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Research Paper

Effect of *indica* pedigree on eating and cooking quality in rice backcross inbred lines of *indica* and *japonica* crosses

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Amylopectin is one of the major determinants of rice (*Oryza sativa* L.) grain quality, and a large difference in amylopectin is found between two subspecies: *japonica* and *indica*. However, the relationship among rice grain quality, *indica/japonica* genetic background, and amylopectin has not been clearly established. In this study, a series of backcross inbred lines derived from the cross between *japonica* (cv. Sasanishiki) and *indica* (cv. Habataki) were used to survey eating and cooking quality (ECQ), rapid visco analyzer (RVA) profiles, and the chain length distribution of amylopectin. The frequency of *indica* pedigree (*Fi*) was calculated to analyze the effects of *Fi* on grain quality and amylopectin. The results showed that the Sasanishiki cultivar was markedly enriched in chain length with DP6-15 and DP34-45 compared to the Habataki. DP34-45 strongly correlated to RVA characteristics, cooking quality, and prolamin content. The *Fi* also has significant correlations to RVA characteristics and ECQ, but only significantly negative correlation to DP34-45. Seven quantitative trait loci (QTLs) corresponding to amylopectin were mapped, of which three were in agreement with previous findings. The results of this study provide valuable information for amylopectin characteristics in the offspring derived from the subspecies cross, and the novel QTLs may provide new insights to the identification of minor starch synthesis-related genes.

Key Words: rice (*Oryza sativa* L.), *indica* pedigree frequency, amylopectin, ECQ.

Introduction

Rice (*Oryza sativa* L.) is one of mankind's major food staples, and more than 2 billion people obtain nutrition and calories from rice and its products (Lin *et al*. 2011, Sun *et al*. 2012). Given recent economic developments and improvements in the standard of living, grain quality has now become the primary consideration of rice customers and breeding programs (Tian *et al*. 2009). Grain quality includes grain appearance quality, milling quality, nutritional quality, and eating and cooking quality (ECQ). ECQ is considered to be the most complex quantitative trait of rice and is not only related to the interaction of the genotype and the environment, but it is also influenced by social factors, such as human habits and living standards.

Given that starch comprises approximately 90% of rice grain, starch biosynthesis is naturally expected to affect ECQ. Starch is composed of linear amylose and branched amylopectin. Rice is grouped into two subspecies, i.e. *japonica* and *indica*, with marked differences in plant architecture as well as agronomic and physiological features (Khush 1997). It is generally said that *japonica* rice has a better palatability and stronger stickiness than *indica* rice, but its swelling power is weaker. The fact that amylose content is usually higher in *indica* rice than that of *japonica* rice has been explained by the two wild-type alleles, Wx^a and Wx^b, which predominate at the *waxy* locus of rice. Most of the *indica* rice cultivars possess the Wx^a allele, while the *japonica* varieties have the Wx^b allele. Amylopectin was the main reason for the difference in rice varieties that had the same or similar amylose content (Derycke *et al.* 2005, Peat *et al*. 1956, Vandeputte *et al*. 2003). Numerous studies have shown that there is a difference in the amylopectin structure between *indica* and *japonica*, in that *japonica* had more short-chain amylopectin but fewer intermediate-size chain amylopectin. Peng *et al*. (2014) used Cheng's index method to divide recombinant inbred lines (RILs) into four types: *indica*, *indicaclinous*, *japonicaclinous*, and *japonica*. The results indicated that the ratios of short chains ($6 \leq DP \leq 11$) of the four types were: *indica* < *indicaclinous* < *japonicaclinous* < *japonica*, and the ratios of the intermediate chains (12

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≤ DP ≤ 24) were: *indica* > *indicaclinous* > *japonicaclinous* > *japonica*. Cheng's index was in good agreement with other studies that used landraces and breeding varieties as materials, while in RILs or backcross inbred lines (BILs) from the crossing of *indica-japonica*, the relatively stable relationship of morphological traits used for classification was disrupted, as the degree of coincidence of the morphological index and molecular marker classification results were significantly reduced (Qian *et al*. 2000). With the development of molecular biology, subspecies-specific DNA markers became an efficient method with which to evaluate the *indica* pedigree frequency (*Fi*) of the offspring derived from the cross between *indica* and *japonica* (Liu *et al*. 2016, Xu *et al*. 2015).

During the past decades, *indica-japonica* hybridization breeding has become one of the important breeding methods in China (Xu *et al*. 2016). The introduction of the *indica* pedigree has brought us a remarkable improvement in rice production, but at the same time, a reduction in the ECQ (Sun *et al*. 2012). The quantitative analysis of the *indica* pedigree in breeding varieties in northeastern China in the last 50 years has shown that the frequency of *indica* alleles in northern *japonica* varieties bred after the 1990s has significantly increased (Sun *et al*. 2012). The ECQ of northeastern *japonica* rice which more *indica* pedigree had been introduced into the *japonica* genetic background was significantly lower than Japanese *japonica*. Thus, there is an urgent demand to understand the relationship among *indica* pedigree, amylopectin, and ECQ.

In this study, the BILs, which had introduced the *indica* pedigree by *indica-japonica* crossing while containing the *japonica* genetic background, were used as materials to analyze the effect of the *indica* pedigree on ECQ and provide a scientific basis for good quality and high-yield hybridization breeding between *indica* and *japonica*.

