Note

Development and characterization of chromosome segment substitution lines derived from backcross between japonica donor rice cultivar Yukihihikari and japonica recipient cultivar Kirara397

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Grain yield-related traits and grain quality-related traits are important for rice cultivars. The quantitative trait loci (QTLs) involved in controlling the natural variation in these traits among closely related cultivars are still unclear. The present study describes the development of a novel chromosome segment substitution line (CSSL) population derived from a cross between the temperate japonica cultivars Yukihihikari and Kirara397, which are grown in Hokkaido, the northernmost limit for rice cultivation. Days to heading, culm length, panicle length, panicle number, brown grain weight per plant, thousand brown grain weight, brown grain length, brown grain width, brown grain thickness, apparent amylose content, and protein content were evaluated. Panicle length, brown grain length and amylose content differed significantly in the parental cultivars. Thirty-five significant changes in the evaluated traits were identified in the CSSLs. A total of 28 QTLs were located on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12. These findings could be useful for breeding rice cultivars in the northernmost limit for rice cultivation.

Key Words: rice, chromosome segment substitution lines, QTL, yield component, grain quality.

Introduction

Rice (Oryza sativa L.) is the staple food for over half the world’s population, mainly in Asian countries. Rice provides not only up to 50% of the dietary caloric supply but also a considerable proportion of the protein intake for millions of people (Muthayya et al. 2014). Many quantitative trait loci (QTLs) for agronomically important traits, such as grain yield, grain quality, disease resistance, and stress tolerance of rice, have been detected using segregated populations derived from crosses between indica and japonica subspecies or with wild relatives.

Chromosome segment substitution lines (CSSLs) are genetic resources, in which the whole genome of a specific donor genotype is represented segment wise (preferably over-lapping) in the genetic background of the recurrent parent. CSSL libraries are developed by advanced backcross, marker-assisted selection (MAS) of donor introgressions, and selfing. CSSLs can simplify the study of complex genetic traits such as yield, and they are valuable resources for the precise identification of quantitative trait loci (QTL) and genes (Ali et al. 2010). In rice, most initial CSSLs were developed by inter-subspecific crosses between indica and japonica cultivars with increased use of wild species as donors (Ali et al. 2010). More recently, CSSLs derived from intra-subspecific crosses between Japanese japonica cultivars were also developed to dissect the natural variation in agronomically important traits among closely related cultivars (Hori et al. 2017, Okada et al. 2018).

Hokkaido prefecture, Japan, is one of the northern limits of rice cultivation in the world. The first good eating quality cultivar in Hokkaido was Yukihihikari, which was released in 1981. A second good eating quality cultivar from Hokkaido, Kirara397, was released in 1987. Yukihihikari is a partial panicle number type cultivar, whereas Kirara397 is a panicle number type with slightly longer grains. The genetic mechanisms underlying agronomically important traits involving plant type and grain shape in these two closely related cultivars remain unclear.

The present study describes the development of a novel population of CSSLs derived from the cross between two rice (O. sativa L. japonica) cultivars, Yukihihikari and Kirara397, and back-crossing Yukihihikari as the donor and Kirara397 as the recipient. A total of 11 yield-related and...
grain quality-related traits were subsequently evaluated in each CSSL.

Materials and Methods

Development of the CSSLs

CSSLs were developed using the rice (*O. sativa* L. *japonica*) cultivar Kirara397 as the recipient and the *japonica* cultivar Yukihikari as the donor. F₁ plants generated from crosses between Yukihikari and Kirara397 were back-crossed to Kirara397 to produce BC₁F₁ generations. The whole genomes of BC₁F₁ and subsequent generations were subsequently subjected to MAS (Fig. 1).

Development and genotyping of InDel markers

The 38 InDel markers used (Kinoshita *et al.* 2016) are shown in Supplemental Table 1, as are the 53 novel InDel markers developed in the present study based on the whole genome resequencing data of the cultivars Yukihikari and Kirara397 (Takano *et al.* 2014). DNA extraction, PCR and gel electrophoresis were performed as described (Kinoshita *et al.* 2016).

