QTL Mapping for the Starch Paste Viscosity of Rice (Oryza Sativa L.) Using a Chromosome Segment Substitution Lines Derived From Two Sequenced Cultivars With Same Wx Allele

Ling Zhao
Jiangsu Academy of Agricultural Sciences
Chunfang Zhao
Jiangsu Academy of Agricultural Sciences
Lihui Zhou
Jiangsu academy of agriculture science
Qingyong Zhao
Jiangsu academy of agricultural sciences
Zhen Zhu
Jiangsu Academy of Agricultural Sciences
Tao Chen
Jiangsu Academy of Agricultural Sciences
Shu Yao
Jiangsu Academy of Agricultural Sciences
Yadong Zhang
Jiangsu Academy of Agricultural Sciences
Cailin Wang (cailin_wang@163.com)
Jiangsu Academy of Agricultural Sciences https://orcid.org/0000-0001-8050-2841

Research article

Keywords: Rice (Oryza sativa L.), Starch paste viscosity, Quantitative trait locus, Chromosome segment substitution lines

Posted Date: January 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-152685/v1

Version of Record: A version of this preprint was published at BMC Genomics on August 5th, 2021. See the published version at https://doi.org/10.1186/s12864-021-07913-7.

Abstract

Background

Eating and cooking qualities (ECQs) of rice (*Oryza sativa* L.) is one of the most important economic characters which affecting production and market value. The starch viscosity profile tested by Rapid visco analyzer (RVA) is the direct measure of ECQs that represents the changes in viscosity associated with starch gelatinization. RVA profiles of rice are controlled by a complex genetic system and affected by environment. It is necessary to identify genetic factors controlled the RVA profile characters for development of rice varieties with excellent ECQs. Although it is known that *Waxy* (*Wx*) is the major gene control the amylose content (AC) and ECQs, there still have other unknown genetic factors effecting on ECQs.

Results

Quantitative trait loci (QTL) for starch paste viscosity of rice was analyzed using chromosome segment substitution lines (CSSLs) developed from 9311 and Nipponbare with same *Wx-b* background. With this CSSLs, the effect of major locus *Wx* could be eliminated and the other locus associated with RVA profile could be identified. QTLs for seven traits of starch RVA profile were tested over 4 years in Nanjing, China. 310 QTLs were identified (from 1 to 55 QTLs for each trait). Among 136 repeated QTLs, 6 QTLs were stable and detected every year. In total of 26 marker intervals detected over 3 years or more, 13 showed pleiotropy with respect to controlling 2 to 6 starch RVA profile properties simultaneously. The stable QTL clusters which we mapped here were overlapped to some of the known starch synthesis–related genes (SSRGs).

Conclusions

Without the effect of major locus *Wx*, many QTLs associated with RVA profile of rice were identified and some of them were stable. Our study illustrated that the hereditary of rice starch RVA profile is complicated, pleiotropic effects and QTL hot spots are the key factors affecting starch RVA traits.

Background

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, with about half of the world's population consuming rice as main food [1]. As such, grain quality is a key factor affecting rice production and market value. In current breeding programs, improvement of rice quality is one of the most important goals, particularly with respect to ECQs.

As the major component of grain, starch and its fine structure determine rice ECQs [2]. The starch viscosity profile tested by the RVA is the direct measure of ECQs that represents the pasting behavior and displays the changes in viscosity associated with starch gelatinization. In recent years, starch RVA profiles have already become increasingly popular for their measurements are easily carried out and only small samples are required [3]. Indeed, starch RVA profiles have been used to estimate ECQs and select or screen new genotypes and lines in breeding programs [2, 4].

Research has demonstrated that starch RVA profiles of rice are controlled by a complex genetic system that involves with QTLs, one or few loci have major effects, and many SSRGs [5]. Linkage mapping using many

different rice populations identified a major QTL in the region that includes the gene *Wx* on chromosome (Chr.) 6. *Wx* encodes the granule-bound starch synthase that is mainly responsible for the longer amylose chains [6-8]. Other locus associated with starch RVA profiles were also found to affect the ECQs of rice [9]. The alkali degeneration (ALK) locus on Chr. 6 encodes starch synthase II a (SSIIa), an SSRG which is the major factor responsible for gelatinization temperature (GT) and the distribution of amylopectin chain length [10-12]. The expression of other SSRGs, such as those encoding debranching enzyme (DBE), isoamylases (ISA), starch branching enzyme (SBE), soluble starch synthase (SSS), and pullulanase (PUL), were found to have only minor effects on starch RVA profiles [13]. The functions of these enzymes have been proposed to control AC, GT or certain other quality traits [14-16].

More than 200 starch RVA trait QTLs were localized using various mapping populations [3, 6, 17-23]. A few QTLs have been cloned or subjected to fine-mapping (http://www.gramene.org/, http://www.ricedata.cn/). Certain varieties with the same allele of *Wx* have different ECQs, implying that any other QTLs effect on ECQs. Because the effects of minor QTLs might be covered by major QTL such as *Wx*, many populations derived from bi-parental with similar AC values or the same *Wx* background have been developed to detect QTLs for starch RVA and eliminate the *Wx* effects [21, 22, 24, 25]. The heredity of starch RVA profile characters is very complex and affected by environmental factors [17, 26]. Moreover, starch RVA parameters vary slightly across years, which has confounded the mapping of consistent QTL.

Association mapping was used successfully to determine the genetic basis on complex traits of rice and the contribution of single-nucleotide polymorphisms that lead to allele variations on starch RVA traits were tested [27]. Allelic diversity in SSRGs explains the genetic basis for the observed diversity in starch physicochemical properties among germplasms, and some SSRGs have been found [13,16]. The distribution of functional alleles is strongly correlated with population structure, which can lead to false results if the population is small [28]. As one of the secondly populations, CSSLs are very useful for precise mapping of QTL and dissection of the genetic basis of complex traits [29]. A set of CSSLs had been developed which derived from two sequenced rice cultivars, indica variety 9311 (recipient) and japonica variety Nipponbare (donor) [30]. The draft genomes of 9311 and Nipponbare were successful sequenced and released by researchers in 2002 [31,32]. It was known that the 9311 and Nipponbare have the same *Wx-b* allele [20]. These CSSLs has increased the accuracy of QTL mapping of starch RVA profiles and facilitated detection of new QTLs which covered by the major *Wx* locus before.