Materials and Methods

Plant materials

Eighty-five BIL lines derived from 'Sasanishiki' (*O. sativa japonica*)/'Habataki' (*O. sativa indica*)//Sasanishiki///Sasanishiki were obtained from the Rice Genome Resource Centre (RGRC). This BIL population was developed by single-seed descent and provided by the National Institute of Agrobiological Sciences, Tsukuba, Japan.

Field experiments were conducted at the Rice Research Institute of Shenyang Agricultural University for two growing seasons during 2014–2015. Seeds were sown in mid-April and transplanted in mid-May. Three rows were planted for each family, 10 plants per row, with a plant spacing of 30 cm × 13.3 cm. The BILs were arranged in a randomized block design with two replications in the field. Cultivation methods and field management varied according to regional cultivation practices. The paddies were harvested at the end of September. Mature rice grains were milled after being harvested, air dried, and stored at room temperature for three months.

Indica-japonica subspecies differentiation measurement

The size of the genetic map was 953.1 centimorgan (cM), including 236 restriction fragment length polymorphism (RFLP) molecular markers. Each chromosome covers 79.28 cM and contains 19.7 markers on average; the mean distance between two markers was 4.03 cM (Nagata *et al*. 2002) (**Fig. 1**). Marker information was available from the RGRC website (http://www.rgrc.dna.affrc.go.jp). The *Fi* was calculated according to Lu *et al*. (2009), with the parents as the controls:

$$Fi = \frac{2\sum_{1}^{N} X_{ii} + \sum_{1}^{N} X_{ij}}{2N},$$

where X_{ii} was the *indica* genotype (II), X_{ij} was the *indica-japonica* hybrid genotype (IJ), and N was the number of specific primers.

ECQ, RVA profile values, AC, GC, and protein composition measurement

The Taste Analyzer (STA-1B, Satake Co. Ltd., Hiroshima, Japan) was used to determine the ECQ of the rice. Milled rice (30 g) was put into an aluminum cup with a cover with holes (rice could not go through these holes). After the lid was put on the cup, milled rice in the cup was washed by flowing water for 3 min. Water was added until the total weight of the milled rice and water was 72 g. The milled rice was soaked for 30 min at room temperature, and the cup was put into a rice cooker. Then, the sample was steamed for about 30 min and kept warm for 10 min. The cooked rice was mixed with a plastic scoop before it was cooled for 20 min. Two hours later, the Taste Analyzer was used to determine the palatability of the rice. The appearance, hardness, stickiness, equilibrium degree, and taste value were used to estimate the ECQ. All of the analyses were performed three times. The viscosity of the cooked rice was analyzed using the Rapid Viscosity Analyzer to obtain RVA profiles (Model No. RVA-4, Newport Scientific, Warriewood, Australia). According to the Standard Method AACC61-02, 3.00 g of rice flour was mixed with 25 mL of water. The sequential temperature curve for a 13-min test was as follows: (1) incubation at 50°C for 1 min; (2) increase in temperature to 95°C and holding for 2.5 min; and (3) cool to 50°C and hold at 50°C until the end of the cycle. RVA profiles were characterized by peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown (BDV) = PKV-HPV, setback (SBV) = CPV-PKV, and consistency (CSV) = CPV-HPV. All of the analyses were performed three times.

The amylose content (AC) and gel consistency (GC) were detected according to The National Standard of the People's Republic of China—GB/T17891-1999. Extraction and measurement of the rice protein composition followed a method described by Li (2000) with modifications.

