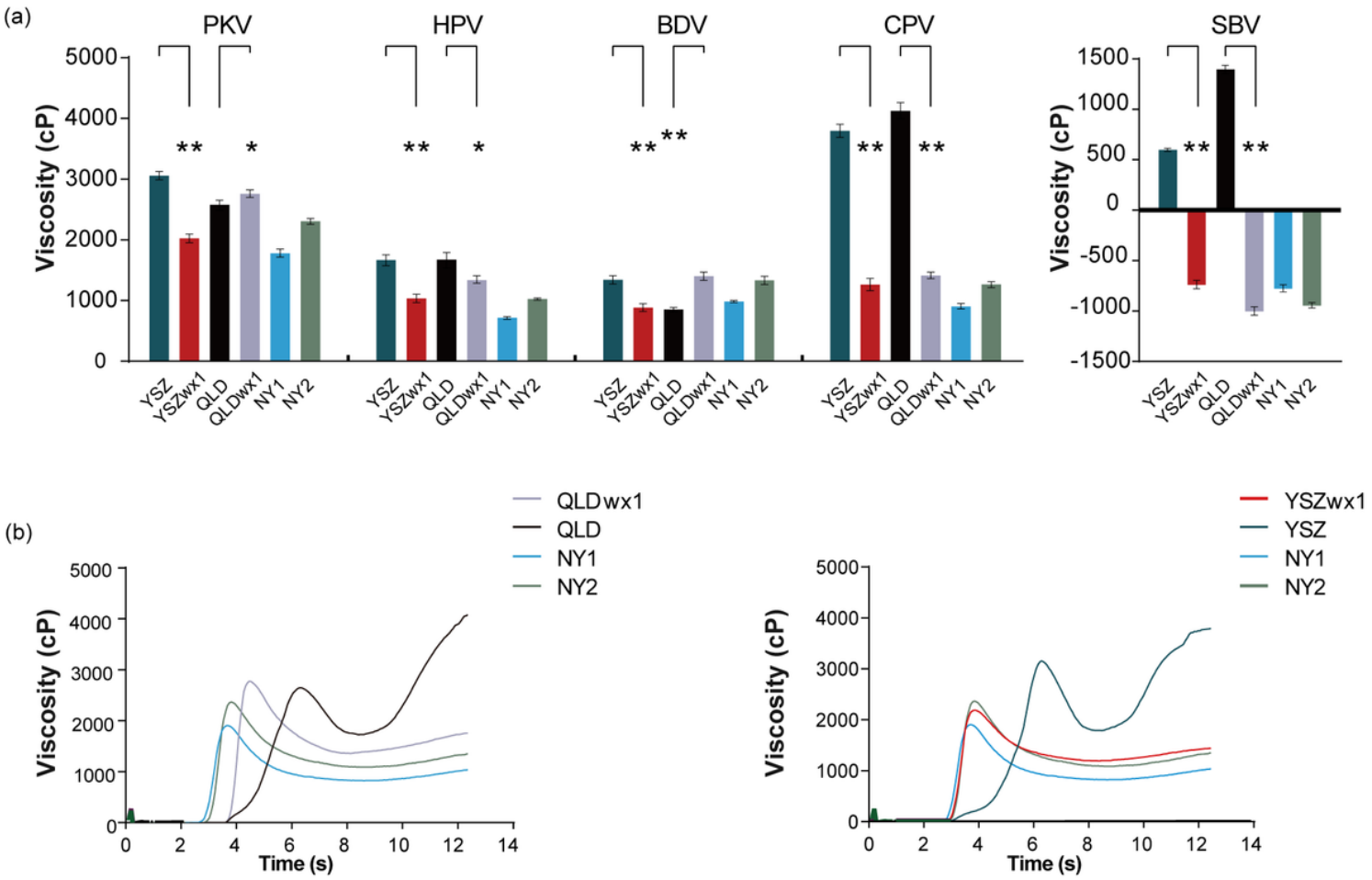

Figure 3

Rapid viscosity analyzer profiles of WT and their wx mutants lines (a) Rapid viscosity analyzer profiles of wx mutant and corresponding their WT. NY1 is japonica waxy rice (in blue), NY is indica waxy rice (in green). (b) Rapid viscosity analyzer profiles curve of wx mutant and corresponding their WT flour. Error bars are mean ± SD (n = 3). Significant differences were determined by Student's t-test (*P < 0.05, **P < 0.01).