Development and genotyping of cleaved amplified polymorphic sequence markers and derived cleaved amplified polymorphic sequence markers

Three cleaved amplified polymorphic sequence (CAPS) markers and two derived CAPS (dCAPS) markers (Kinoshita *et al.* 2016) were used in the present study. In addition, two CAPS markers were developed to fill the gaps between the above-mentioned molecular markers (Supplemental Table 2). PCR, restriction enzyme digestion and gel electrophoresis were performed as described (Kinoshita *et al.* 2016).

Field trial and trait measurements

The CSSLs were evaluated relative to Kirara397 for grain yield-related and grain quality-related traits. These traits included days to heading (DTH), culm length (CL), panicle length (PL), panicle number (PN), brown grain weight per plant (BGW), thousand brown grain weight (TBGW), brown grain length (BGL), brown grain width (BGW), apparent amylose content (AAC), and protein content (PC). Plants were grown in the experimental field of Kamikawa Agricultural Experiment Station (KAES, Pippu, 43°51’N, 142°48’E). Seeds were sown in a greenhouse at Obihiro University of Agriculture and Veterinary Medicine on 25 April 2018. Seedlings aged 36 days were transplanted into the paddy fields of KAES on 31 May 2018. All seedlings were transplanted at densities of one plant per hill and a spacing of 30 × 15 cm (22.2 plants/m²). Forty plants of each triplicate parental line and of each CSSL were grown. Plants were fertilized with 8 kg N/10 a, 9.7 kg P₂O₅/10 a and 6.9 kg K₂O/10 a. Traits were determined as described (Kinoshita *et al.* 2017).

Detection of QTLs

QTL analysis was performed on CSSLs that showed significantly different traits compared with the recurrent parent Kirara397, based on Dunnett’s multiple comparison tests at 95% confidence interval (*p* < 0.05). QTLs were subsequently assigned to the chromosome regions of these CSSLs. A QTL detected in only one CSSL was regarded as located on non-overlapping chromosome segments, whereas QTLs detected in multiple CSSLs were regarded as located on overlapping chromosome segments.
Results

Development of CSSLs

The CSSL development procedure is summarized in Fig. 1 and Supplemental Text 1. Based on the physical locations and genotypes of the 99 molecular markers, including 92 InDel and 7 SNP markers, in the 26 Yukihikari-Kirara397 CSSLs (YKCSS), a physical map of each CSSL was constructed (Fig. 2).

Putative QTLs controlling agronomic traits in the CSSLs

The agronomic and grain traits of the CSSLs under experimental field conditions are presented in Table 1. Thirty-five significant changes in the evaluated traits were identified in the CSSLs, and 28 QTLs were found to be located on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12 (Table 2, Fig. 3).

Days to heading

The DTH values were 96.7 days for Kirara397 and an average 97.7 days for Yukihikari. The DTH of three CSSLs differed significantly from that of Kirara397. YK3CSSL-8.1, which has segments of Yukihikari chromosomes 2, 3, 8, 9 and 12, had the shortest DTH, 80.0 days; YK3CSSL-5.4, which has segments of Yukihikari chromosomes 3, 4, 5 and 12, had a DTH of 81.3 days; and YKCSSL-6.1, which has segments of Yukihikari chromosomes 4 and 6, had a DTH approximately 20 days longer than Kirara397. Because the DTH of YKCSSL-6.1 was too long for seed maturation, this CSSL was eliminated from further trait evaluation.