Albumin: Rice flour (100 mg) was put into a 1.5 centrifugal

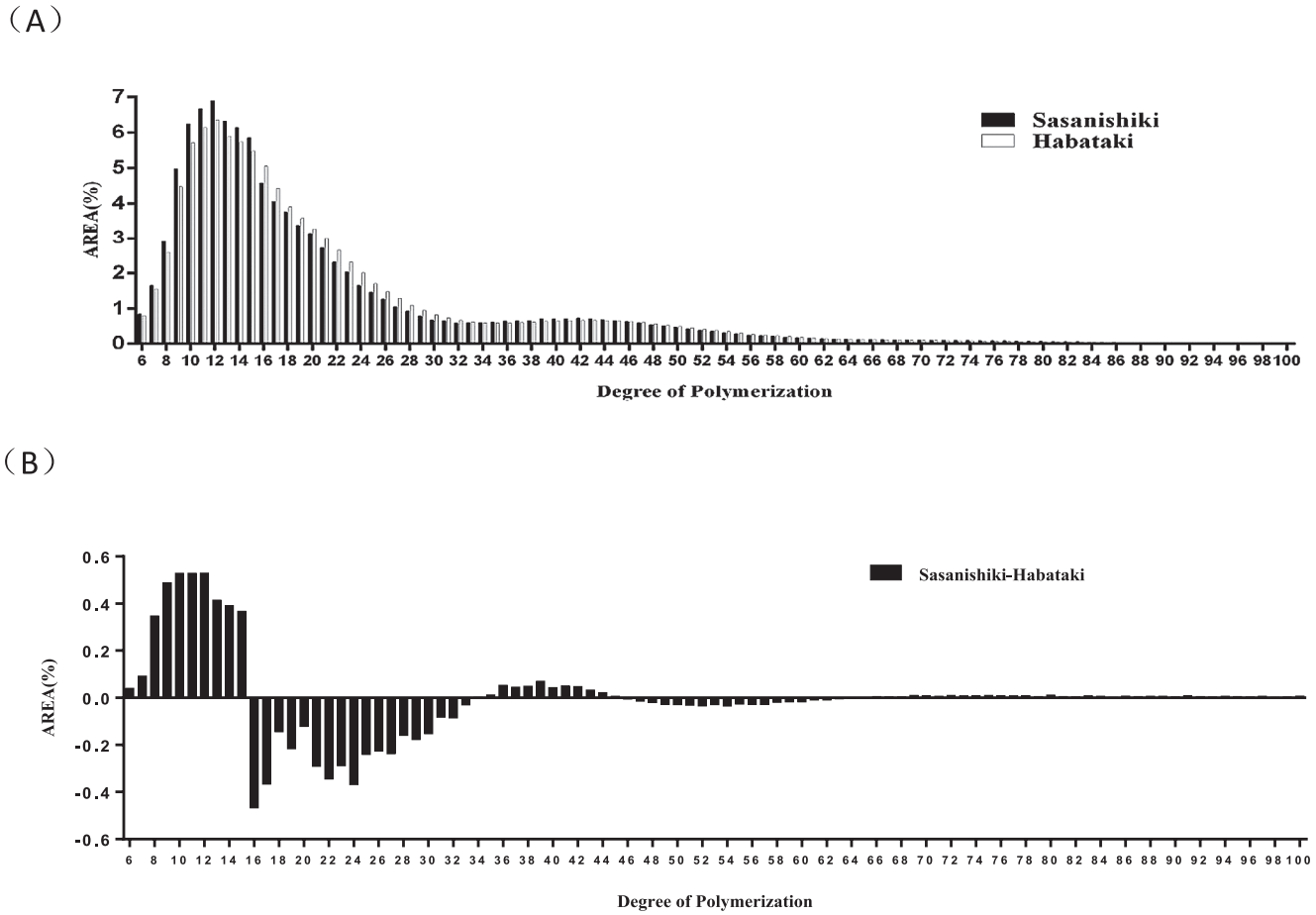

Fig. 1. Comparison (A) and difference (B) in amylopectin chain length distribution between Sasanishiki and Habataki.

tube, then extracted by shaking with 1 mL of distilled water for 4 h, and centrifuged at 10,000 rpm for 20 min. The supernatants were put into the centrifugal tube. The extraction was repeated three times in order to extract all of the albumin. All of the supernatants were combined to be detected by the Modified Bradford Protein Assay kit.

Globulin, prolamin, glutelin: After water extraction, the flour was extracted with 1 mL of 5% NaCl for 4 h and centrifuged at 10,000 rpm for 20 min (globulin extract). The flour was then extracted for prolamin with 1 mL of 70% ethanol for 4 h and centrifuged at 10,000 rpm for 20 min, followed by glutelin extraction with 1 mL of 0.2% NaOH for 4 h and centrifugation at 10,000 rpm for 20 min. The following extraction and determination of globulin and prolamin was the same as that for albumin. The content of glutelin was higher than the others, therefore, the supernatants needed to be diluted before being measured. All of the samples were analyzed three times.

Chain length distribution of amylopectin measurement

Starch was extracted from the flour and debranched following a method described by Hasjim *et al*. (2010) with modifications. The debranched starch was labeled using 8-aminopyrene-1,3,6,trisulfonic acid following a procedure described by Wu *et al*. (2014) and then separated with a carbohydrate separation buffer (Beckman-Coulter, Brea, CA, USA). The chain length distribution (CLD) of debranched amylopectin was characterized using a PA-800 Plus FACE System (Beckman-Coulter), coupled with a solid-state laser-induced fluorescence detector and an argon-ion laser as the excitation source. Samples were analyzed in duplicate.

Quantitative trait locus and statistical analysis

The BIL population from the cross between Sasanishiki and Habataki, comprising 85 lines, was grown in a paddy field in Shenyang and subjected to QTL (quantitative trait locus) analysis with 236 RFLP molecular markers. QTLs were identified with simple interval mapping and composite interval mapping using the software QTL Cartographer ver. 2.5. LOD thresholds for the presence of QTLs were estimated by a 1,000-times permutation test using QTL Cartographer. The LOD-score peak was used to estimate the most likely QTL position on the linkage map. The data were analyzed with SPSS 17.0, and figures were constructed by GraphPad prism software. As same trends were observed in both 2014 and 2015 (data not shown), we used average data for the analysis in this study.

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Results

Difference of starch-related traits between Sasanishiki and Habataki

In order to avoid the influence of amylose on grain quality, we screened a series of BILs in the attempt to find the BIL population that was derived for the cross between *indica* and *japonica* and had the same type of allele in the *waxy* locus. We found that the *indica* variety Habataki and the *japonica* variety Sasanishiki shared the same Wx^b type allele (**Supplemental Fig. 1**). Thus, we could then focus on the effect of amylopectin on grain quality. To compare the structural differences of starch-related traits between the *japonica* rice variety Sasanishiki and the *indica* rice variety Habataki and the parental lines of BILs, the distribution of the chain length of amylopectin was analyzed (**Fig. 1**). The amylopectin chain length profiles of Sasanishiki and Habataki were clearly different in that Sasanishiki had more short chains, particularly with DP6-15. **Fig. 1B** compares the chain length distribution of amylopectin from Sasanishiki with those from Habataki in the range of DP6-100. Sasanishiki was markedly enriched in short chains with DP6-15 and DP34-45 and depleted in the intermediated-sized chains with DP16-33 and DP46-60 compared to Habataki. There was no significant difference of amylopectin chain length distribution between Sasanishiki and Habataki when DP was over 60 (**Fig. 1**). Consequently, the amylopectin chain length was divided into four groups based on the difference between the two parent lines in this study: $6 < DP < 15$, $16 < DP < 33$, $34 < DP < 45$, and $46 < DP < 60$. The total chain length distribution proportion for each group was represented as: ∑DP6-15, ∑DP16-33, ∑DP34-45, and ∑DP46-60. The average chain length for each group was represented as: ACLDP6-15, ACLDP16-33, ACLDP34-45, and ACLDP46-60, respectively.