In the study presented here, we aimed to map the locus underlying parameters of starch RVA profiles and discovered some stable and new QTLs over 4 years using these CSSLs. Our results will establish the foundation for fine mapping and subsequent cloning of these QTLs and molecular breeding research aimed at improving rice quality.

Results

Performance of starch RVA profiles of parents and CSSLs in different years

The starch RVA profiles of two parents, 9311 and Nipponbare, differed significantly over 4 years of the study. Most of the RVA parameters varied greatly among 4 years, except PeT and PaT which were less affected by

environment (Table 1). For *indica* rice 9311, almost all parameters were much smaller than those in Nipponbare, with the exception of SB and PeT.

The starch RVA profiles were continually distributed in the CSSLs over the different years except for PaT, which exhibited a double-peak distribution (Figure 1). Among the various RVA parameters, the maximum value of peak viscosity (PV) was greater than the other parameters as consequence of greater kurtosis. The starch RVA profiles of CSSLs showed partial separation for some parameters in 2016 and 2017, such as PV, final viscosity (FV) and pasting temperature (PaT). Among the CSSLs, the PV, FV and PaT phenotypic values exhibited greater distribution than the other traits over the 4 years, whereas breakdown viscosity (BD), setback viscosity (SB) and peak time (PeT) were stable and varied little. The mean values for the various RVA parameters of the CSSLs in each year were near the mid-parent value, but some values were not (Table 1). In addition, the phenotypic values for all starch RVA profiles had bidirectional ultra-parental genetic types in the CSSL population, suggesting that those traits were of quantitative traits and controlled by polygenes.

Correlation analysis using the average value of four years as variable revealed that PV was positively correlated with trough viscosity (TV), BD and FV, but negatively correlated with SB and PaT. TV was positively correlated with FV and PeT. FV was positively correlated with PeT and PaT but negatively correlated with SB. BD was negatively correlated with all other RVA parameters. SB was positively correlated with PeT and PaT. These correlations were highly significant ($P < 0.01$). Among the 4 years, the relationship between SB to TV and FV was changed (Supplementary table). SB correlated with FV except in 2013. TV was significantly positively correlated with SB in 2016 and 2017.

Microclimate

Because of the variation in daily maximum temperature and average temperature, mean of daily maximum temperature during recorded periods ranged from 28.7 ˚C to 34.3 ˚C among four years in our research. The daily average temperatures during observational periods in 2013, 2016 and 2017 were about 28.0 ˚C, higher than 25.1 ˚C in 2014 (Figure 2). The average of daily maximum temperature showed the same tendency as the daily average temperatures among those four years. In 2017, it had the highest recorded temperature among those years. The relative humidity (RH) of those four years showed big difference with the temperature. The average daily RH of observational periods in 2014 and 2017 were about 91%, higher than 80.5% and 82.5%, those in 2013 and 2016.

QTL analysis

To elucidate the interference of environmental factors on starch viscosity among the CSSLs, we mapped QTLs that influenced the starch RVA profile characteristics over the 4 years of the study. In total, 310 QTLs were detected for all seven RVA characteristics. We detected 44 PV-related QTLs, 42 TV and 43 FV QTLs. 43, 49, 34 and 55 QTLs were identified that controlled BD, SB, PeT and PaT, respectively.

Among 310 QTLs, 22 loci for PV, 15 for TV, 25 for FV and 11 for BD were detected in more than one year, and 50%, 35.7%, 58.1% and 25.6% of those QTLs were detected in different years that control those four starch RVA profile characters. For SB, PeT and PaT, 28, 8 and 27 QTLs, respectively, were mapped in different years. Among all 310 QTLs, 81 and 50 QTLs were mapped in 2 and 3 years, respectively. Five constant QTLs that control TV,

FV and PaT, were identified in all 4 years (Table 2). The other 174 QTLs appeared in only 1 year. These results implied that, although the QTLs showed large variation in different years, there still exits some constant QTLs related to starch RVA profile characters. Such as the area located on 16.9–19 Mb of Chr. 2, which was found to affect both FV and TV each year. *qPaT7* and *qTV7*, which are located near RM432 on Chr. 7, and *qFV9*, located near RM219 on Chr. 9, were also mapped over the 4 years. A QTL near RM3827 on Chr.6, which was associated with SB, and a QTL near RM1812 on Chr.11 associated with BD, is not been previously identified in those particular locations (Table 3). Those 6 QTLs were not affected by environmental factors in our research, there are very important for the RVA characteristics of rice starch.

We observed pleiotropism of QTLs for starch RVA profile characteristics. In total, 26 marker intervals corresponding to 55 QTLs were identified in more than 3 years in which QTL were associated with starch RVA profiles. Among those constant QTLs, 13 chromosomal intervals showed pleiotropy with more than one starch RVA profile characteristic (Table 3). In particular, the region near RM219 on Chr. 9 contained a QTL that was associated with all starch RVA profile characteristics except TV. Moreover, marker interval RM469 to RM587 on Chr. 6 was found to be associated with five parameters, and the interval near marker RM3795 on Chr. 2 as well as markers RM289 and RM178 on Chr. 5 and marker 10-1.63 on Chr. 10 were found to be associated with four RVA parameters.

Several studies have reported on QTLs that corresponded to starch RVA profiles of rice [17, 19, 20, 22, 33]. In our study present here, those 26 intervals detected more than 3 times were compared with QTL mapped previously. Except for two intervals, all the other intervals were found to overlap with QTLs identified for starch RVA profiles among different mapping populations and different environments (Table 3). Certain hot spots were identified such as region RM469 to RM587 on Chr. 6 and RM1375 on Chr. 10. In the region RM469 to RM587 on Chr. 6, we mapped QTLs associated with SB, PV, BD, PeT and PaT. Many QTLs associated with PeT, TV, FV, BD and SB were identified in the same genomic regions reported previously [1, 3, 6, 19, 22]. *qBD10*, located near RM1375 (15.9–18 Mb) on Chr. 10, was in a similar chromosomal region as certain starch RVA profile QTLs which related with FV, SB, PeT, TV, PaT and PV [3, 22, 33].