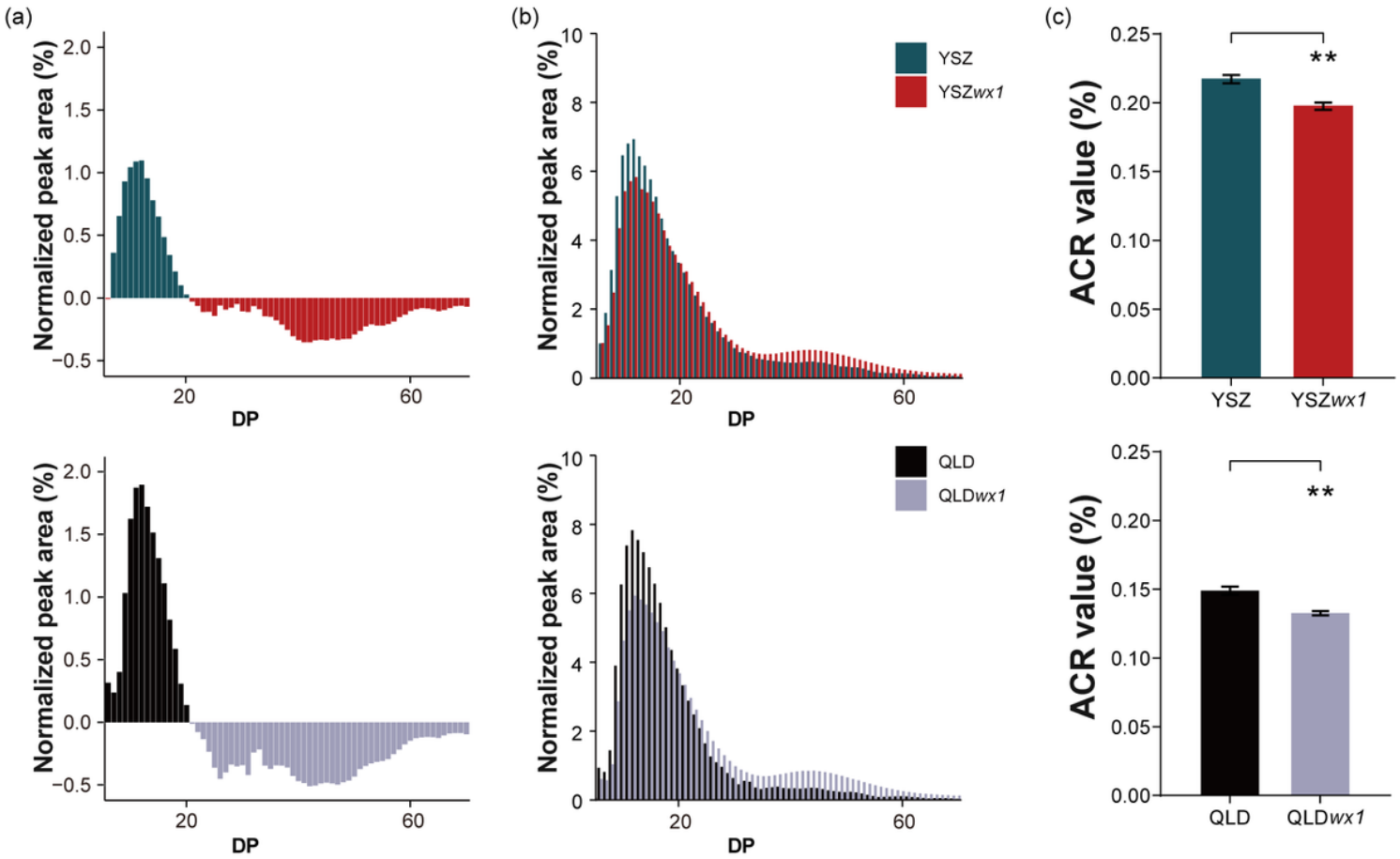

Figure 4

Differences in amylopectin structure between WT and wx mutant strach (a) Comparison of percentage distribution of HPAEC–PAD chromatograms of amylopectin chain length from wx mutant and corresponding their WT. (b) Difference in chain-length distribution of amylopectin between wx mutant and corresponding their WT. (c) the$\Sigma DP \leq 10/\Sigma DP \leq 24$ value of wx mutant and corresponding their WT. (the amylopectin chain ratio, ACR value). Error bars are mean ± SD (n = 3). Significant differences were determined by Student's t-test (*P < 0.05, **P < 0.01).