ECQ and amylopectin distribution in BILs

The *Fi* of parents, Sasanishiki and Habataki, were 0 and 1, respectively. The performance of BILs was similar to that of the *japonica* parent as the BILs were derived from the backcrossing of Sasanishiki crossed with Habataki, followed by the offspring being backcrossed twice with Sasanishiki. The *Fi* was successively distributed from 0.028 to 0.39 (**Supplemental Figs. 2**, **3**). Therefore, the *japonica* standard could be used to detect and analyze ECQ. The appearance, hardness, stickiness, equilibrium degree, and taste value were used to comprehensively evaluate the ECQ of rice. The equilibrium degree quantified the ratio of stickiness to hardness. The scoring criteria of the appearance, hardness, stickiness, and equilibrium degree were 0–10 points, while that of the taste value was 0–100 points. The ECQ distribution of BILs showed that the appearance scores ranged from 2.5 to 8.6 points and Habataki and Sasanishiki were 4.4 and 7 points. Hardness scores ranged from 5.5 to 8.4 points and the parents were 7.5 and 6.3 points. Scores of stickiness and equilibrium degree ranged from 2.3 to 8.8 points and 2.3 to 8.7 points. Parents were 4.6 and 7.5 points and 4.3 and 7.1 points, respectively. Taste value ranged from 43 to 84.2 points, and the parents were 55.8 and 73.4 points. The differences among the lines in appearance, stickiness, equilibrium degree, and taste value were significant. In most lines, the appearance, hardness, stickiness, equilibrium degree, and taste value were mid-parental and in small lines exhibited transgressive distributions (**Fig. 2**).

The analysis showed that relative percentages of ∑DP6-15 ranged from 44.44% to 49.44%, with Habataki and Sasanishiki being 45.69% and 48.45%, respectively. In most lines, the relative percentages of ∑DP6-15, were mid-parental and there were transgressive distributions in some lines. The relative percentages of ∑DP16-33 ranged from 35.28% to 39.77%, with Habataki and Sasanishiki being 38.24% and 35.52%, respectively. ∑DP34-45 ranged from 6.90% to 8.14%, with 7.65% and 8.00% for Habataki and Sasanishiki, respectively. The relative percentages of ∑DP34-45 in most of the BIL lines were lower compared to that of the parents, while that in a small amount of lines exhibited transgressive distributions. The relative percentages of ∑DP46-60 ranged from 5.10% to 6.62%, with Habataki and Sasanishiki being 6.61% and 6.15%, respectively (**Fig. 2**).

Relationship between Fi and amylopectin

Consistent with the *japonica* variety Sasanishiki, it was enriched in DP6-15 and DP34-45, but depleted in DP16-33 and DP46-60 compared to Habataki. The *Fi* was negatively correlated with the average chain length of DP6-15 and DP34-45, but positively correlated with the average chain length of DP16-33 and DP46-60. However, only the correlation efficiency between *Fi* and the average chain length of DP34-45 reached a significant level (**Fig. 3**). Unexpectedly, no significant correlation between *Fi* and the chain length distribution properties was detected (**Supplemental Fig. 4**). In order to make an in-depth analysis of the relationship among amylopectin, ECQ, and *Fi*, we conducted a correlation analysis for DP6 to DP60. The result showed that only DP35 had a significant positive correlation to *Fi*. Interestingly, the same region also showed significantly negative correlation to taste value (**Supplemental Fig. 5**).

Correlations of Fi to RVA characteristics, AC, GC, protein composition, and ECQ in BILs

Table 1 shows that *Fi* had significantly negative relationships with PKV and BDV, whereas it had significantly positive relationships with CSV, gelatinization temperature (GT) and SBV. At the same time, *Fi* had extremely significant negative and positive correlations with GC and prolamin content, respectively, which indicated that the introduction of the *indica* pedigree reduced PKV, BDV, and GC, but increased CSV, SBV, and prolamin content in the BILs. The correlation analysis showed that *Fi* had significantly negative relationships with appearance, stickiness, equilibrium degree, and taste value and a significantly positive relationship with hardness (**Table 1**). It indicated that the