Our stable QTL clusters shows overlapping to some of the known SSRGs compared with previous studies (Table 4). All of these QTL clusters were found in regions where some of the SSRGs are, such as *GBSSI (Wx)*, *SSIII-1*, *SSIV-2* and *SBE3.* Besides the QTL in the interval 0.56–2.86 Mb on Chr. 6, which overlapped with *Wx*, the region 3.24–5.38 Mb and 30.45–32.65 Mb on Chr. 4, 24.59–26.37 Mb on Chr.5, and 16.9–20.95 Mb on Chr.2 contained *PUL*, *SSIII-1*, *SSIV-2,* and *SBE3*, respectively. *ADPlar, ADPsma*, *SSII-1*, and *ISA* were near our starch RVA QTLs. For *ADPlar*, *ADPiso*, *GBSSII*, *SSII-2*, *SSIII-2*, *SSIV-1* and *PUL*, the QTLs near or overlapped these genes were detected in only 1 or 2 years, implying that those QTLs were unstable and easily affected by environment. No QTL associated with starch RVA traits was detected in regions that contained *SSI,SSII-3*, *SBE1* and *SBE4*. The region between RM6748 to RM5473 on Chr. 4 where the gene *SSIII-2* situated is related to SB. *SSIII-2* is one of the essential gene controlling Pat and PeT [5]. However, the effort of this region on Pat and PeT did not tested in our research.

The clusters closed to *SSII-1* and *SSII-3* were reported that had major effects on the PT, and minor effects on gel consistency (GC), AC, PV, CPV, BD, and SB [2]. In this research, we found that the QTL near *SSII-1* was associated with BD. Yan reported the QTL clusters near the S*BE3* locus on the Chr. 2, which had 4 major QTLs

associated with HPV, consistency viscosity, viscosity at 95℃ and BD were detected in 2 years [25]. We also found QTL clusters overlapped with *SBE3* gene, which was associated with FV, TV, SB and PaT.

Discussion

Recent studies have confirmed that environmental variation largely affected rice starch PV, TV and FV, while AC and other starch RVA parameters were mainly influenced by genotype [1, 10]. In this research, starch RVA profiles of parents and CSSLs varied greatly in different years, especially in 2016 and 2017. Possible reason for this phenomenon is the high temperature of grain filling happened in the summer of these years [34, 35, 36]. In this research, PV positively correlated with TV, BD and FV, but negatively correlated with SB and PaT. Same relationships were found by other researchers [1, 3, 33]. We also established that for PV, FV, TV, BD, PaT and PeT, consistent correlations with each other existed irrespective of their great variations across different years, which indicated they were mainly affected by genotypic variance for their tendency of correlation analysis were same with different P value. There existed main effect loci to control these starch RVA profile characteristics. The interaction between genotype and environment was important in SB, because the correlations of SB to other parameters except PV were different in 4 years.

The effort of environment and existence of major effort gene *Wx* make it difficult to identify other QTLs associated with starch RVA profiles. Only a few genes and QTLs related to starch RVA profiles were cloned successful, such as *qAC2, qGC6, ALK, Chlalk5* and *Dul* [5]. In this study, the mapping population is CSSLs derived from Nipponbare and 9311 under same *Wx-b* background [20]. New QTLs that exert minor effects on starch RVA profiles could be identified without the influence of major *Wx* gene. In our research, although 310 QTLs were detected for 7 paste viscosity properties of rice starch RVA profile, only 136 QTLs were mapped in more than one years. It indicated that the RVA profile characters were affected by environment, consistent with other researcher's report [17, 26]. Compared with previous studies, those stable and novel loci for all starch RVA parameters are very important and require further validation, especially 6 QTLs been mapped every year. The new and stable QTLs that are not reported before, such as *qSB6* and *qBD11,* might be meaningful for the research and marker-assisted selection of ECQs.

Based on our mapping, clustering phenomenon of QTL controlling RVA traits indicate that pleiotropic effects and QTL hot spots are the key factors affecting starch RVA traits in rice. This pleiotropic were also reported before [http://qtaro.abr.affrc.go.jp/, 3, 22]. QTLs with high correlations are often grouped in the same or adjacent marker intervals on chromosome [37]. Mostly starch RVA traits showed significant correlation with each other and those correlations confirmed the linkage or pleiotropism of the corresponding loci. The association between our mapping QTLs and known SSRGs is also existed widely in this research (Table 4). The possible reason for other known SSRGs not being responsible for the starch RVA characters in this research is the lack of polymorphism between the two parents, 9311 and Nipponbare.

Conclusions

We mapped the QTLs associated with starch viscosity profile which is one of the most important factors of ECQs in rice. The effect of major locus *Wx* could be eliminated and the other locus associated with RVA profile could be identified using CSSLs developed from 9311 and Nipponbare with same *Wx-b* background. QTLs for

RVA profile were tested over 4 years and 136 repeated QTLs were identified. Among of them, 6 stable QTLs were detected every year which are very important for the RVA characteristics of rice starch. 13 intervals which were detected over 3 years showed pleiotropy with respect to controlling 2 to 6 starch RVA profile properties simultaneously. There are four intervals, such as RM469 to RM587 on Chr. 6, found to be associated with more than four RVA parameters at the same time. Pleiotropic effects and QTL hot spots are the key factors affecting starch RVA traits in rice. Further research will be done about those stable QTLs and hot clusters in the future.

Methods

Plant materials and field planting

An advanced backcross population was developed by our lab using the *indica* variety '9311' (recipient) and the *japonica* variety 'Nipponbare' (donor). 9311 were got from its breeder, Yangzhou Institute of Agricultural Sciences, Jiangsu Academy of Agricultural Sciences. Nipponbare were got from Jiangsu Provincial Platform for Conservation and Utilization of Agricultural Germplasm.

The backcrossed population was consisted by 119 BC_4F_2 lines. Backcrossing and simple sequence repeat marker selection were performed as described in detail by Zhu and Zhao [26,30]. Each introgression line in the population contained 1 to 7 segments that came from Nipponbare. The 119 lines contained 318 substituted segments with an average of 2–7 segments per line and covered 84.0% of the whole rice genome [30].

The CSSLs and two parents were planted in fields at the Jiangsu Academy of Agricultural Sciences (32°02'N, 118°52'E; elev. 10 m) in Nanjing in 2013, 2014, 2016 and 2017. Each year, all seeds were planted within two blocks on May 15 and transplanted on June 20. Each line was planted in three rows with a row-to-row distance of 30 cm and plant-to-plant distance of 13.3 cm. The seeds of five plants were sampled from each CSSL line and natural drying. The paddy was grinded into powder and then passed through a 100-mesh sieve. After drying at 4°C in an oven**,** each powder sample was balanced for 2 days at room temperature and then kept at 4°C for three months, and then paste viscosity was measured. For each line, five samples were used to analyze the starch RVA profile respect.