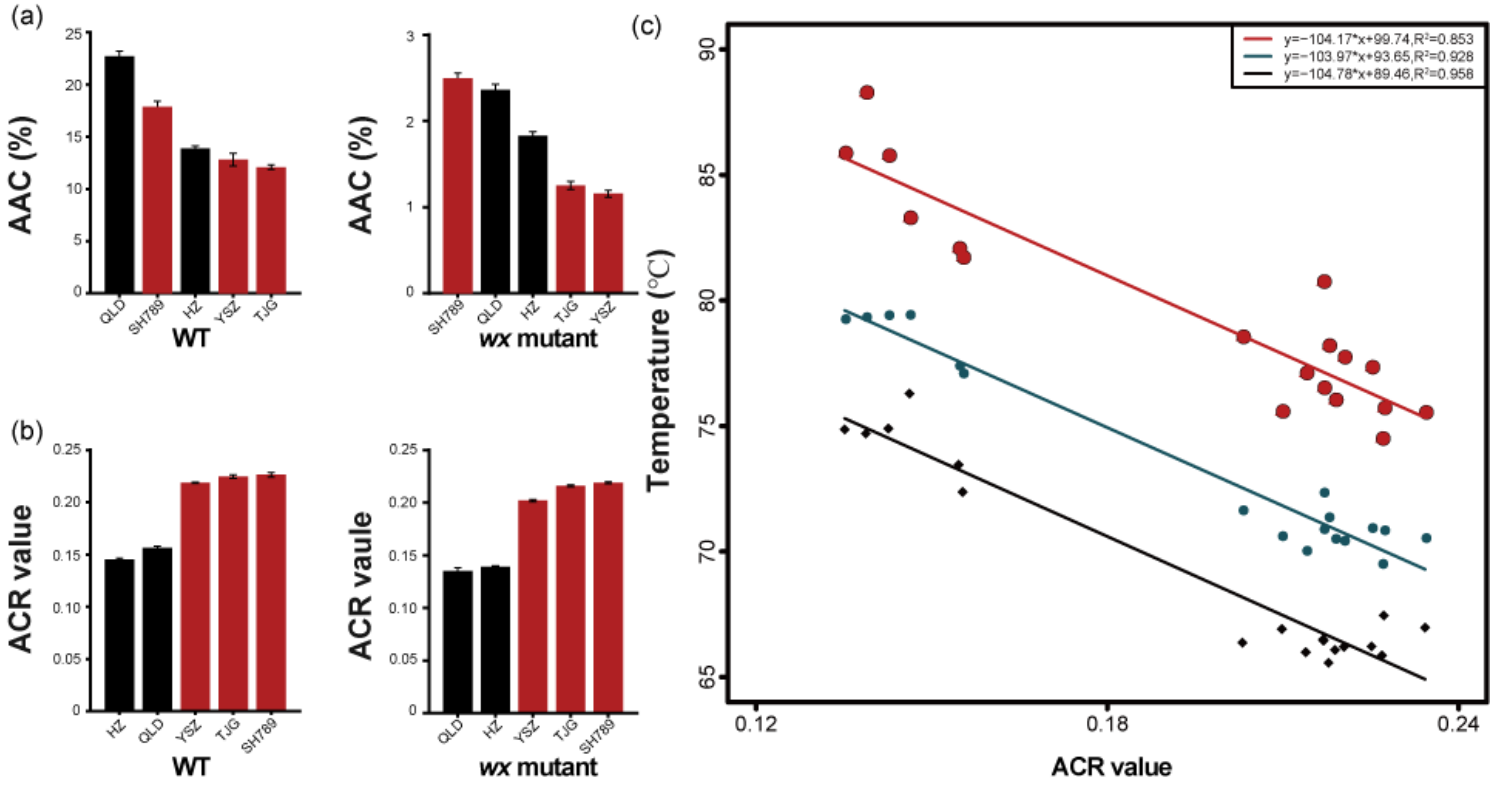

Figure 5

The apparent amylose content and ACR value of five wx mutant and corresponding their WT. (a) Apparent amylose content of five wx mutant and corresponding their WT. (b) ACR of of five wx mutant and corresponding their WT. (c) Relationship between ACR value and gelatinization temperature, Each dot represents the ACR value of a rice variety. Black is onset temperature (To), bluish-black is peak temperature (Tp), red is conclusion temperature (Tc). Error bars are mean ± SD (n = 3).