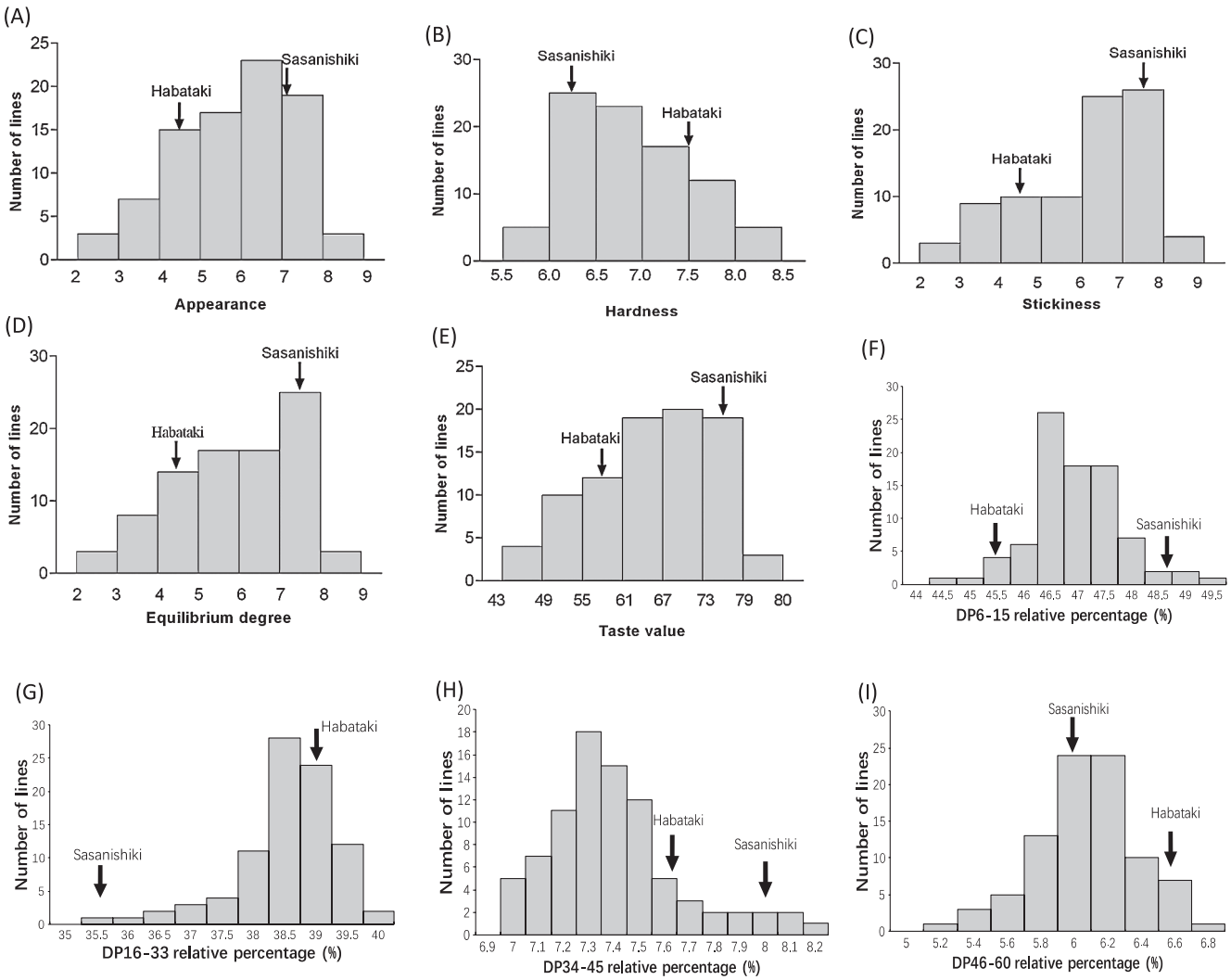

Fig. 2. The ECQ and amylopectin distribution in BILs. (A) Appearance, (B) hardness, (C) stickiness. (D) equilibrium degree, (E) taste value, (F) relative percentage of DP6-15, (G) relative percentage of DP16-33, (H) relative percentage of DP34-45, and (I) relative percentage of DP46-60.

appearance, stickiness, equilibrium degree, and taste value decreased while the hardness increased with the *indica* pedigree introduced in the BILs.

Correlations of amylopectin to ECQ, RVA characteristics, GC, and protein composition

Correlation analysis showed that DP6-15 had a significant relationship with almost all RVA characteristics except for CSV and GT (**Table 2**). DP16-33 had a weak effect on RVA characteristics, and only ∑DP16-33 showed a significant positive correlation to PKV and a significant negative correlation to SBV. The average chain length of DP34-45 had a significant correlation to all of the characteristics of RVA, whereas the ∑DP34-45 had no significant relationship to the RVA characteristics. DP46-60 had negligible influence on RVA. For the traits of appearance, hardness, stickiness, equilibrium degree, and taste value, only the average chain length of DP34-45 showed a significant relationship to these traits. Moreover, the average chain length of DP34-45 showed a significantly positive relationship to GC and a significantly negative relationship to prolamin, whereas other amylopectin characteristics showed a weak correlation to these traits. In summary, the average chain length of DP34-45 was pivotal to the RVA characteristics, GC, ECQ, and protein composition.

QTL analysis of starch-related traits

A total of 236 RFLP molecular markers were used to construct a linkage map and detect QTLs for amylopectin-related traits (**Fig. 4**). We found three QTLs corresponding to ∑DP, the QTLs on Chr. 1 among marker C1370 and G393 corresponding to both ∑6-15 and ∑34-45, two QTLs for ∑16-33 were detected on Chr. 2 among R418 and C1221, as well as on Chr. 8 between C277 and C1121, respectively. Four QTLs of average chain length were detected: one QTL on Chr. 3 between R250 and C136, one QTL on Chr. 4 between R288 and C891, two QTLs on Chr. 7 between G1068 and C145, and between C847 and R1789.