Microclimate

The climate parameters of the site (air temperature, RH) were measured in the field using Thermo Recorder TR-72U (T & D Corp, Japan) every year. The sensor was placed at the height of 170 cm; air temperature and RH were collected every 10 mint. The climate parameters were collected from the flowing time to fully mature every year.

Starch RVA profile

Starch paste viscosity was measured with a Rapid Visco Analyser (Tecmaster, Perten) according to the American Association of Cereal Chemists Standard Method (AACC 61-02) with TCW software 3 (Thermal Cycle for Windows) [38]. The method required 3 g rice flour (12% m. b.) in 25 ml water. The heat profile was set as follows: (1) the temperature was held at 50℃ for 1 min; (2) the temperature was linearly ramped up to 95℃ for 3.75 min; (3) the temperature was held at 95℃ for 2.5 min; (4) the temperature was linearly ramped down to

50℃ for 3.75 min; (5) the temperature was held at 50℃ for 1.4 min. The RVA paddle speed was at 960 rpm for the first 10 s of the test, after which the speed was at 160 rpm.

Starch paste viscosity characteristics of rice were described by five important parameters: peak viscosity (PV), trough viscosity (TV), final viscosity (FV), peak time (PeT), and pasting temperature (PaT). Breakdown viscosity (BD) and setback viscosity (SB) were calculated as: BD = PV – TV, and SB = FV – PV [39]. Correlations among the seven RVA parameters of each year were analyzed by IBM SPSS Statistics v22.

QTL mapping

Genotype data for 250 polymorphic loci, including 211 simple sequence repeat and 39 sequence tag site markers, were used for QTL detection. Molecular linkages were established using composite interval mapping with version 3.3 of QTL Icimapping software [40].

The QTL was detected according to the method described by Eshed and Zamir [41]. The significance of each QTL was determined by comparing the mean RVA profile values of a CSSL line with the recipient parent 9311 using analysis of variance and Dunnett's test. The QTL was considered present when a CSSL line exhibited a significant difference compared with 9311 with corresponding probability value of $P < 0.05$. If more than three CSSLs showed differences simultaneously, then the QTL was estimated as being located within the chromosomal region shared by those CSSLs [20]. QTL nomenclature was followed as that of McCouch et al [42].

Abbreviations

ECQs: Eating and cooking qualities; QTL: Quantitative trait loci; RVA: Rapid Visco Analyzer; CSSLs: Chromosome segment substitution lines; SSRG: Starch synthesis related gene; AC: Amylose content; GC: Gel consistency; GT: Gelatinization temperature; PV: Peak viscosity; TV: Trough viscosity; FV: Final viscosity; PaT: Pasting temperature; PeT: Peak time; BD: Breakdown viscosity; SB: Setback viscosity; ALK: Alkali degeneration; DBE: debranching enzyme; ISA: Isoamylases; SBE: Starch branching enzyme ; SSS: Soluble starch synthase; PUL: Pullulanase; RH: Relative humidity.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the materials analyzed during the current study are available in the Jiangsu Provincial Platform for Conservation and Utilization of Agricultural Germplasm (http://jagis.jaas.ac.cn/CL_crop.aspx). The deposition

numbers of 9311 and Nipponbare are SD_JAAS_12364 and SD_NJAU_149, respectively. The deposition numbers, raw phenotype data and genotype data of CSSLs will be provided on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work supported by grants from the Natural Science Foundation of Jiangsu Province of China (BK20180302), the Agriculture Research System of China (CARS-01-62), Jiangsu Agriculture Science and Technology Innovation Fund (CX(18)1001), the Provincial Key Laboratory of Agrobiology, Jiangsu Academy of Agricultural Sciences, China. The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

LZ, CZ and CW designed the experiments. LZ and SY prepared and the powers of grain and measured the RVA profile. LZ, TC, QZ, ZZ, YZ developed the CSSLs populations and CZ identified the CSSLs by molecular markers. TC and ZZ managed the planting of materials in field. QZ performed analyses. LZ wrote the manuscript which was modified by YZ. All authors read and approved the final manuscript.

Acknowledgements

We thanks Dr. Wunyin Zhu and Miss Jin Lin for their word at development of CSSLs.

Author details

1Institute of food crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China Jiangsu High Quality Rice R&D Center / Nanjing Branch of China National Center for Rice Improvement, Nanjing 210014, China

2 Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing 210095, China

References

1. Tong C, Chen Y, Tang F, Xu F, Hung Y, Cheng H, et al. Genetic diversity of amylose content and RVA pasting parameters in 20 rice accessions grown in Hainan, Chin Food Chem. 2014;161:239–45.
2. Cozzolino D. The use of the Rapid Visco Analyser (RVA) in breeding and selection of cereals. J Cereal Sci. 2016;70:282–90.
3. Yang Y, Ni D, Song F, Li L, Lu X, Li Z, et al. Identification of QTLs for rice starch RVA profile properties in different ecological sites. Acta Agronomica Sinica. 2012;38(2):264–74(in Chinese with English abstract).
4. Bao J, Shen S, Xia Y. Analysis of genotype environment interaction effects for starch pasting viscosity characteristics in Indica rice. Acta Genetica Sinica. 2006;33:1007–13 (in Chinese with English abstract).