Culm length and panicle length

CL, which was 64.3 cm in Kirara397 plants, was 22.6% shorter in YK3CSSL-5.4 and 13.7% shorter in YK3CSSL-8.1. PL was 16.0 cm in Kirara397 and 9.4% longer in Yukihikari. In addition, four CSSLs had significantly different PLs than Kirara397. YK3CSSL-4.1, which has segments of Yukihikari chromosomes 2 and 4, had PLs 14.4% longer than Kirara397; and YK3CSSL-12.3, which has segments of Yukihikari chromosomes 2, 3, 4 and 12, had 10.6% longer PLs than Kirara397. In contrast, PLs of YK3CSSL-8.1 and YK3CSSL-5.4 were 16.3% and 10.0% shorter,

![Fig. 2. Graphical representation of the genotypes of the 26 CSSLs. White bars and gray bars indicate homozygous chromosomal segments derived from Kirara397 and Yukihikari, respectively. The InDel and SNP (CAPS and dCAPS) markers used for MAS are indicated by their physical positions on each chromosome.](image-url)
respective, than those of Kirara397 plants. After removing the extremely early heading genotypes YK3CSSLs-5.4 and -8.1, we found that YK3CSSL-3.1, which has segments of Yukihikari chromosomes 2, 3 and 9, showed a 11.9% increase in PL.

**Panicle number**

Kirara397 plants had a PN of 21.8, but this number was 32.6% greater in YK3CSSL-8.1 plants. After removing extremely early heading genotypes YK3CSSLs-5.4 and -8.1, we found that YK3CSSL-4.2, which has segment of Yukihikari chromosome 4, showed a 28.9% reduction in PN.

**Brown grain weight per plant and thousand brown grain weight**

Kirara397 had a BGW of 27.96 g/plant and a TBGW of 24.41 g/plant. Neither of these values differed significantly in any of the CSSLs.

**Brown grain length**

BGL was 5.27 mm in Kirara397, but was 4.7% shorter in Yukihikari. Two and three of the CSSLs had significantly longer and shorter average BGLs, respectively, than Kirara397. YK3CSSL-9.2, which harbors Yukihikari chromosome 9, had the longest BGL, 5.38 mm, 2.1% longer than Kirara397. BGL was also 1.9% longer in YK3CSSL-8.1 than in Kirara397. YK3CSSL-8.2, which harbors segments of Yukihikari chromosomes 1 and 8, had the shortest BGL, 5.3% shorter than Kirara397. Compared with Kirara397, BGL was 2.1% shorter in YK3CSSL-10.2, which has segments of Yukihikari chromosomes 1, 7 and 10, and 1.9% shorter in YK3CSSL-11.1, which has segments of Yukihikari chromosomes 9 and 11.

**Brown grain width**

The BGWI of Kirara397 was 2.92 mm, similar to that of Yukihikari. Four CSSLs had had significantly greater and three had significantly smaller average BGWIs than

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### Table 1. Average phenotypic values of parents and comparisons of each CSSL with Kirara397