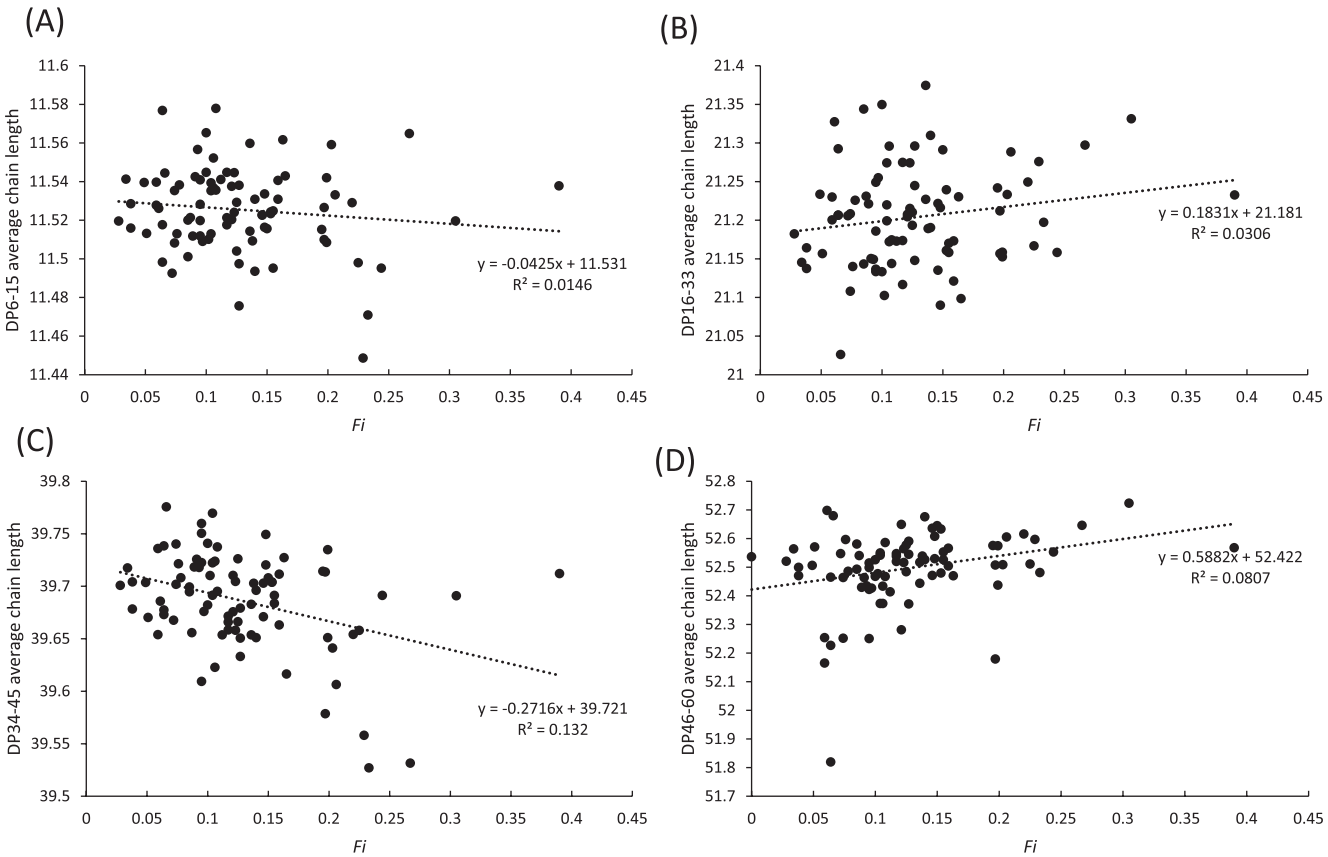

Fig. 3. The correlation between *Fi* and average chain length of (A) DP6-15, (B) DP16-33, (C) DP34-45, and (D) DP46-60.

Table 1. The correlation of *Fi* to RVA characteristics, GC, protein composition and ECQ in BILs

Traits	Correlation efficiency	Traits	Correlation efficiency
PKV	–0.302*	Hardness	0.352*
HPV	–0.184	Stickiness	–0.361*
BDV	–0.281*	Equilibrium degree	–0.359*
HPV	–0.094	Taste value	–0.359*
CPV	0.248*	Albumin	–0.158
GT	0.342*	Globulin	0.125
SBV	0.343*	Prolamin	0.312*
AC	0.098	Glutelin	0.172
Appearance	–0.355*	GC	–0.253*

*P < 0.05.

These two QTLs also corresponded to the PKV traits. The QTL on Chr. 3 between C1452 and R2170 was pleiotropic to taste value, hardness, appearance, and stickiness. The QTL of GT with 61.5% PVE on Chr. 12 between R1957-C1336 was pleiotropic to prolamin. A QTL for both hardness and ECQ was detected on Chr. 12 between S1436 and R1709. A QTL for GC was detected on Chr. 9 between R1164 and R1751. As ∑DP35 showed significantly positive correlation to *Fi* and significantly negative correlation to ECQs, we conducted a QTL analysis for ∑DP35 in particular. The results showed that a QTL with 44.2% PVE was detected on Chr. 3 between C721 and R2778.