5. Yan C, Tian Z, Fang Y, Yang Y, Li J, Zeng S, et al. Genetic analysis of starch paste viscosity parameters in glutinous rice *Oryza sativa* Theor Appl Genet. 2011;122:63–76.
6. Bao J, Zheng X, Xia Y, He P, Shu Q, Lu X, et al. QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa*). Theor Appl Genet. 2000;100: 280–4.
7. Lapitan V, Redona E, Abe T, Brar D. Mapping of quantitative trait loci using a double–haploid population from the cross of indica and japonica cultivars of rice. Crop Sci. 2009;49:1620–8.
8. Zhang C, Chen S, Ren X, Lu Y, Liu D, Cai X, et al. Molecular structure and physicochemical properties of starches from rice with different amylose contents resulting from modification of OsGBSI activity. J Agr Food Chem.2017;65(10): 2222–32.
9. Su Y, Rao Y, Hu S, Yang Y, Gao Z, Zhang G, et al. Map-based cloning proves *qGC-6*, a major QTL for gelconsistency of japonica/indica cross, responds by *Waxy* in rice (*Oryza sativa*). Theor Appl Genet. 2011;123: 859–67.
10. Bao J, Sun M, Zhu L. Corke H. Analysis of quantitative trait locus for some starch properties in rice (*Oryza sativa*), thermal properties, gel texture, swelling volume. J Cereal Sci. 2004;39:379–85.
11. Fan C, Yu X, Xing Y, Xu C, Luo L,Zhang Q. The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled–haploid line population. Theor Appl Genet. 2005;110:1445–52.
12. Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y. Mapping of a gene responsible for the difference in amylopectin structure between japonica–type and indica–type rice varieties. Theor Appl Genet. 2002;104:1–8.
13. Tian Z, Quian Q, Liu Q, Yan M, Liu X, Yan C, et al. Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proc Natl Acad Sci USA. 2009;106:21760–5.
14. Xu F, Bao J, He Q, Park Y. Genome–wide association study of eating and cooking qualities in different subpopulations of rice (*Oryza sativa*). BMC Genomics. 2016;17:663.
15. Xiang X, Kang C, Xu S, Yang B. Combined effects of *Wx* and *SSIIa* haplotypes on rice starch physicochemical properties. J Sci Food Agri. 2017;97:1229–34.
16. Yang F, Chen Y, Tong C, Huang Y, Xu F, Li K, ,et al. Association mapping of starch physicochemical properties with starch synthesis–related gene markers in nonwaxy rice (*Oryza sativa*). Mol Breeding. 2014;34:1747–63.
17. Zhang Q, Zhan Y, Zhu Z, Zhao L, Zhao Q, Xu L, et al. Inheritance analysis and QTL mapping of rice starch viscosity (Rapid Visco Analyzer profile) characteristics. Rice Sci. 2008;15:186–94.
18. Liu X, Wan X, Ma X, Wan J. Dissecting the genetic basis for the effect of rice chalkiness, amylose content, protein content, and rapid viscosity analyzer profile characteristics on the eating quality of cooked rice using the chromosome segment substitution line population across eight environments. 2010;54:64–80.
19. Zhang Y, Jiang L, Liu X, Liu S, Chen L, Zhai H, et al. Analysis of QTLs for starch RVA profile properties in the superior rice cultivar Koshihikari. China J Rice 2010;24(2):137–44 (in Chinese with English abstract).
20. Zhang C, Bing H, Zhu K, Zhang H, Leng Y, Tang S, et al. QTL mapping for rice RVA properties using high-throughput resequenced chromosome segment substitution lines. Rice Science. 2013;20(6):407–14.

21. Liu X, Zhu K, Zhang C, Hong R, Sun P, Tang S, et al. Mapping of minor QTLs for rice gelatinization temperature using chromosome segment substitution lines from indica 9311 in the japonica background. Acta Agronomica Sinica. 2014;40(10):1740–7 (in Chinese with English abstract).
22. Yao X, Wang J,Liu J, Zhang J, Ren C, Ma D, et al. Mapping quantitative trait loci associated with starch paste viscosity in rice (*Oryza sativa*) under different environmental conditions. Plant Breed, 2017;136:591–602.
23. Shar T, Sheng Z, Ali U, Fiaz S, Wei X, Xie L, et al. Mapping quantitative trait loci associated with starch paste viscosity attributes by using double haploid populations of rice (*Oryza sativa*). J Integr Agric. 2020;19(7):1691–703.
24. Bao J, Wu Y, Hu B, Wu P, Cui H, Shu Q. QTL for rice grain quality based on a DH population derived from parents with similar apparent amylose content. Euphytica. 2002;128:317–24.
25. Yan B, Tondi Y, Chen J, Wang Y, Gao G, Zhang Q, et al. Analysis of minor quantitative trait loci for eating and cooking quality traits in rice using a recombinant inbred line population derived from two indica cultivars with similar amylose co Mol Breed. 2014;34:2151–63.
26. Zhu W, Lin J, Yang D, Zhao L, Zhang Y, Zhu Z, et al. Development of chromosome segment substitution lines derived from backcross between two sequenced rice cultivars, indica recipient 93-11 and japonica donor Nipponbare. Plant Mol Biol Rep. 2009;27:126–31.
27. Lu F, Park Y. Sequence variations in OsAGPase significantly associated with amylose content and viscosity properties in rice (*Oryza sativa*). Genet Research. 2012;94:179–89.
28. Yu J, Buckler E. Genetic association mapping and genome organization of maize. Curr Opin Chem Biol. 2006;17(2):155–60.
29. Xu J, Zhao Q, Du P, Xu C, Wang B, Feng Q, et al. Developing high throughput genotyped chromosome segment substitution lines based on population whole–genome re–sequencing in rice (*Oryza sativa*). BMC Genomics. 2010;11:656.
30. Zhao C, Zhou L, Yu X, Zhao Q, Chen T, Yao S, et al. Identification of tiller angle quantitative trait loci based on chromosome segment substituted lines and high-density physical map in rice. Bull Botany. 2012;47(6):1–8 (in Chinese with English abstract).
31. Yu J, Hu S N, Wang J, Wong G, Li S, Liu B, et al. A draft sequence of the rice genome (*Oryza sativa* ssp. *indica*). Sience,2002;296:79–92.
32. Goff S A, Ricke D, Lan T H, Presting G, Wang R, Dunn M, et al. A draft sequence of the rice genome (*Oryza sativa* ssp. *japonica*). Sience,2002;296:92–100.
33. Zhang J, Zheng L, Cai Y, You X, Kong F, Wang G, et al. Correlation analysis and QTL mapping for starch RVA profile properties and amylose and protein contents in rice. China J Rice Sci.2017;31(1):31–9 (in Chinese with English abstract).
34. Mei M, Jiang Y, Wan Z, Liu L, Ye D, Wang Y, et al. National Climate Centre. Climatic characteristics and major meteorological events over China in 2016. Meteorological Monthly. 2017;43(4):468–76 (in Chinese with English abstract).
35. Feng A, Zeng H, Yin Y, Song Y, Liu Y, Wang Y, et al. Climatic characteristics and major meteorological events over China in 2017. Meteorological Monthly. 2018;44(4):548–55 (in Chinese with English abstract).