| Genotype     | DTH     | CL (cm) | PL (cm) | PN      | BGW (g/plant) | TBGW (g) | BGL (mm) | BGWI (mm) | AAC (%) | PC (%) |
|--------------|---------|---------|---------|---------|---------------|----------|----------|-----------|---------|--------|
| Kirara397    | 96.7    | 64.3    | 16.0    | 21.8    | 27.96         | 24.41    | 5.27     | 2.92      | 22.7    | 6.5    |
| Yukihikari   | 97.7    | 64.2    | 15.9    | 21.3    | 27.09         | 22.99    | 5.20     | 2.83      | 22.4    | 6.0**  |
| YK3CSSL-1    | 98.7    | 63.4    | 15.7    | 23.2    | 27.12         | 23.85    | 5.22     | 2.91      | 22.7    | 6.6    |
| YK3CSSL-3    | 96.6    | 64.4    | 18.3*** | 15.9    | 29.04         | 25.06    | 5.35     | 2.93      | 22.5    | 6.1    |
| YK3CSSL-4    | 97.7    | 65.1    | 16.8    | 15.5**  | 27.53         | 24.05    | 5.20     | 2.91      | 22.3    | 6.3    |
| YK3CSSL-5.1  | 97.7    | 62.3    | 16.5    | 27.76   | 24.61         | 5.20     | 2.98**   | 2.03***   | 22.6    | 6.4    |
| YK3CSSL-5.2  | 97.7    | 62.8    | 16.7    | 28.07   | 24.60         | 5.27     | 2.94      | 2.02      | 22.5    | 6.2    |
| YK3CSSL-5.3  | 96.7    | 59.6    | 16.5    | 18.8    | 29.06         | 24.68    | 5.32     | 2.96      | 2.03    | 22.3    |
| YK3CSSL-5.4  | 121.3***| 49.8*** | 14.4*   | 27.2    | 27.82         | 24.61    | 5.21     | 2.96      | 2.06    | 21.9***|
| YK3CSSL-6.1  | 120.3***| nd      | nd      | nd      | nd            | nd       | nd       | nd        | nd      | nd     |
| YK3CSSL-6.2  | 95.0    | 62.7    | 16.3    | 23.2    | 30.17         | 23.94    | 5.26     | 2.90      | 2.00    | 21.9**  |
| YK3CSSL-7.1  | 97.3    | 64.4    | 16.3    | 21.1    | 27.52         | 23.30    | 5.25     | 2.91      | 2.02    | 22.7    |
| YK3CSSL-7.2  | 97.7    | 65.3    | 16.8    | 19.4    | 28.18         | 24.47    | 5.27     | 2.93      | 2.02    | 22.6    |
| YK3CSSL-8.1  | 80.0*** | 55.5**  | 13.4*** | 28.9*   | 24.55         | 24.83    | 5.37*    | 2.88      | 2.04    | 22.7    |
| YK3CSSL-8.2  | 96.3    | 59.1    | 16.0    | 22.2    | 27.44         | 23.05    | 4.99***  | 1.99***   | 22.3    | 6.4    |
| YK3CSSL-8.3  | 97.0    | 62.8    | 16.2    | 21.1    | 28.95         | 24.37    | 5.27     | 2.92      | 2.03    | 22.8    |
| YK3CSSL-9.1  | 96.3    | 62.3    | 16.6    | 22.5    | 27.59         | 24.53    | 5.25     | 2.96      | 2.04    | 22.9    |
| YK3CSSL-9.2  | 98.0    | 65.8    | 16.3    | 17.9    | 28.82         | 25.05    | 5.38**   | 2.97*     | 2.03*   | 23.2    |
| YK3CSSL-10.1 | 96.7    | 62.5    | 16.2    | 20.3    | 23.79         | 24.65    | 5.27     | 2.94      | 2.04    | 22.7    |
| YK3CSSL-10.2 | 96.0    | 61.6    | 15.7    | 23.4    | 27.51         | 23.81    | 5.16**   | 2.93      | 2.03    | 22.3    |
| YK3CSSL-11.1 | 96.7    | 61.4    | 16.3    | 20.1    | 26.20         | 24.52    | 5.17*    | 2.98**    | 2.04**  | 22.5    |
| YK3CSSL-11.2 | 97.7    | 66.2    | 16.9    | 20.1    | 27.99         | 23.92    | 5.34     | 2.87*     | 1.99*   | 22.4    |
| YK3CSSL-12.1 | 97.0    | 65.9    | 16.9    | 21.9    | 28.61         | 23.90    | 5.28     | 2.92      | 2.00    | 22.6    |
| YK3CSSL-12.2 | 97.7    | 66.4    | 17.1    | 20.2    | 27.27         | 23.70    | 5.22     | 2.90      | 2.02    | 22.4    |
| YK3CSSL-12.3 | 97.7    | 69.6    | 17.7*   | 19.2    | 28.98         | 24.88    | 5.36     | 2.93      | 2.03    | 22.7    |

Dunnett’s multiple comparison test was conducted for each trait to compare Kirara97 with each CSSL, and “*”, “**”, and “***” represented significant at \( p < 0.05 \), \( p < 0.01 \) and \( p < 0.001 \), respectively.