Discussion

Indica-japonica hybridization breeding with the integration of subspecies advantages and utilization of heterosis has become one of the most efficient breeding methods in China. The introduction of the *indica* pedigree has contributed to the great improvement of rice production in northeastern China. However, the *indica* pedigree has also dragged down the ECQ of rice varieties (Sun *et al*. 2012). A large number of previous studies have shown that amylose content directly influenced ECQ, and the amylose content was mainly determined by the *waxy* gene (Huang and Lai 2014, Tian *et al*. 2009). Later studies have found that amylopectin was the main reason for the difference in rice varieties that had the same or similar amylose content (Derycke *et al*. 2005, Peat *et al*. 1956, Vandeputte *et al*. 2003). Although the rice varieties in northeastern China have an *indica* pedigree, the genetic background remained based on *japonica*. Thus, most of the rice varieties in northeastern China share the Wx^b allele (Tian *et al*. 2009). Consequently, to understand the factors influencing the ECQ of rice varieties in northeastern China, the experimental material should be derived from the cross between *indica* and *japonica*, but based on the *japonica* genetic background. Moreover, the material should share the same allele of the *waxy* gene to avoid the effects of amylose on ECQ. To achieve this, the

Table 2. The correlation of amylopectin to ECQ, RVA characteristics, GC, and protein composition

Amylopectin	PKV	HPV	BDV	CPV	CSV	SBV	GT
ACLDP6-15	0.487*	0.380*	0.339*	0.384*	–0.035	–0.306*	–0.097
∑DP6-15	–0.447*	–0.312*	–0.351*	–0.280*	0.132	0.358*	0.034
ACLDP16-33	0.036	–0.065	0.146	–0.037	0.082	–0.092	0.079
∑DP16-33	0.250*	0.148	0.230	0.098	–0.152	–0.263*	–0.028
ACLDP34-45	0.559*	0.443*	0.373*	0.366*	–0.265*	–0.431*	–0.296*
∑DP34-45	0.046	0.128	–0.087	0.132	–0.007	0.073	–0.070
ACLDP46-60	0.062	–0.008	0.115	0.048	0.143	–0.040	0.174
∑DP46-60	0.182	0.104	0.125	0.122	–0.002	–0.165	–0.078
	Appearance	Hardness	Stickiness	Equilibrium degree	Taste value	AC	GC
ACLDP6-15	0.077	–0.096	0.065	0.076	0.076	–0.225*	–0.061
∑DP6-15	–0.113	0.123	–0.105	–0.113	–0.113	0.342*	0.026
ACLDP16-33	–0.121	0.119	–0.125	–0.124	–0.122	–0.305*	–0.192
∑DP16-33	0.145	–0.158	0.124	0.141	0.140	–0.211	–0.012
ACLDP34-45	0.425*	–0.439*	0.424*	0.433*	0.430*	–0.207	0.331*
∑DP34-45	–0.113	0.118	–0.086	–0.103	–0.103	–0.095	–0.059
ACLDP46-60	–0.132	0.140	–0.145	–0.143	–0.141	–0.039	–0.015
∑DP46-60	0.061	–0.069	0.062	0.063	0.064	–0.069	0.034
	Albumin	Globulin	Prolamin	Glutelin			
ACLDP6-15	–0.201	0.070	–0.206	0.197			
∑DP6-15	0.203	0.204	0.136	–0.133			
ACLDP16-33	–0.154	–0.257	0.116	0.089			
∑DP16-33	–0.053	–0.186	–0.007	0.046			
ACLDP34-45	0.003	–0.121	–0.365*	–0.073			
∑DP34-45	–0.051	0.073	0.019	0.074			
ACLDP46-60	–0.112	–0.067	–0.067	0.174			
∑DP46-60	–0.271	–0.216	–0.204	0.154			

*P < 0.05.

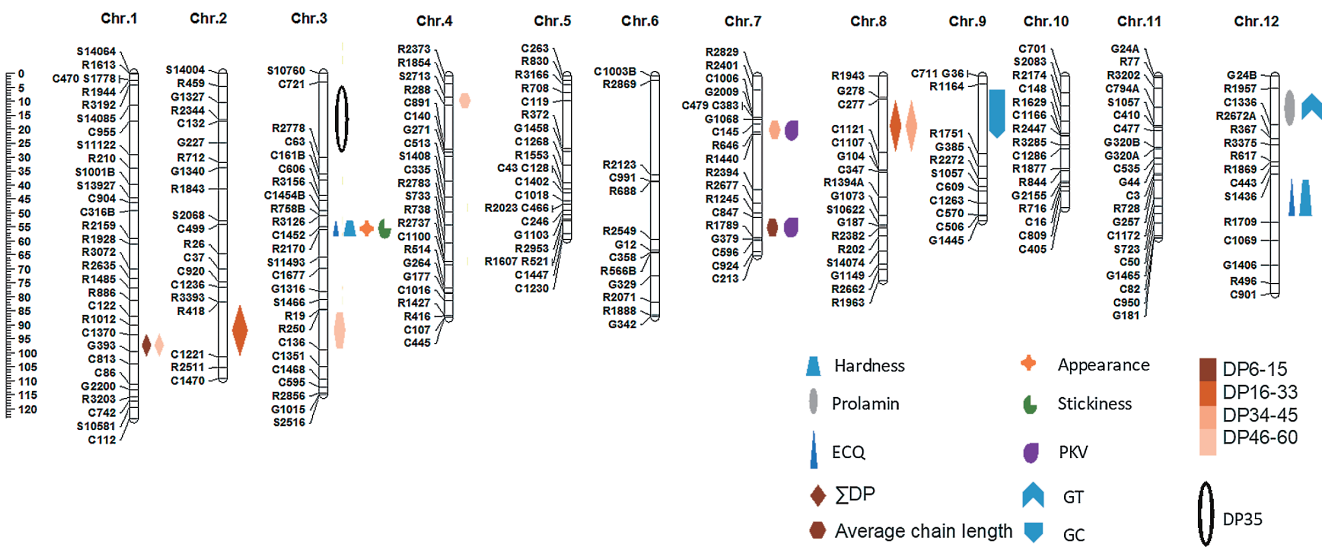

Fig. 4. QTL analysis of amylopectin, hardness, appearance, PLA, stickiness, taste value, PKV, GT, and GC.