36. Jiang H, Dian W, Wu P. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. Phytochemistry. 2003;63:53–9.
37. Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar H, Zhuang J, et al. Identification of QTL for growth and grain yield–related traits in rice across nine locations of Asia. Theor Appl Genet. 2003;107:679–90.
38. Bao J, Xia Y. Genetic control of the paste viscosity characteristics in indica rice (*Oryza sativa*). Theor Appl Genet. 1999;98:1120–4.
39. Chen L, Yan X, Wang L, Gao W, Yang J, Chen S, et al. Pasting properties, grain-filling characteristics and allelic variation linked to the grain quality in diverse rice. Euphytica. 2017;213:29–44.
40. Wang J, Li H, Zhang L, Meng L. 2013. Users' Manual of QTL IciMapping. The Quantitative genetics group, Institute of crop science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6–641, 06600 Mexico, DF, Mexico.
41. Eshed Y, Zamir D. An introgression line population of *Lycopersican pennelli* in the cultivated tomato enables the identification and fine mapping of yield associated QTL. Genetics. 1995;141(3):1147–62.
42. McCouch S, Cho Y, Yano M, Paul E, Blinstrub M, Morishima H, et al. Report on QTL nomenclature. Rice Genetics Newsletter. 1997;14:11–3.
43. Shao X, Chen Z, Liu Z, Ding C, Tang S, Li G, et al. QTL mapping for rice RVA properties using chromosome segment substitution lines derived from a cross between Koshihikari and 9311. Mol Plant Breed. 2015;2:261–8 (in Chinese with English abstract).
44. Hsu Y, Tseng M, Wu Y, Lin M, Wei F, Hwu K, et al. Genetic factors responsible for eating and cooking qualities of rice grains in a recombinant inbred population of an inter-subspecific cross. Mol Breed. 2014;34:655–73.

Tables

Table 1 Phenotypic variations of starch RNA characteristics among 9311, Nipponbare and their CSSL populations

Traits		Parent		CSSLs			
	Year	Nipponbare	9311	Average	Range	Kurtosis	Skewness
PV/cP	2013	3765.2±50.9	3372.4±14.0	3573.4	3003.0~4074.0	0.98	−0.22
	2014	3243.6±44.5	2828.2±61.1	3046.4	2610.0~3863.0	2.4	0.47
	2016	2978.5±29.2	2197.9±22.6	1993.6	920.0~2459.0	2.68	−1.48
	2017	3372.3±38.7	2162.5±76.2	1977.7	471.0~2678.0	2.26	−1.28
TV/cP	2013	2010.6±16.3	1950.0±13.2	1965.2	1719.0~2189.0	−0.38	0.13
	2014	1372.4±37.3	1294.3±23.5	1490.5	1232.0~1992.0	1.09	0.92
	2016	2273.0±25.7	1467.5±24.1	1231.4	403.0~1901.0	−0.73	2.29
	2017	2373.3±124.2	1520±141.2	1376.4	224.0~2157.0	−0.80	1.29
FV/cP	2013	3395.5±48.1	3336.8±30.5	3381.6	3077.0~3702.0	−0.44	0.12
	2014	2705.9±61.3	2657.1±58.2	2859.2	2477.0~3280.0	−0.49	0.21
	2016	2961.4±36.2	2239.4±30.7	2283.0	978.0~2954.0	4.29	−1.55
	2017	3311.5±24.9	2798.3±149.3	2550.4	668.0~3562.0	3.35	−1.43
BD/cP	2013	1766.4±28.6	1422.1±37.2	1608.2	1284.0~1964.0	0.32	0
	2014	1571.6±65.4	1533.7±40.5	1547.8	1024.0~1973.0	0.06	−0.25
	2016	1063.2±49.2	872.3±26.5	762.2	335.0~1116.0	0.23	−0.37
	2017	999.2±51.4	742.5±65.3	601.3	247.0~911.0	−0.31	−0.45
SB/cP	2013	−370.1±28.1	−35.6±5.5	−191.7	−604.0~225.0	0.17	0.14
	2014	−338.3±59.1	−170.5±30.6	−178.2	−659.0~489.0	−0.43	0.19
	2016	−160.6±36.7	235.8±12.3	289.5	−171.0~639.0	0.87	−0.18
	2017	−60.5±38.5	523±73.4	572.7	184.0~1060.0	1.05	0.13
PeT/min	2013	6.2±0.1	5.9±0.1	6	5.7~6.3	1.79	0.58
	2014	6.2±0.1	6.1±0.0	6.1	5.7~6.5	−0.51	0.12
	2016	6.5±0.1	6.2±0.1	6.2	5.5~6.8	0.78	−0.10
	2017	6.6±0.1	6.6±0.2	6.6	5.9~7.0	0.37	−0.35
PaT/℃	2013	74.7±0.9	86.3±3.1	81.9	73.6~87.1	−1.31	−0.69
	2014	76.7±0.5	74.4±0.6	77.9	71.95~88.6	−1.48	0.63
	2016	76.0±0.3	75.2±1.2	76.4	71.95~92.6	4.74	2.48
	2017	77.1±0.4	76.8±0.4	79.3	72.85~94.9	0.92	1.65

Table 2 Analysis of QTL for starch RVA profile properties

Trait	No. of QTLs				
	Mapped over 2 years	Mapped over 3 years	Mapped over 4 years	Total mapped QTLs	Repeatedly detected QTL (%)
PV/cP	18	4	0	44	50
TV/cP	10	3	3	42	35.7
FV/cP	13	10	2	43	58.1
BD/cP	3	8	0	43	25.6
SB/cP	15	13	0	49	57.1
PeT/min	5	3	0	34	23.5
PaT/℃	17	9	1	55	49.1
Total	81	50	6	310	43.9