PL and PN of Kirara97 was compared with that of each CSSL, except for YK3CSSLs-5.4 and -8.1, using Dunnett’s multiple comparison tests.

\( \ast p < 0.05 \), \( \ast\ast p < 0.01 \),

“nd” represents no data.

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Table 2. QTLs for grain yield and grain quality related traits

| Trait | QTL       | Chr | Position (bp) | Representative CSSL | Positive allele | Related loci (references) |
|-------|-----------|-----|---------------|---------------------|-----------------|--------------------------|
|       |           |     | start-end     | YK3CSSL             |                 |                          |
| DTH   | qDTH5     | 5   | 20,382,329    | YK3CSSL-5.4        | Yukihikari      |                          |
|       | qDTH6     | 6   |               | YK3CSSL-6.1        | Yukihikari      |                          |
|       | qDTH8     | 8   | 17,027,670    | YK3CSSL-8.1        | Kirara397      |                          |
|       |           |     |               |                     | (Hori et al. 2015) |                          |
| CL    | qCL5      | 5   | 30,073,438    | YK3CSSL-5.4        | Yukihikari      |                          |
|       | qCL8      | 8   | 17,027,670    | YK3CSSL-8.1        | Kirara397      |                          |
|       |           |     |               |                     |                 | Hg/Drh8/DTH8 (Lin et al. 2003, Yan et al. 2011, Wei et al. 2010) |
| PL    | qPL3      | 3   | 14,660,164    | YK3CSSL-3.1        | Yukihikari      | qPL3-1, qPL3-2, qPL3-3 (Marathi et al. 2012) |
|       | qPL4      | 4   | 19,680,181    | YK3CSSL-4.1        | Yukihikari      |                          |
|       | qPL5      | 5   | 30,073,438    | YK3CSSL-5.4        | Yukihikari      |                          |
|       | qPL8      | 8   | 17,027,670    | YK3CSSL-8.1        | Kirara397      |                          |
|       |           |     |               |                     |                 | Hg/Drh8/DTH8 (Lin et al. 2003, Yan et al. 2011, Wei et al. 2010) |
| PN    | qPN4      | 2   | 36,060,856    | YK3CSSL-4.2        | Yukihikari      | D17 (Umehara et al. 2008) |
|       | qPN8      | 8   | 17,027,670    | YK3CSSL-8.1        | Kirara397      |                          |
|       |           |     |               |                     |                 | Hg/Drh8/DTH8 (Lin et al. 2003, Yan et al. 2011, Wei et al. 2010) |
| BGL   | qBGL9     | 9   | 15,334,065    | YK3CSSL-8.1        | Yukihikari      | qGL9-1, qGL9-2 (Nagata et al. 2015) |
|       | qBGL10    | 10  | 23,703,438    | YK3CSSL-10.2       | Kirara397      |                          |
| BGW1  | qBGW11    | 1   | 7,058,375     | YK3CSSL-1          | Kirara397      | qGW1-3, qGW1-4 (Nagata et al. 2015) |
|       |           |     | 9,871,380     | YK3CSSL-9.2        |                 |                          |
|       |           |     |               |                     |                 |                          |
|       |           |     |               |                     | Kirara397      |                          |
|       |           |     |               |                     |                 |                          |
|       |           |     |               |                     | Kirara397      |                          |
|       |           |     |               |                     |                 |                          |
| BGT   | qBGT2     | 2   | 23,133,290    | YK3CSSL-2          | Yukihikari      | qGW2-1, qGW2-2, qGW2-3 (Nagata et al. 2015), qGWh2 (Okada et al. 2018) |
|       |           |     |               |                     |                 |                          |
|       | qBGT3     | 5   | 5,805,036     | YK3CSSL-5.1        | Yukihikari      | qGW5-1 (Nagata et al. 2015) |
|       | qBGT9     | 9   | 23,895,721    | YK3CSSL-9.2        | Yukihikari      | qGW9 (Zhang et al. 2020) |
|       | qBGT11    | 11  | 31,219,694    | YK3CSSL-11.2       | Kirara397      | qGW11-2 (Nagata et al. 2015) |
| AAC   | qAAC5     | 5   | 30,073,438    | YK3CSSL-5.4        | Kirara397      | qAC5 (Wang et al. 2017) |
|       | qAAC6     | 6   | 21,217,349    | YK3CSSL-6.2        | Yukihikari      |                          |
|       |           |     | 21,040,044    | YK3CSSL-12.2       | Kirara397      |                          |
| PC    | qPC1      | 1   | 7,058,375     | YK3CSSL-1          | Kirara397      | qPC1 (Kinoshita et al. 2017) |
|       |           |     | 9,871,380     | YK3CSSL-9.2        |                 |                          |
|       | qPC5      | 5   | 30,073,438    | YK3CSSL-5.4        | Yukihikari      |                          |
|       | qPC8      | 8   | 17,027,670    | YK3CSSL-8.1        | Kirara397      | qPC8 (Kinoshita et al. 2017) |
|       | qPC12     | 12  | 10,194,933    | YK3CSSL-12.1       | Kirara397      |                          |
|       |           |     | 5,752,634     | YK3CSSL-12.2       |                 |                          |

a The Rice Annotation Project (RAP) database (build5).

Kirara397. BGWI was 2.4% greater in YK3CSSL-2, which has segments of Yukihikari chromosomes 2, 11 and 12, than in Kirara397. Two CSSLs, YK3CSSL-5.1, which has segments of Yukihikari chromosomes 3, 4 and 5, and YK3CSSL-11.1, had 2.1% greater BGWI, and YK3CSSL-9.2 had 1.7% greater BGWI than Kirara397. YK3CSSL-1, which has Yukihikari chromosome 1, had the smallest BGWI, 3.1% less than that of Kirara397. Relative to Kirara397, BGWIs of YK3CSSL-8.2 and YK3CSSL-11.2 were smaller, by 2.4% and 1.7%, respectively.
Brown grain thickness

BGT of Kirara397 was 2.02 mm, similar to the 2.00 mm BGT of Yukihikari. Four CSSLs had significantly thicker and two had significantly thinner BGTs than Kirara397. YK3CSSL-2 and YK3CSSL-11.1 had the thickest BGTs, 0.99% greater than that of Kirara397, whereas YK3CSSL-5.1 and YK3CSSL-9.2 had BGTs 0.5% greater than that of Kirara397. In contrast, BGT was 1.5% lower in YK3CSSL-8.2 and YK3CSSL-11.2 than in Kirara397.

Apparent amylose content

The AAC of Yukihikari was 23.4%, significantly higher than that of Kirara397, which was 22.7%. The AACs of YK3CSSL-5.4 and YK3CSSL-6.2 were 21.9%, significantly lower than that of Kirara397.

Protein content

The PC of Kirara397 was 6.5%, similar to that of Yukihikari. Two CSSLs had significantly higher and three had significantly lower PC than Kirara397. YK3CSSL-5.4 and YK3CSSL-8.1 had the highest PCs, averaging 7.4%. YK3CSSL-1 had the lowest PC, 6.0%. YK3CSSL-12.1 and YK3CSSL-12.2 had PCs of 6.1% and 6.3%, respectively.

Discussion

The present study identified two types of heading characteristics on a genetic background of Kirara397: the extremely early genotypes YK3CSSL-5.4 and YK3CSSL-8.1 and the extremely late genotype YK3CSSL-6.1. qDTH5 and qDTH8 for early heading overlapped with other QTLs (Hori et al. 2015, Lin et al. 2003, Wei et al. 2010, Yan et al. 2011), whereas qDTH6 overlapped with Hd1, Hd17, RFT1 and Hd3a (Kojima et al. 2002, Matsubara et al. 2012, Ogiso-Tanaka et al. 2013, Yano et al. 2000). Because Hd1 was shown to be a key determinant for adaptability of rice cultivars to growth in Hokkaido (Fujino et al. 2019a), it is necessary to determine whether Hd1 and/or other(s) contribute to the extremely late heading associated with qDTH6.

Kirara397 has shorter panicles than those in Yukihikari. PL strongly affects grain yield by altering several panicle-related traits, including the numbers of primary and secondary branches per panicle and the grain density per panicle (Jang et al. 2018, Peng et al. 2014). Alternatively, panicle architecture may be associated with grain filling nature properties, contributing to their high quality (Fujino et al. 2019b). The present study identified four QTLs for PL, one of which, qPL4, was novel, with PL being longer in Yukihikari. qPL3 was found to be positioned close to qPL3-1, qPL3-2 and qPL3-3 (Marathi et al. 2012), with PL again being longer in Yukihikari. Alternatively, early heading genotypes at qDTH5 and qDTH8 (Hd5) from Yukihikari reduced PL. PN is a component that directly influences rice yield, with the present study identifying qPN4, which was close to D17 (Umehara et al. 2008), and qPN8. The early heading genotypes at qDTH8 (Hd5) from Yukihikari increased PN. Additional studies are needed to clarify the genotype that provides desirable panicle architecture and number for grain yield and grain quality, together with suitable earliness.

Grain shape directly influences grain yield and quality.
Kirara397 had longer grains than Yukihikari, with the two having similar BGWI and BGT. Comparisons of Kirara397 and each CSSL showed 16 differences, five for BGL, six for BGWI and five for BGT, indicating that grain shape in these closely related two cultivars is subject to complex genetic regulation. Eleven QTLs for grain appearance quality traits were detected. Yukihikari harbored positive effects at qBGL9, qBGWI2, qBGWI5, qBGWI9, qBGT2, qBGT5 and qBGT9, whereas Kirara397 harbored positive effect at qBGL10, qBGWI11, qBGWI11, and qBGT11. Of the 11 QTLs, eight overlapped with those previously reported (Nagata et al. 2015, Okada et al. 2018, Zhang et al. 2020), whereas three, qBGT2, qBGT5, and qBGT11, were novel. Further studies are needed to clarify the dosage-pyramiding effects of the favorable alleles derived from each cultivar on grain yield and quality. YK3CSSL-8.2 had the shortest BGL, along with smaller BGWI and BGT compared with Kirara397. However, we could not assign a putative QTL to each trait by comparisons with the graphical genotypes of the CSSL library. This line carries segments of Yukihikari chromosomes 1 and 8. Two possibilities were hypothesized. First, an uncovered chromosomal region might contribute to the genetic effect detected in YK3CSSL-8.2. Second, the genetic interaction among QTLs on chromosomes 1 and/or 8 might reduce grain appearance quality. To differentiate between these possibilities, we are planning QTL mapping of the progeny of a cross between YK3CSSL-8.2 and Kirara397 combined with additional DNA markers.

The eating quality of Yukihikari might be achieved by pyramiding spontaneous mutation(s) and/or pre-existing gene(s) in the Hokkaido gene pool (Fujino et al. 2019b, Shinada et al. 2014). The present study identified two QTLs for AAC, qAAC5 and qAAC6, with the Yukihikari allele associated with reduced AAC. The QTL qAAC5 was adjacent to the previously reported QTL qAC5 (Wang et al. 2017), whereas qAAC6 was novel. The AAC of Kirara397 was significantly lower than that of Yukihikari, perhaps due to as yet undetected QTL(s) for AAC. This undetected QTL may be located on segment of chromosome 6.

The present CSSL populations could be used for fine mapping and cloning the present novel QTL and for clarifying the pyramiding effect of these QTLs.

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