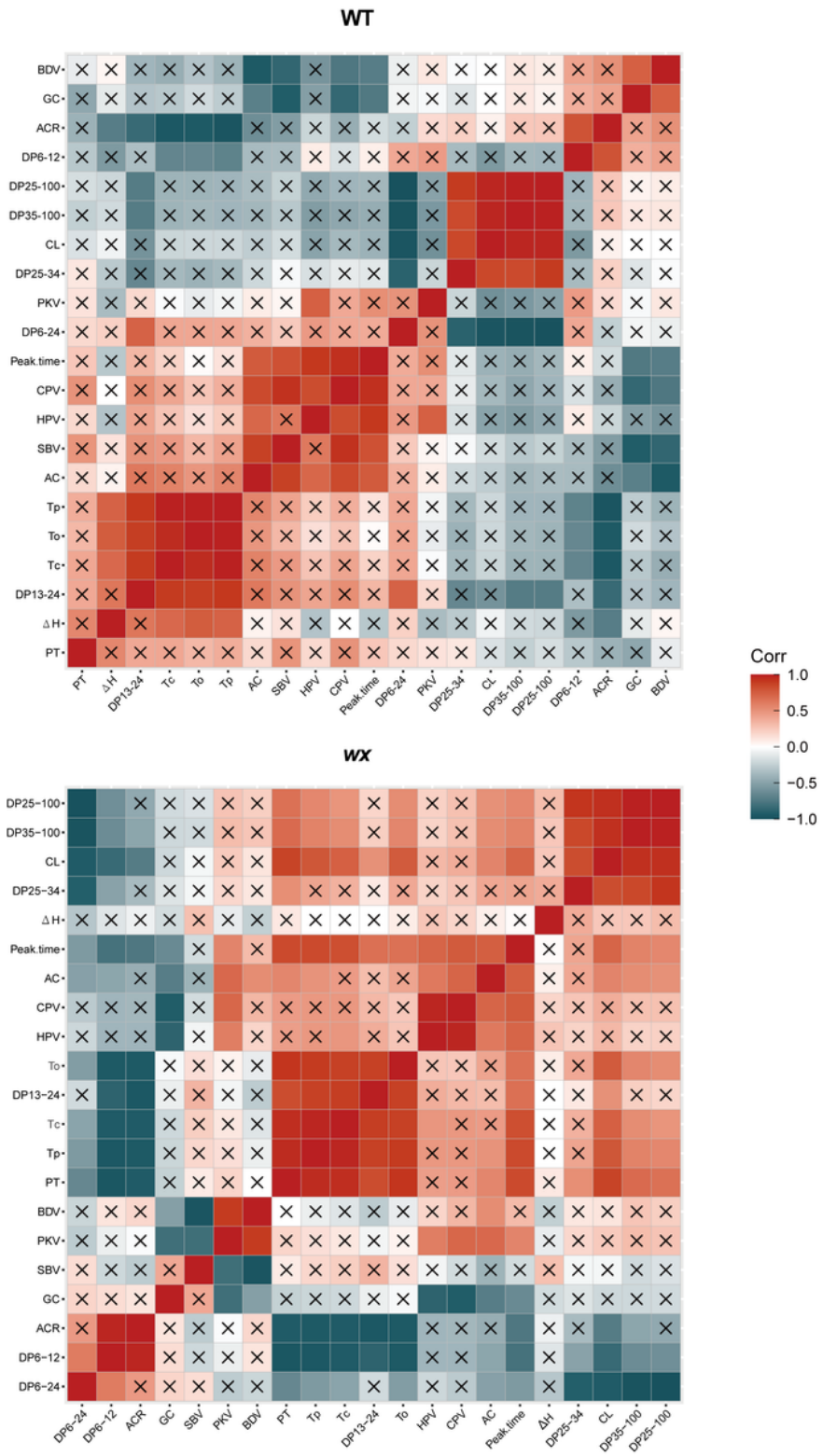

Figure 6

Pearson's correlation analysis of amylopectin structure relationships of WT and wx. '×' means 'no correlation'. The stronger the red color, the more significant positive relationship, the stronger bluish black, the more Significant negative relationship.

Supplementary Files

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