BILs that had an *Fi* ranging from 0.028 to 0.39 were used in this study. We also found that Sasanishiki and Habataki shared the same *Wx*^{b} allele. In summary, the BILs that were derived from the cross between Sasanishiki and Habataki were the ideal experimental materials to analyze the relationship among *Fi*, amylopectin and ECQ.

The RVA profile values can reflect the ECQ of rice as the PKV, BDV and SBV are closely related to the ECQ (Hsu *et al*. 2014, Reddy *et al*. 1994, Sun *et al*. 2011). GC is one of the indicators of rice softness. It is generally considered that the longer the gummosis length, the softer the GC and the better mobility and ductility. Our study showed that the introduction of the *indica* pedigree decreased PKV, BDV, and GC and increased the alkali spreading value, CSV and SBV. The protein and its components in rice not only are the key indicators for determining the nutritional quality of rice, but they also have a great effect on the ECQ (Baxter *et al*. 2010, 2014, Derycke *et al*. 2005, Lyon *et al*. 2000). The albumin,

globulin, and glutelin, which have excellent amino acid compositions and rich nutrition, do not affect the ECQ, while prolamin, which impedes the development of the starch mesh structure and has almost no gastrointestinal absorption, causes a decrease in ECQ (Xia *et al*. 2012). Our study indicated that with the *indica* pedigree introduced, the prolamin content increased, lowering the ECQ in the BILs.

Numerous studies have shown that *japonica* varieties are enriched in the amylopectin chain length of DP < 11 and depleted in the chain length of 12 < DP < 24 and that DP < 11 and 12 < DP < 24 are the main factors impacting the pasting temperature and relative crystallinity (Hanashiro *et al*. 1996, Umemoto *et al*. 2002). In this study, we found that the amylopectin of Sasanishiki and Habataki was distinguished by enrichment in DP6-15 and DP34-45, and depletion in DP16-33 and DP46-60. The *Fi* only showed a significant negative correlation to the average chain length of DP34-45, yet it showed a significant correlation to almost all of the characteristics of RVA and cooking quality, indicating that the *Fi* may affect the ECQ indirectly through amylopectin. Interestingly, the average chain length of DP34-45 had a significant correlation with all of the RVA characteristics. Moreover, only the average chain length of DP34-45 had a significant correlation with the traits of ECQ and protein composition. In summary, the DP34-45 exhibited a much stronger effect on ECQ than that of DP6-15 and DP16-33. These opposite results in comparison to those of the previous studies may be due to the genetic background of Sasanishiki and Habataki.

In the QTL analysis, seven QTLs corresponding to amylopectin were detected. Among them, the QTL on Chr. 1 between C1370 and G393 overlaps the region of *SSIV-1*, which is involved in amylopectin chain elongation (Tian *et al*. 2009). The QTL on Chr. 2 between R418 and C1221 contains the area of *SSII-2*, and the QTL on Chr. 8 between C277 and C1121 shares the region of *SSIII-2*. *SSII-2* and *SSIII-2* also belong to the amylopectin chain elongation regulated family (Tian *et al*. 2009). Unexpectedly, the major amylopectin-regulated gene, *alk*, was not detected in our QTL analysis (Umemoto *et al*. 2002). Thus, we conducted a marker assistance selection of *alk* for the parent lines according to the protocol from a previous study (Tian *et al*. 2010). The results showed that Sasanishiki and Habataki shared the same *japonica*-type allele of the *alk* gene (**Supplemental Fig. 1B**). The same allele of the *alk* allele in the parent lines may explain why the short chains and intermediate-size chains have less effect on the ECQ in this study. QTL mapping provides a useful tool for identifying genetic loci, but it is usually difficult to isolate genes with minor effects that play a minor role due to narrow germplasms used in a single experiment. Nevertheless, the same allele of *waxy* and *alk* in the parent lines provided us with a unique opportunity to detect novel factors which affect ECQ or respond to *Fi*. In the present study, we found that DP35 has a significantly positive correlation to *Fi* and a significantly negative correlation to taste value, and a QTL with 44.2% PVE was detected on Chr. 3 between C721 and R2778. Consequently, the QTL for DP35 may help us to find a novel gene that regulates the long chain length of amylopectin among different subspecies, thus further fine mapping and gene cloning is in urgent need of being done.

Amylopectin and its structure are the key determinant factors for rice ECQ. Currently, studies on amylopectin are at the molecular level and the enzymes and genes involved in the regulation of amylopectin synthesis have been confirmed. The mechanism of the effect of amylopectin on rice ECQ is not clear, which prompted us to associate the fine structure of amylopectin with synthesis-related enzymes, expand the study materials, and experiment under different ecological conditions to explore the genetic and eco-physiological mechanisms of the relationship between amylopectin structure and quality traits to provide a scientific basis for good quality and high-yield hybridization breeding between *indica* and *japonica*.

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