Table 3 Stable RVA QTLs that were detected more than 3 times

Chr.	Marker	Interval (Mb)	Traits	Other RVA QTL overlapped
1	RM488	25.75–27	SB	*qBDV1* [17]*; qBDV1* [22]
1	RM3143	28.05–29	BD	*qPKV1* [22]
2	RM5390	10.15–11.11	PaT, TV	*qCPV2, qSBV2* [33]*; qBDV2a, qCSV2a, qTD2* [21]
2	RM3795	16.9–20.95	FV*, TV*,SB, PaT	*qPKV2, qCPV2, qSBV2* [33]*; qPeT2* [14]
2	RM191	25.75–29.3	FV	*qBDV2* [43] *; qHPV2* [33]; *qPaT* [25]
2	RM1342	28.65–29.3	PV, PaT	*qHPV2* [43]*; qPKV2* [17]
4	RM518	1.1–3.24	SB	*qPKV4* [25]
4	RM6748–RM5473	30.45–32.65	SB	*qPT4* [43]*; qASV* [39]
5	RM289	7.13–8.55	BD, PaT, SB, FV	*qSBV5, qCSV5* [33]; *qHPV5* [43]; *qPV5*, *qHPV5, qPeT5* [14]
5	RM178	24.59–26.37	BD, FV, SB ,PaT	*qPKV5, qHKV5* [20]
6	RM469–RM587	0.56–2.86	SB, PV, BD, PeT, PaT	*qPeT6* [3]*; qTPV6, qFPV6, qBDV6, qSBV6, qPKT6* [19]; *qHPV6–1, qCPV6, qCS6, qSBV6* [6]; *qPKV6, qBDV6, qSBV6, qPeT6* [22]*; qBD6, qSB6* [1]; *qTV6, qCPV6, qSBV6, qPeT6, qPKV6* [23]
6	RM527	9.31–10.98	SB, FV	*qPeT6* [22]
6	RM3827	22.55–23.8	SB	
6	RM3628	23.8–24.8	PV	*qHPV6* [1]
6	RM412	30.85–31.59	SB	*qBDV6, qCSV6, qCPV6, qHPV6, qSBV6* [17]
7	RM542	12.41–14.5	PeT	*qGT7* [21]
7	RM432	18.6–20.55	PaT*, TV*	*qCPV7* [17]; *qHPV7, qBDV7, qCPV7* [22]; *qGT7–1* [21]; *qPaT-7, qPeT-7* [23]
8	RM5485	23.32–25.4	FV	*qBDV8, qPeT8* [33]; *qPKV8* [22]; *qPKV8, qHPV8, qHPV6* [3]

9	RM219	7.39–9.11	BD, PV, SB, PaT, PeT, FV*	*qPaT9* [14]; q*PKV9,qBDV9-b* [23]
9	RM566	14.63–16.15	PV, PeT	*qPKV10* [33]*; qHPV9, qHPV9* [1]*; qCPV9, qCS9* [14]
10	10–1.63	0.8–2.12	BD, FV, SB, TV	*qCPV10, qSBV10, qCSV10* [3]; *qBDV10, qPeT10* [44]
10	10–9.12	8.76–9.57	TV*,FV, BD	*qPKV10, qHPV10, qCPV10, qSBV10, qPeT10, qCSV10* [3]; *qSB10* [25]
10	RM1375	15.89–18.04	BD	*qCPV10; qSBV10; qPeT10, qHPV10, qPKV10* [3]; *qPET10, qPAT10, qPKV10, qPKV10* [33]
11	RM1812	1.35–2.61	BD	*qBDV11, qPKV11,qPaT11* [23]
12	RM1261	15.52–18.07	FV	*qGT12* [21]; *qPaT12* [14] ; *qBDV12, qPaT12* [23]
12	RM1227	27.4–27.6	FV, SB	*qGT12* [21]
*Note. * indicates the QTL was detected in each of the 4 years.*				

Table 4 Overlap of known SSRGs with the starch RVA QTLs detected in this study

Gene	Acc. no.	Position (Mb)	Traits associated with QTL in this research	Interval of QTL (Mb)
ADPlar	LOC_Os05g50380	Chr.5, 28.87	BD, FV	27.95–28.85
ADPiso	LOC_Os01g44220	Chr.1, 25.35	BD, PT, PeT	23.85–25.75
ADPsma	LOC_Os09g12660	Chr.9, 7.24	BD, PV, SB, PaT, PeT, FV*	7.39–9.11
GBSSI (Wx)	LOC_Os06g04200	Chr.6, 1.70	SB, PV, BD, PeT, PaT	0.56–2.86
SSI	LOC_Os06g06560	Chr.6, 3.08	–	–
SSII–1	LOC_Os10g30156	Chr.10, 15.67	BD	15.89–18.04
SSII–2	LOC_Os02g51070	Chr.2, 31.23	PK, PK	30.45–34.75
SSII–3	LOC_Os06g12450	Chr.6, 6.75	–	–
SSIII–1	LOC_Os04g53310	Chr.4, 31.76	SB	30.45–32.65
SSIII–2	LOC_Os08g09230	Chr.8, 5.35	BD, FV, PK, TV	4.75–6.28
SSIV–1	LOC_Os01g52250	Chr.1, 30.04	PK, ST, TV	29.75–35.1
SSIV–2	LOC_Os05g45720	Chr.5, 26.48	BD, FV, SB ,PaT	24.59–26.37
SBE1	LOC_Os06g26234	Chr.6, 15.33	–	–
SBE3	LOC_Os02g32660	Chr.2, 19.36	FV*, TV*,SB, PaT	16.9–20.95
SBE4	AB023498	Chr.4, 20.12	–	–
ISA	AB093426	Chr.8, 25.89	FV	23.32–25.4
PUL	D50602	Chr.4, 4.40	BD, PeT, ST	3.24–5.38
*Note. * indicates the QTL was detected in each of the 4 years.*				
_indicates the QTL was detected only one or two times.				

Figures

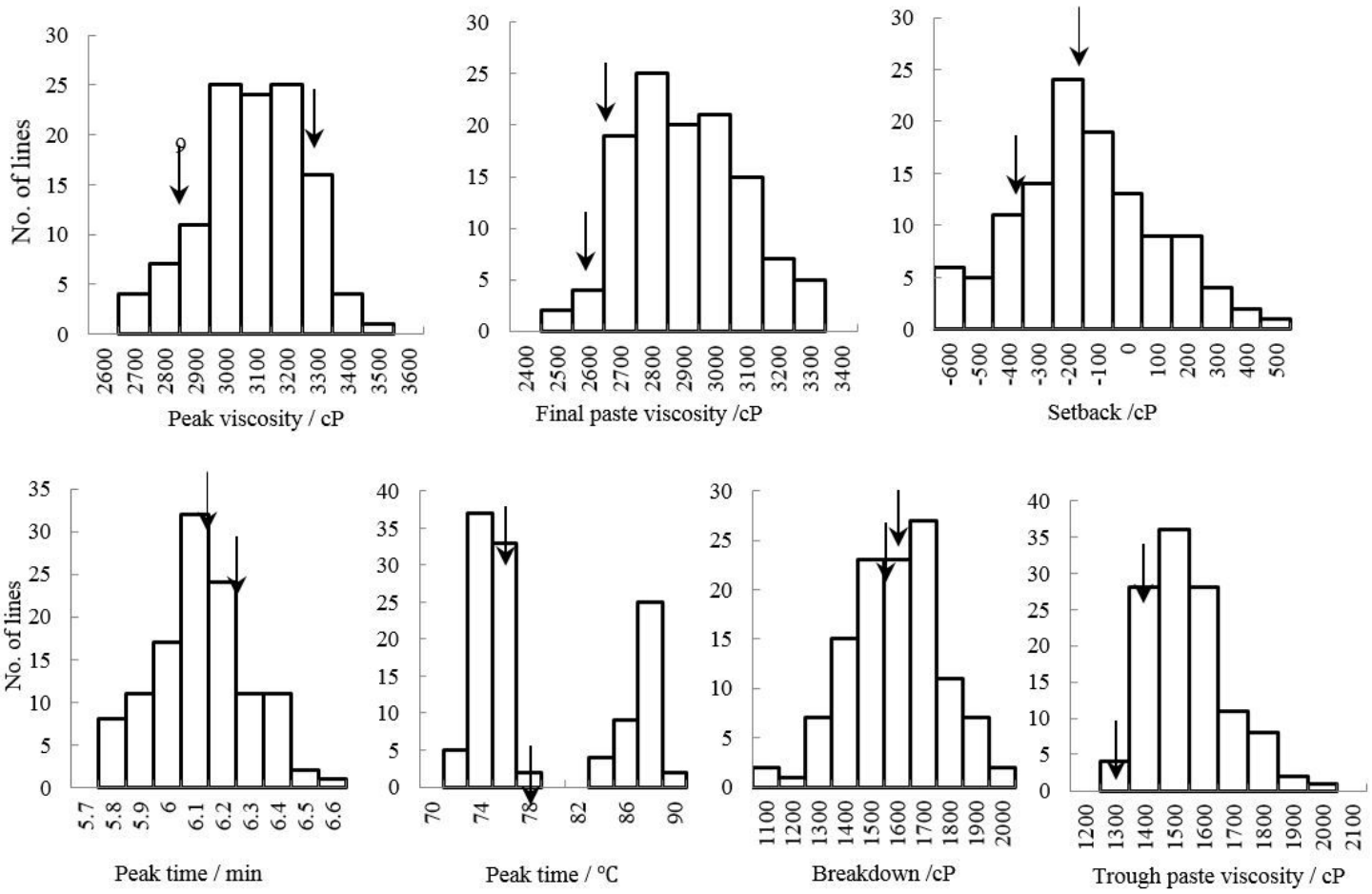

Figure 1

Distribution of starch RVA profile characteristics in Nipponbare/9311 CSSL population in 2014. Note. 9 indicates 9311 and N indicates Nipponbare.

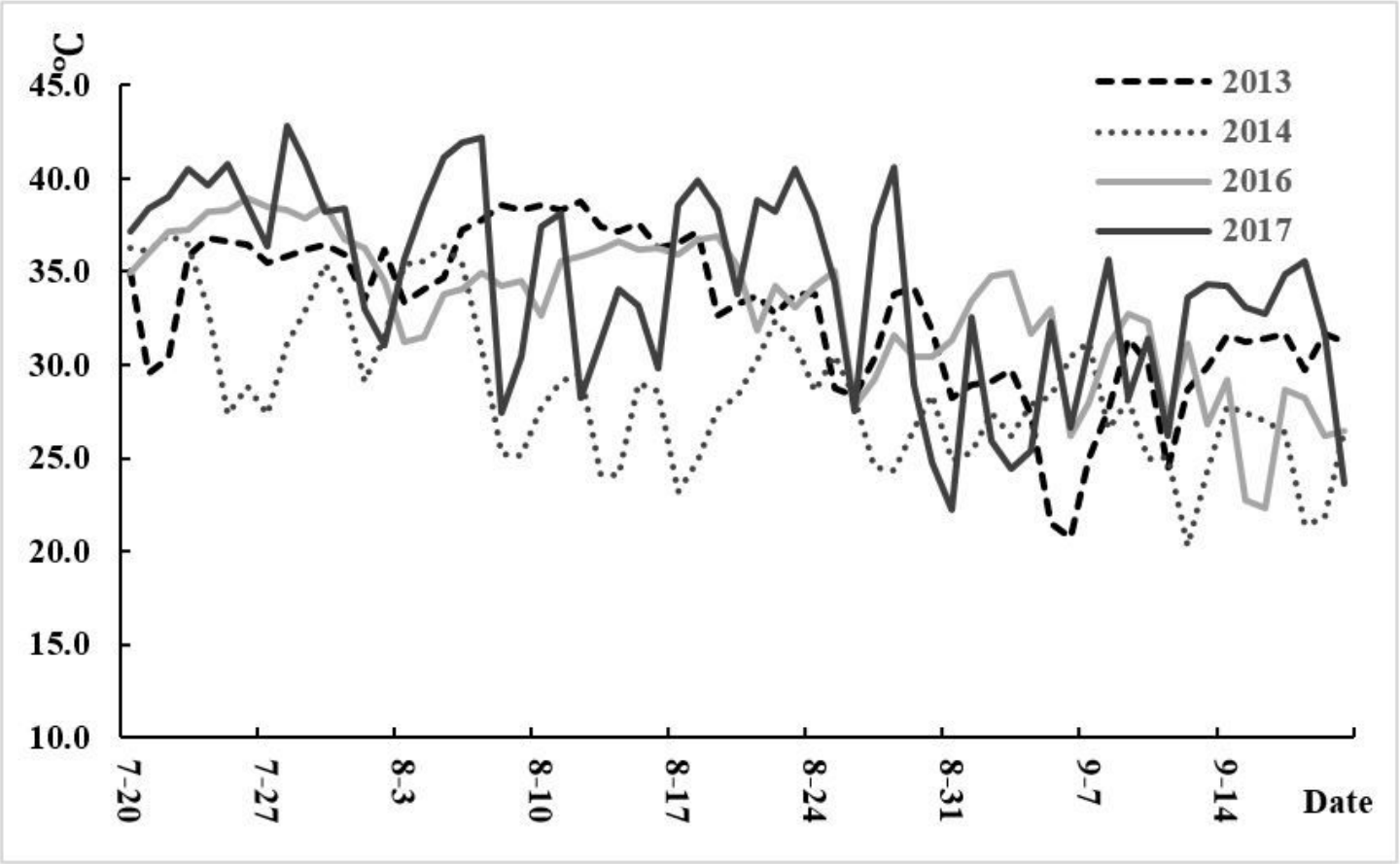

Figure 2

Daily maximum temperature recorded during observational periods over four years.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx