

Article

Mapping of QTLs for Brown Rice Traits Based on Chromosome Segment Substitution Line in Rice (*Oryza sativa* L.)

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Abstract: Brown rice traits are critical to both grain yield and quality. In the present study, the chromosome segment substitution lines (CSSLs) population derived from a cross between *japonica* Koshihikari and *indica* Nona Bokra was used to analyze the brown rice length (BRL), brown rice width (BRW), length–width ratio of brown rice (BLWR), brown rice thickness (BRT), brown rice perimeter (BRP), brown rice area (BRA), thousand-grain weight of brown rice (BRGW), brown rice ratio (BRR), taste value of brown rice (BTV), and water content of brown rice (BWC). Correlations analysis showed that most of the brown rice traits had significant correlations with each other, except for BRR, BTV, and BWC. A total of sixty-one QTLs for these traits were identified under three environments, which mapped to chromosomes 1, 2, 3, 5, 6, 7, 8, 10, 11, and 12, with the LOD ranging from 2.52 to 16.68 and accounting for 2.60 to 25.38% of the total phenotypic variations. Moreover, thirty pairs of epistatic interactions for BRL, BRW, BLWR, BRP, and BRA were estimated and distributed on all chromosomes except 10. These findings will provide a further understanding of the genetic basis of brown rice traits and facilitate the genetic improvement of rice yield and quality through breeding.

Keywords: rice (*Oryza sativa* L.); brown rice traits; QTLs; CSSLs



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1. Introduction

Rice is one of the most important staple foods for more than a half of the world's population. To meet the increasing demand of this consumption, enhancing grain yield and improving grain quality have become the two main goals of rice breeders. Rice grain consists of brown rice and a hull that are reciprocally interlocked by the lemma and palea [1]. Brown rice is composed of bran, an embryo, and endosperm. The bran and embryo are the two outer layers of the brown rice, which supply most of the vitamins and minerals in the grain, and endosperm forms the interior layer that mainly provides carbohydrates. Although rice hulls play an important role in grain yield, the brown rice is directly related to grain yield and quality. Therefore, paying more attention to the genetic basis of brown rice traits is useful for the improvement of varieties.

Brown rice traits are complex and quantitative. These mainly include nutritional components, milling traits, physical appearance, cooking and taste properties, and so on. The nutritional component is one of the most important traits of brown rice. Brown rice can provide essential amino acids, fibers, minerals, flavonoids, and other valuable phytochemicals [2]. To date, hundreds of QTLs controlling the nutritional component of brown rice have been reported on 12 chromosomes of rice. For example, Tan et al. [3] identified two protein QTLs using recombinant inbred lines (RILs) derived from Zhenshan 97 and

Minghui 63. Using RILs and backcross inbred lines (BILs), Dong et al. [4] found two QTLs and one QTL for folate content, respectively. Hu et al. [5] identified 17 QTLs for mineral contents in brown rice using a Xieqingzao B and Dongxiang wild rice (*Oryza rufipogon* Griff.) BC₂F_{4.5} population. Some of the QTLs involved in the nutritional component of brown rice have been cloned. Peng et al. [6] cloned a grain protein content (GPC), QTL *qPC1*, in rice. *qPC1* encodes a putative amino acid transporter, OsAAP6, which functions as a positive regulator of GPC in rice. *qGPC10* encodes a glutelin type-A2 precursor, which positively regulates GPC content and has a pleiotropic effect on rice grain quality [7]. Zhou et al. [8] performed a GWAS of oil-related traits and confirmed four genes (*PAL6*, *LIN6*, *MYR2*, and *ARA6*) involved in oil metabolism. Milling traits are another important component of brown rice. These mainly include brown rice recovery (BBR), milled rice recovery (MRR), and head milled rice recovery (HMR). In the past few decades, a number of QTL mapping studies for milling traits have been conducted using different bi-parental populations [9–12] or genome-wide association (GWA) mapping approaches [13]. However, few of these QTLs have been cloned. Using a DH population of the rice varieties Chunjiang 06 and Taichung Native 1, Ren et al. [12] found four QTLs for BRR located on chromosomes 1, 8, 9, and 10, respectively. They further mapped *qBBR10* to a 39.5 kb region on chromosome 10, and two candidate genes, *LOC_Os10g32124* and *LOC_Os10g32190*, showed significantly differential expression in TN1 and CSSL1-2 compared with CJ06. Apart from nutritional components and milling traits, there is less that is known about the genetic mechanisms of physical appearance and cooking and taste properties of brown rice, with only a few QTLs known to be associated with these traits. For instance, Malik et al. [14] identified three QTLs for rough rice length, two QTLs for rough rice breadth, and three QTLs for a rough rice length-to-breadth ratio.

Chromosome segment substitution lines (CSSLs) are chromosome segments that are introduced into the donor parent in the recipient parent that eliminate the interference of genetic backgrounds and improve the accuracy of QTL mapping. Based on a CSSLs population, a large number of QTLs have been identified for agronomic traits. For example, Li et al. [15] identified one QTL for BRR using a CSSL population constructed between an *indica* restorer line, Xihui 18, and a *japonica* donor parent, Huhan 3. Furuta et al. [16] identified 15 major effective QTLs for six traits using an *O. rufipogon*, W0106, and a *japonica* cultivar, Koshihikari. Bian et al. [17] identified 41 QTLs for four yield traits under two environments using a CSSL population derived from an elite *japonica* cultivar, C418, and an elite *indica* cultivar, 9311. Further, it was confirmed that the QTL with the largest effect, *qTGW7*, is co-segregated with RM22034 on the short arm of chromosome 7. In the current study, a CSSL population derived from the *japonica* cultivar Koshihikari and *indica* cultivar Nona Bokra was used to analyze BRL, BRW, BLWR, BRT, BRP, BRA, BRGW, BRR, BTV, and BWC under three environments. The main effect QTL and epistatic QTL for ten brown rice traits were estimated. The aim of this study was to explore and utilize important QTLs related to brown rice traits for improving grain yield and quality in rice breeding.

2. Materials and Methods

2.1. Plant Materials

A CSSL population comprised of 154 lines, which was established in our laboratory, was used in this study [18]. This population was developed by a cross of the *japonica* Koshihikari and the *indica* Nona Bokra. Uniform seeds of all CSSLs and their parents were soaked in distilled water in the dark at 30 °C for 2 days and then germinated in distilled water at 35 °C for 1 day. The germinated seeds were sown in a paddy field in the experimental farm of Yangzhou University in Lingshui and Yangzhou in 2020, 2021, and 2022, respectively. Approximately 30 days after sowing, the plants were transplanted to the field. Trials were laid out in a randomized complete block design (RCBD). The parents and each CSSL were planted in four rows, with each row containing six plants. The plant density was 18 cm × 18 cm. Field management was performed as described by Wang et al. [19]. The field plots were supplemented with about 150 kg N ha⁻¹ urea in a

ratio of 5:3:2 at the basal, tillering, and earing stages, respectively, with 130 kg K₂O ha⁻¹ in a ratio of 1:1 at the basal and earing stages, respectively, and 60 kg P₂O₅ ha⁻¹ at the basal stage. After harvesting, the grains of each line with three replicates were sampled for the evaluation of brown rice traits.

2.2. Evaluation of Brown Rice Traits

The freshly harvested seeds were stored at room temperature for one month before testing. Ten brown rice traits, including brown rice length (BRL), brown rice width (BRW), length–width ratio of brown rice (BLWR), brown rice thickness (BRT), brown rice perimeter (BRP), brown rice area (BRA), thousand-grain weight of brown rice (BRGW), brown rice ratio (BRR), taste value of brown rice (BTV), and water content of brown rice (BWC), were measured in this study. The BRL, BRW, BRP, BRA, and BRGW traits were analyzed using the SC-G automatic seed testing system (Hangzhou WSeen Detection Technology Co., Ltd., Hangzhou, China) following the manufacturer’s instructions. About 200–300 grains for the parents and each line were evaluated. BLWR was calculated as the ratio of BRL and BRW. BRT was measured with a vernier caliper, and 10 grains for the parents and each line were evaluated. BRR was calculated as the ratio of brown rice weight and grain weight. BWC and BTV were analyzed using a Satake Taste Analyzer (Satake, Tokyo, Japan).

2.3. Identification of QTLs

A genetic linkage map was constructed using 126 polymorphism markers on the 12 rice chromosomes [20]. QTL mapping for brown rice traits was conducted using QTL IciMapping v4.2 based on an inclusive composite interval mapping (ICIM) model [21,22]. A likelihood of odds (LOD) threshold of 2.5 was set to declare the presence of a putative QTL. The contribution of each QTL to the phenotypic variance, genetic parameters, and additive effect for relative traits was also estimated. The QTL nomenclature followed the standard of McCouch et al. [23]. The genetic map was drawn by MapChart v 2.32 [24].

2.4. Statistical Analysis

One-way ANOVA of the parents and the CSSLs population was analyzed using Excel software (Microsoft Office 2020). $p < 0.05$ was defined as a significant difference. Population distribution and correlation analysis were performed using the ggplot2 and corplot package in R, respectively.

3. Results

3.1. Phenotypic Variation of Brown Rice Traits in Parents and CSSLs

Figure 1 and Table 1 summarize the brown rice traits of the parents and CSSLs population under three environments. All brown rice traits showed significant differences between parents in Lingshui 2020. The value of BRL, BRW, BLWR, BRT, BRP, BRA, BRGW, and BWC in Nona Bokra was significantly higher than that of Koshihikari, whereas the value of BRR and BTV in Nona Bokra was significantly lower than that of Koshihikari. Compared with the brown rice traits of Koshihikari in the three environments, it was found that values of BRT, BRGW, BRR, and BTV in Lingshui were higher than those in Yangzhou, and the other six traits were lower than in Yangzhou.

The brown rice traits varied widely throughout the CSSLs population (Figure 1, Table 1). The frequency distribution of brown rice traits exhibited a continuous variation in the three test environments, and transgressive variation was also found in the CSSLs population. The absolute values of skewness and kurtosis were less than one, except for BBR and BWC in Lingshui 2020 and BWC in Yangzhou 2022, suggesting that these traits were suitable for QTL analysis (Figure 1, Table 1).

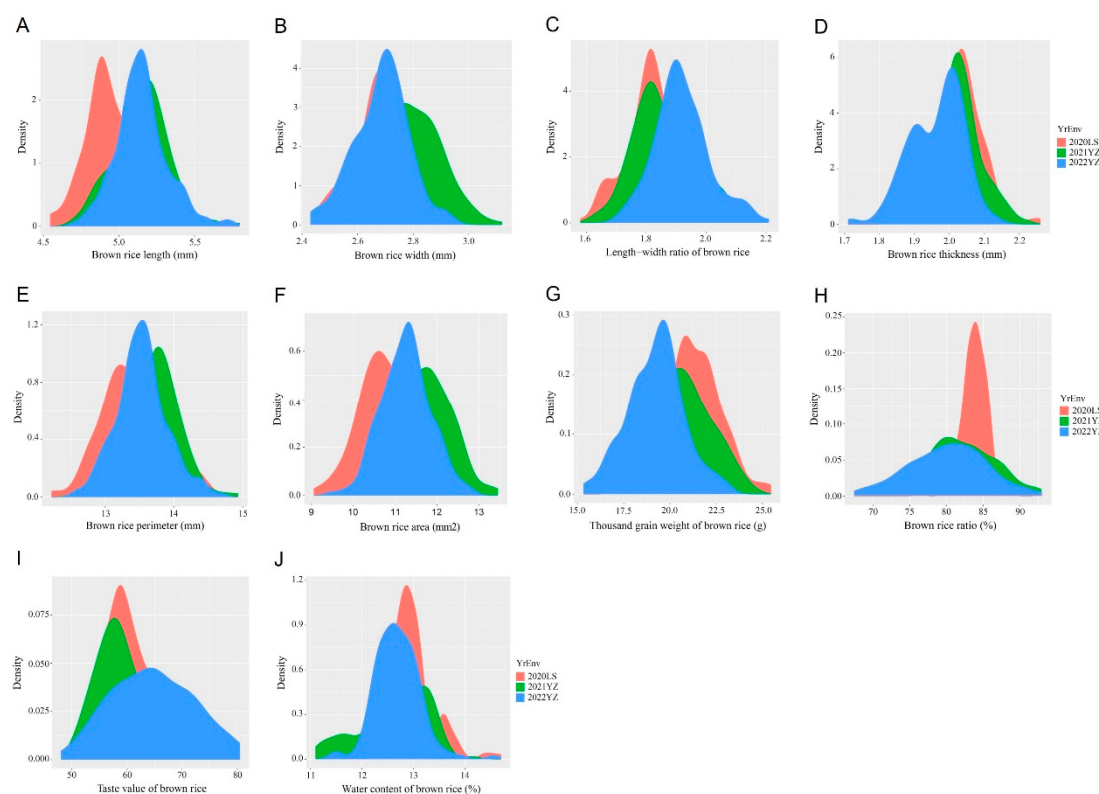


Figure 1. Frequency distributions of brown rice traits in CSSLs population under three environments. LS: Lingshui; YZ: Yangzhou. (A–J) The frequency distributions of (A) Brown rice length, (B) brown rice width, (C) length–width ratio of brown rice, (D) brown rice thickness, (E) brown rice perimeter, (F) brown rice area, (G) thousand-grain weight of brown rice, (H) brown rice ratio, (I) taste value of brown rice, (J) water content of brown rice.

Table 1. Description of brown rice traits of the parents and their CSSLs population under three environments.

Year and Location	Traits	Parents (Mean $\pm$ SD)			CSSLs Population		
		Koshihikari	Nona Bokra	Mean $\pm$ SD	Range	Skewness	Kurtosis
2020 Lingshui	BRL (mm)	4.95 $\pm$ 0.31	6.14 $\pm$ 0.29 **	4.95 $\pm$ 0.18	4.54~5.50	0.59	0.67
	BRW (mm)	2.76 $\pm$ 0.20	2.86 $\pm$ 0.18 *	2.72 $\pm$ 0.13	2.46~3.12	0.42	−0.02
	BLWR	1.80 $\pm$ 0.05	2.15 $\pm$ 0.07 **	1.83 $\pm$ 0.10	1.58~2.17	0.47	1.00
	BRT	2.05 $\pm$ 0.02	2.09 $\pm$ 0.03 **	2.02 $\pm$ 0.07	1.86~2.26	0.14	0.51
	BRP (mm)	13.41 $\pm$ 0.71	16.11 $\pm$ 0.81 **	13.37 $\pm$ 0.44	12.22~14.53	0.31	0.16
	BRA (mm ²)	10.87 $\pm$ 1.03	14.36 $\pm$ 1.22 **	10.80 $\pm$ 0.70	9.05~13.11	0.41	0.42
	BRGW(g)	21.61 $\pm$ 0.39	25.98 $\pm$ 0.08 **	21.20 $\pm$ 1.55	17.46~25.46	0.06	0.06
	BRR (%)	83.60 $\pm$ 0.50	81.99 $\pm$ 0.56 *	83.94 $\pm$ 1.81	76.42~93.02	0.41	5.15
	BTB	57.67 $\pm$ 1.37	52.67 $\pm$ 1.53 **	59.75 $\pm$ 4.79	50.00~77.00	0.55	0.54
2021 Yangzhou	BWC (%)	12.34 $\pm$ 0.86	13.48 $\pm$ 0.39 *	12.91 $\pm$ 0.50	11.40~14.70	0.32	2.23
	BRL (mm)	5.16 $\pm$ 0.20	—	5.15 $\pm$ 0.18	4.71~5.80	0.18	0.75
	BRW (mm)	2.80 $\pm$ 0.20	—	2.77 $\pm$ 0.12	2.46~3.10	−0.13	−0.25
	BLWR	1.84 $\pm$ 0.01	—	1.86 $\pm$ 0.11	1.64~2.17	0.50	−0.13
	BRT	1.94 $\pm$ 0.02	—	2.01 $\pm$ 0.08	1.75~2.22	−0.30	0.49
	BRP (mm)	13.67 $\pm$ 0.61	—	13.65 $\pm$ 0.41	12.60~14.93	−0.04	0.15
	BRA (mm ²)	11.70 $\pm$ 1.10	—	11.63 $\pm$ 0.68	9.78~13.45	−0.04	−0.02
	BRGW (g)	19.58 $\pm$ 0.75	—	20.31 $\pm$ 1.78	15.97~24.33	−0.08	−0.43
	BRR	76.96 $\pm$ 3.45	—	81.84 $\pm$ 4.82	68.40~92.70	−0.04	−0.20
	BTB	51.67 $\pm$ 2.08	—	61.07 $\pm$ 6.37	51.00~77.50	0.71	−0.33
	BWC (%)	13.13 $\pm$ 0.12	—	12.62 $\pm$ 0.59	11.10~14.15	−0.50	0.18

Table 1. Cont.

Year and Location	Traits	Parents (Mean $\pm$ SD)		CSSLs Population			
		Koshihikari	Nona Bokra	Mean $\pm$ SD	Range	Skewness	Kurtosis
2022 Yangzhou	BRL (mm)	5.21 $\pm$ 0.29	—	5.15 $\pm$ 0.17	4.71~5.74	0.51	1.02
	BRW (mm)	2.79 $\pm$ 0.23	—	2.68 $\pm$ 0.10	2.43~2.93	−0.20	0.15
	BLWR	1.87 $\pm$ 0.01	—	1.92 $\pm$ 0.09	1.71~2.21	0.51	0.51
	BRT	1.92 $\pm$ 0.02	—	1.96 $\pm$ 0.08	1.71~2.14	−0.52	0.08
	BRP (mm)	13.70 $\pm$ 0.71	—	13.53 $\pm$ 0.37	12.57~14.68	0.25	0.45
	BRA (mm ²)	11.36 $\pm$ 1.42	—	11.24 $\pm$ 0.59	9.48~12.76	0.00	0.12
	BRGW (g)	19.22 $\pm$ 0.39	—	19.23 $\pm$ 1.52	15.33~23.08	−0.03	0.08
	BRR	74.57 $\pm$ 0.50	—	80.17 $\pm$ 5.18	67.43~92.48	−0.12	−0.26
	BTV	53.00 $\pm$ 2.00	—	64.61 $\pm$ 7.40	48.00~80.50	0.09	−0.66
	BWC (%)	13.03 $\pm$ 0.15	—	12.64 $\pm$ 0.59	11.50~14.55	0.63	2.75

BRL: Brown rice length; BRW: brown rice width; BLWR: length–width ratio of brown rice; BRT: brown rice thickness; BRP: brown rice perimeter; BRA: brown rice area; BRGW: thousand-grain weight of brown rice; BRR: brown rice ratio; BTV: taste value of brown rice; BWC: water content of brown rice. * and ** show significances at the level of 5% and 1%, respectively.

3.2. Correlation Analysis of Brown Rice Traits

A correlation analysis of ten brown rice traits under the three environments is presented in Figure 2. Most of the brown rice traits were significantly correlated with each other, except for BRR, BTV, and BWC. BRP showed the highest significant positive correlation with BRA, and the correlation coefficients were 0.91 in Lingshui 2020, 0.88 in Yangzhou 2021, and 0.88 in Yangzhou 2022, respectively, indicating that BRP was the most important contributor to BRA. Similarly, BRW showed the highest significant negative correlation with BLWR. The correlation coefficients were −0.84 in Lingshui 2020, −0.86 in Yangzhou 2021, and −0.81 in Yangzhou 2022, respectively. The correlations in brown rice between BRW and BLWR were the same as the correlations between grain width and length–width ratio [20].

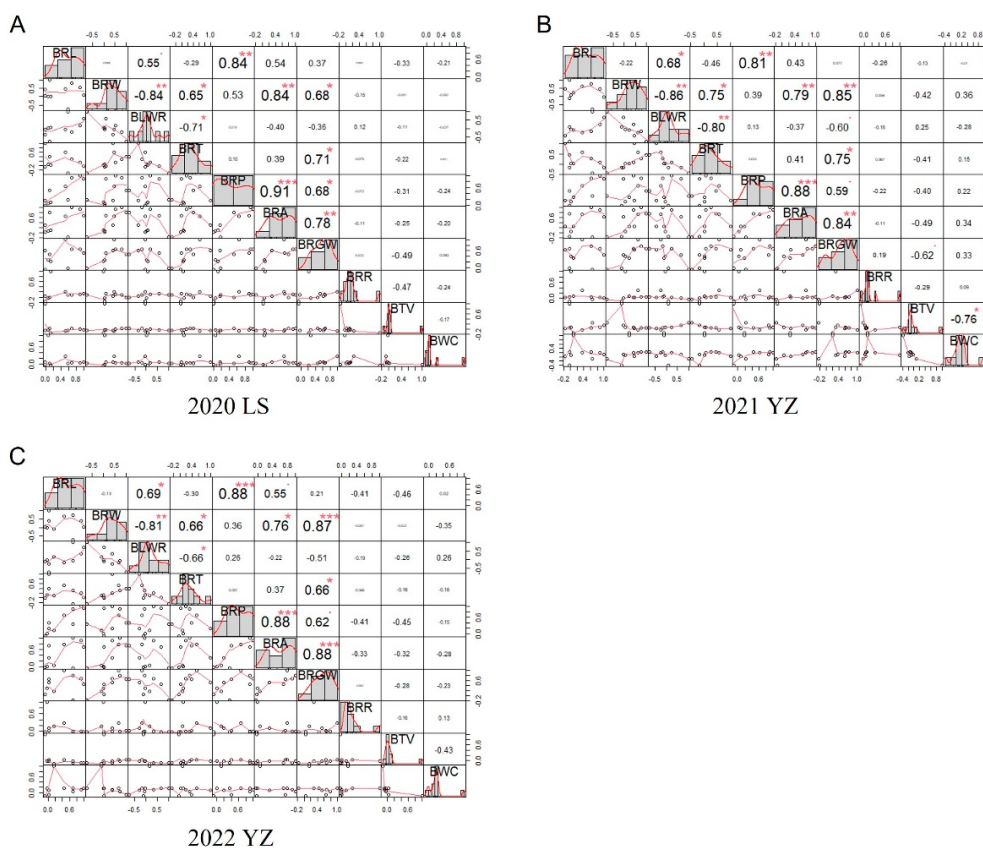


Figure 2. Correlation coefficients of brown rice traits in CSSLs populations under three environments. LS: Lingshui; YZ: Yangzhou; Correlation coefficients of brown rice traits in 2020 Lingshui (A), 2021

Yangzhou (B), and 2022 Yangzhou (C). BRL: brown rice length; BRW: brown rice width; BLWR: length–width ratio of brown rice; BRT: brown rice thickness; BRP: brown rice perimeter; BRA: brown rice area; BRGW: thousand-grain weight of brown rice; BRR: brown rice ratio; BTV: taste value of brown rice; BWC: water content of brown rice. *, **, and *** show significances at the level of 5%, 1%, and 0.1%, respectively.

3.3. QTL Analysis for Brown Rice Traits

A total of sixty-one QTLs for brown rice traits were detected in the CSSLs population under the three environments (Table 2). These QTLs were distributed on chromosomes 1, 2, 3, 5, 6, 7, 8, 10, 11, and 12 (Figure 3). The LOD values of the main effect QTLs spanned from 2.52 to 16.68, and the phenotypic variation ranged from 2.60 to 25.38% (Table 2).

Table 2. Quantitative trait loci for brown rice traits in CSSLs population.

Traits	QTL	Chr.	Marker	Position (cm)	LOD			Additive Effect			PVE (%)		
					2020LS	2021YZ	2022YZ	2020LS	2021YZ	2022YZ	2020LS	2021YZ	2022YZ
BRL	<i>qBRL1-1</i>	1	STS1-12	132.60	4.56	—	—	0.16	—	—	8.41	—	—
	<i>qBRL1-2</i>	1	SSR1-104	167.85	—	—	4.99	—	—	0.10	—	—	6.49
	<i>qBRL2-1</i>	2	SSR2-24	92.20	—	—	3.46	—	—	−0.07	—	—	4.38
	<i>qBRL2-2</i>	2	SSRW2-309	96.09	—	2.73	—	—	−0.07	—	—	8.06	—
	<i>qBRL3-1</i>	3	SSR3-272	1.46	3.34	—	—	0.13	—	—	6.05	—	—
	<i>qBRL3-2</i>	3	STS3-1	113.84	5.78	—	11.58	0.10	—	0.14	10.86	—	16.67
	<i>qBRL3-3</i>	3	SSR3-337	132.32	—	—	4.31	—	—	−0.09	—	—	5.55
	<i>qBRL3-4</i>	3	SSR3-36	147.67	—	2.57	—	—	−0.17	—	—	7.59	—
	<i>qBRL5-1</i>	5	SSR5-1	14.19	—	—	3.51	—	—	0.10	—	—	4.46
	<i>qBRL5-2</i>	5	SSR5-126	76.21	3.18	—	—	0.07	—	—	5.74	—	—
	<i>qBRL8</i>	8	SSR8-390	111.97	3.74	—	4.22	0.09	—	0.09	6.81	—	5.41
BRW	<i>qBRW2</i>	2	SSR2-24	92.20	—	—	3.18	—	—	−0.04	—	—	5.89
	<i>qBRW3</i>	3	SSR3-120	41.63	—	6.93	6.90	—	−0.07	−0.06	—	19.48	13.53
	<i>qBRW7</i>	7	SSR7-145	20.13	9.81	—	—	0.09	—	—	21.93	—	—
	<i>qBRW8</i>	8	SSR8-417	90.25	4.43	3.51	—	0.06	0.06	—	9.10	9.35	—
	<i>qBRW12</i>	12	SSR12-107	66.43	—	—	6.26	—	—	−0.07	—	—	12.15
BLWR	<i>qBLWR1-1</i>	1	SSR1-442	159.81	11.61	—	—	−0.10	—	—	11.90	—	—
	<i>qBLWR1-2</i>	1	SSR1-104	167.85	16.68	3.62	3.30	0.11	0.05	0.04	18.55	5.11	5.60
	<i>qBLWR3-1</i>	3	SSR3-288	17.12	6.80	—	—	0.07	—	—	6.47	—	—
	<i>qBLWR3-2</i>	3	SSR3-120	41.63	9.40	14.96	11.52	0.06	0.08	0.07	9.31	25.38	22.30
	<i>qBLWR3-3</i>	3	STS3-1	113.84	—	4.78	—	—	0.05	—	—	6.87	—
	<i>qBLWR7-1</i>	7	SSR7-76	56.11	2.89	—	—	−0.03	—	—	2.60	—	—
	<i>qBLWR7-2</i>	7	SSR7-174	99.43	—	2.96	—	—	0.04	—	—	4.14	—
	<i>qBLWR10</i>	10	SSR10-169	80.71	—	5.64	4.63	—	0.06	0.05	—	8.22	8.05
	<i>qBLWR12</i>	12	STS12-8	57.18	—	4.05	4.21	—	0.04	0.04	—	5.76	7.28
BRT	<i>qBRT2</i>	2	SSR2-213	106.43	—	2.68	3.56	—	0.03	0.04	—	5.50	8.15
	<i>qBRT3</i>	3	SSR3-120	41.63	—	3.51	—	—	−0.03	—	—	7.30	—
	<i>qBRT6</i>	6	SSR6-111	6.25	3.27	—	—	0.03	—	—	8.77	—	—
	<i>qBRT7</i>	7	SSR7-174	99.43	2.82	3.93	—	−0.04	−0.04	—	7.51	8.22	—
	<i>qBRT11</i>	11	SSR11-235	3.61	—	4.23	3.52	—	0.04	0.04	—	8.90	8.05
BRP	<i>qBRP1</i>	1	STS1-12	132.60	—	—	3.34	—	—	0.25	—	—	4.07
	<i>qBRP2</i>	2	SSR2-24	92.20	4.53	4.81	5.95	−0.21	−0.21	−0.18	8.44	11.28	7.52
	<i>qBRP3-1</i>	3	STS3-1	113.84	4.51	—	12.00	0.22	—	0.30	8.40	—	16.65
	<i>qBRP3-2</i>	3	SSR3-337	132.32	—	—	5.47	—	—	−0.22	—	—	6.87
	<i>qBRP3-3</i>	3	SSR3-36	147.67	—	4.08	—	—	−0.45	—	—	9.45	—
	<i>qBRP5</i>	5	SSR5-1	14.19	—	—	3.20	—	—	0.21	—	—	3.88
	<i>qBRP7</i>	7	SSR7-145	20.13	4.34	—	—	0.19	—	—	8.06	—	—
	<i>qBRP8</i>	8	SSR8-390	111.97	3.24	2.52	4.92	0.21	0.19	0.20	5.92	5.70	6.13
	<i>qBRP12</i>	12	SSR12-107	66.43	4.19	—	—	−0.25	—	—	7.8	—	—
BRA	<i>qBRA2</i>	2	SSR2-24	92.20	4.05	5.97	4.94	−0.31	−0.38	−0.30	7.60	11.81	11.02
	<i>qBRA3-1</i>	3	SSR3-105	53.44	—	2.70	—	—	−0.31	—	—	5.07	—
	<i>qBRA3-2</i>	3	SSR3-36	147.67	—	3.56	3.13	—	−0.67	−0.49	—	6.79	6.78
	<i>qBRA7</i>	7	SSR7-145	20.13	7.74	—	—	0.42	—	—	15.36	—	—
	<i>qBRA8</i>	8	SSR8-417	90.25	3.04	4.24	—	0.27	0.33	—	5.61	8.16	—
	<i>qBRA12</i>	12	SSR12-107	66.43	4.63	—	4.25	−0.41	—	−0.36	8.77	—	9.36

Table 2. Cont.

Traits	QTL	Chr.	Marker	Position (cm)	LOD			Additive Effect			PVE (%)		
					2020LS	2021YZ	2022YZ	2020LS	2021YZ	2022YZ	2020LS	2021YZ	2022YZ
BRGW	<i>qBRGW2</i>	2	SSR2-24	92.20	3.93	—	—	−0.72	—	—	8.06	—	—
	<i>qBRGW6</i>	6	SSR6-111	6.25	4.41	—	—	0.66	—	—	9.13	—	—
	<i>qBRGW8</i>	8	SSR8-417	90.25	4.71	—	—	0.82	—	—	9.80	—	—
BTV	<i>qBTV5</i>	5	SSR5-83	99.01	3.35	5.69	5.67	1.80	3.19	3.56	5.94	13.32	15.87
	<i>qBTV6-1</i>	6	STS6-1	32.33	—	—	2.66	—	—	3.93	—	—	6.96
	<i>qBTV6-2</i>	6	SSR6-71	38.71	—	5.96	—	—	5.56	—	—	14.01	—
	<i>qBTV6-3</i>	6	SSR6-20	52.21	—	—	3.05	—	—	3.20	—	—	8.07
	<i>qBTV6-4</i>	6	SSR6-248	107.23	7.65	—	—	3.07	—	—	14.61	—	—
	<i>qBTV6-5</i>	6	SSR6-135	115.54	3.86	—	—	−2.17	—	—	6.91	—	—
	<i>qBTV7</i>	7	SSR7-174	99.43	4.22	2.73	4.51	2.55	2.66	3.95	7.59	6.08	12.28
BWC	<i>qBWC3</i>	3	SSR3-337	132.32	—	3.42	—	—	−0.38	—	—	9.4	—
	<i>qBWC6</i>	6	SSR6-71	38.71	—	3.07	—	—	−0.40	—	—	8.33	—
	<i>qBWC7-1</i>	7	SSR7-145	20.13	3.42	—	—	−0.25	—	—	10.9	—	—
	<i>qBWC7-2</i>	7	SSR7-76	56.11	—	—	2.99	—	—	−0.18	—	—	8.71
	<i>qBWC7-3</i>	7	STS7-5	82.36	—	—	5.55	—	—	−0.33	—	—	16.13
	<i>qBWC12</i>	12	STS12-4	3.37	—	—	3.10	—	—	0.34	—	—	8.52

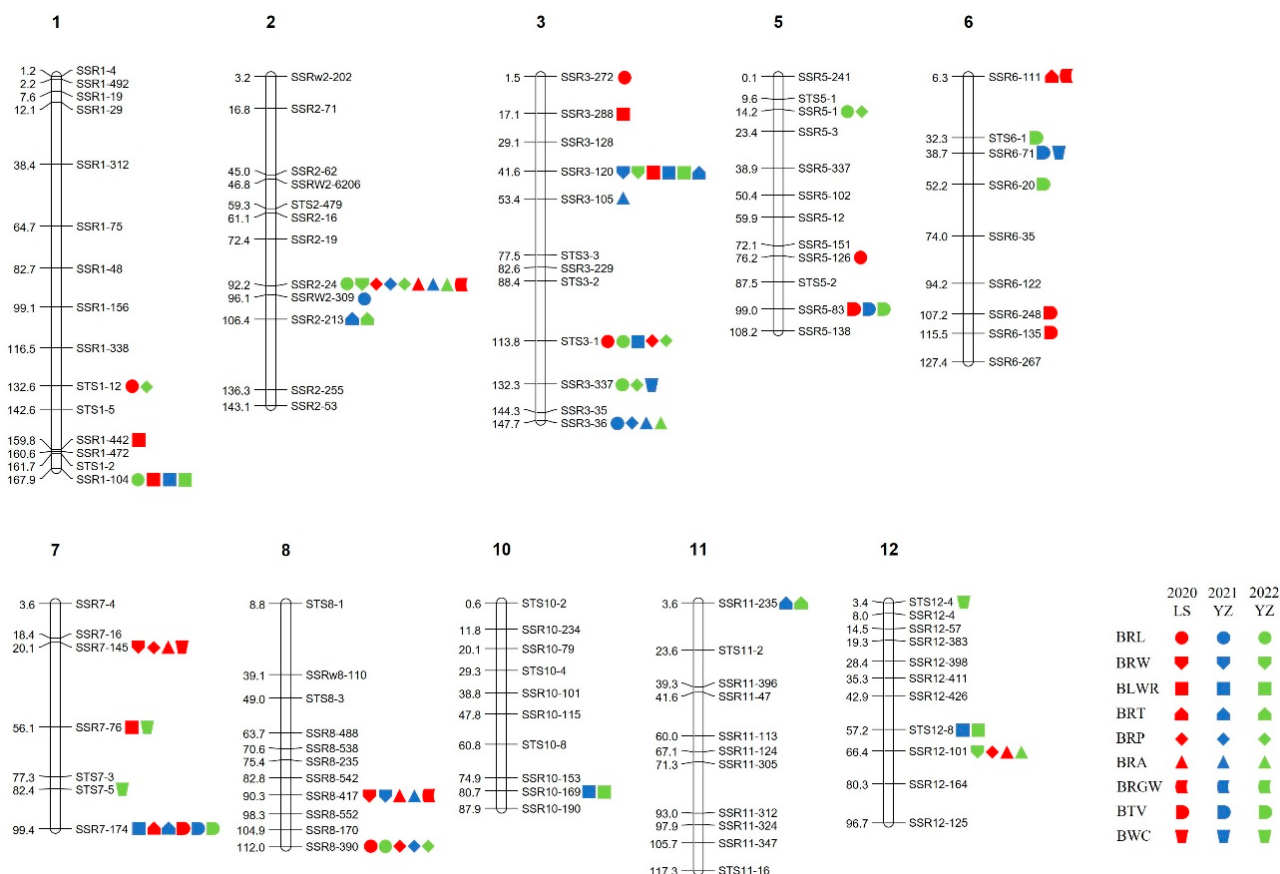


Figure 3. Chromosomal locations of putative QTLs for brown rice traits in CSSLs population under three environments. LS: Lingshui; YZ: Yangzhou; BRL: brown rice length; BRW: brown rice width; BLWR: length–width ratio of brown rice; BRT: brown rice thickness; BRP: brown rice perimeter; BRA: brown rice area; BRGW: thousand-grain weight of brown rice; BTV: taste value of brown rice; BWC: water content of brown rice.

For BRL, a total of eleven QTLs, including five in Lingshui 2020, two in Yangzhou 2021, and six in Yangzhou 2022, were identified on chromosomes 1, 2, 3, 5, and 8 (Table 2). *qBRL3-2* and *qBRL8* were detected in two environments, explaining the phenotypic variance that ranged from 5.41 to 16.67%. Another nine QTLs were only detected in one environment and explained 4.38 to 8.41% of the phenotypic variance.

Five QTLs for BRW were detected on chromosomes 2, 3, 7, 8, and 12 (Table 2). The LOD values of these QTLs ranged from 3.18 to 9.81 and explained 5.89 to 21.93% of the phenotypic variation (Table 2). *qBRW3* and *qBRW8* were identified under two environments; the phenotypic variation of the two QTLs was 19.48% and 13.53% and 9.10% and 9.35%, respectively. The remaining three QTLs, *qBRW2*, *qBRW7*, and *qBRW12*, were identified in one environment. The LOD values of these QTLs ranged from 3.18 to 9.81, explaining the phenotypic variance that ranged from 5.89 to 21.93%.

Nine QTLs for BLWR were detected on chromosomes 1, 3, 7, 10, and 12 (Table 2). *qBLWR1-2* and *qBLWR3-2* were identified on chromosome 1 and 3 under the three environments, which individually accounted for 18.55%, 5.11%, and 5.60% and 9.31%, 25.38%, and 22.30% of the phenotypic variation, respectively. The allele of *qBLWR1-2* and *qBLWR3-2* from Nona Bokra increased the BLWR by about 0.11%, respectively. *qBLWR10* and *qBLWR12* were both detected under two environments and explained 8.22% and 8.05% and 5.76% and 7.28% of the phenotypic variance in Yangzhou 2021 and 2022, respectively. The alleles that increased the BLWR were contributed by Nona Bokra. The remaining five QTLs for BLWR were only detected in one environment, and *qBLWR1-1* exhibited the highest phenotypic variation in Lingshui 2020.

Five QTLs were identified for BRT on chromosomes 2, 3, 6, 7, and 11 (Table 2). These QTLs had LOD values of 2.68 to 4.23 and explained 5.50% to 8.90% of the phenotypic variation. The QTL *qBRT11*, identified on chromosome 11, had the highest phenotypic variation in Yangzhou 2021. The allele of *qBRT11* from Nona Bokra increased the BRT by about 0.04%. *qBRT2*, *qBRT7*, and *qBRT11* were detected under two environments, respectively, and explained the phenotypic variance that ranged from 5.50 to 8.90%. *qBRT3* and *qBRT6* were only detected in one environment. *qBRT6* showed the highest phenotypic variation for BRT, reaching 8.77%.

A total of nine QTLs were detected on chromosomes 1, 2, 3, 5, 7, 8, and 12 for BRP (Table 2). *qBRP2* and *qBRP8* were identified on chromosome 2 and 8 under the three environments, with individually accounted for 8.44%, 11.28%, 7.52% and 5.92%, 5.70%, 6.13% of the phenotypic variation, respectively. The highest phenotypic variation of those two QTLs was detected in Yangzhou 2021 and 2022, which reaching to 11.28% and 6.13%, respectively. The allele of *qBRP2* from Koshihikari decreased the BRP about 0.21%, and the allele of *qBRP8* from Nona Bokra increased the BRP by 0.20%, respectively. *qBRP3-1* was detected in Lingshui 2020 and Yangzhou 2022. It explained the variation of 8.40% and 16.65%, respectively. The remaining four QTLs were only detected in one environment, with the variation ranging from 3.88% to 9.45%.

Six QTLs were identified for BRA on chromosomes 2, 3, 7, 8, and 12 (Table 2). *qBRA2* was detected on chromosome 2 under the three environments. The allele from Koshihikari decreased the BRA by about 0.38%. *qBRA3-2*, *qBRA8*, and *qBRA12* were detected under two environments and explained 6.79% and 6.78%, 5.61% and 8.16%, and 8.77% and 9.36% of the phenotypic variance, respectively. The remaining two QTLs, *qBRA3-1* and *qBRA7*, were only detected in one environment. *qBRA7* exhibited the highest phenotypic variation for BRA, reaching 15.36%.

Three QTLs for BRGW were identified on chromosomes 2, 6, and 8. These were *qBRGW2*, *qBRGW6*, and *qBRGW8*, respectively (Table 2). These QTLs were only detected in Lingshui 2020 and had a LOD score of 3.93 to 4.71, resulting in a variation of 8.06 to 9.80%. Among them, *qBRGW8*, the major locus for BRGW, was located on chromosome 8, which displayed the highest LOD score with 9.80% phenotypic variation.

A total of seven QTLs were detected on chromosomes 5, 6, and 7 for BTV (Table 2). *qBTV5* and *qBTV7* were detected under the three environments. The phenotypic variation of the two QTLs was 5.94%, 13.32%, and 15.87% and 7.59%, 6.08%, and 12.28%, respectively. The allele of *qBTV5* and *qBTV7* from Koshihikari increased the BTV by about 3.56% and 3.59%, respectively. The remaining five QTLs were all located on chromosome 6. The LOD values of these QTLs ranged from 2.66 to 7.65.

Six QTLs located on chromosomes 3, 6, 7, and 12 were identified for BWC in one environment (Table 2). These QTLs had a LOD score of 2.99 to 5.55 and explained 8.33% to 16.13% of the phenotypic variation. The QTL *qBWC7-3* was identified on chromosome 7. It had the highest phenotypic variation in Yangzhou 2022. The allele of *qBWC7-3* from Koshihikari decreased the BWC by about 0.33%.

3.4. Digenic Epistasis QTLs for Brown Rice Traits

In order to understand the genetic components of the brown rice traits, we further estimated the digenic epistatic effects of the BRL, BRW, BLWR, BRT, BRP, BRA, BRGW, BRR, BTV, and BWC. Thirty pairs of digenic epistatic QTLs for brown rice traits were detected, except for BRT, BRGW, BRR, BTV, and BWC, which indicated that the main effect QTL is the primary genetic basis for those traits (Table 3). Most of the epistasis interactions that occurred between loci had main effects at the single-locus level and a locus that did not show a significant main effect. The epistatic interaction explained 2.40 to 26.50% of the phenotypic variance, with the effect ranging from -0.57 to 0.24 . Four pairs of digenic epistasis QTLs were detected for BRL, which explained 5.01 to 5.17% of the phenotypic variation in Yangzhou 2022. Ten pairs of epistatic QTLs for BRW were estimated and accounted for 4.18 to 5.44% of the phenotypic variation in Lingshui 2020. A total of six pairs of digenic epistasis QTLs were detected for BLWR under the three environments, which included one pair of digenic epistasis QTLs in Lingshui 2020, three pairs of digenic epistasis QTLs in Yangzhou 2021, and two pairs of digenic epistasis QTLs in Yangzhou 2022, respectively. For BRP, seven pairs of digenic epistasis QTLs were detected in Yangzhou 2022, which explained the phenotypic variation that ranged from 2.40 to 2.49%. Three pairs of digenic epistasis QTLs were detected for BRA, which explained 7.95 to 8.78% of the phenotypic variation in Lingshui 2020.

Table 3. Epistasis effect for brown rice traits in CSSLs population.

Traits	Environment	Chr.	Marker	Chr.	Marker	LOD	Epistasis (AA)	PVE (%)
BRL	2022YZ	1	SSR1-338	3	STS3-1	13.12	−0.08	5.17
	2022YZ	3	STS3-1	3	SSR3-35	12.64	0.08	5.01
	2022YZ	3	STS3-1	4	SSR4-274	12.92	−0.08	5.11
	2022YZ	3	STS3-1	4	SSR4-302	12.98	−0.08	5.12
BRW	2020LS	2	SSR2-255	7	SSR7-145	11.38	0.08	4.20
	2020LS	2	SSR2-53	7	SSR7-145	11.32	0.08	4.18
	2020LS	5	SSR5-241	7	SSR7-145	13.42	−0.09	4.81
	2020LS	5	STS5-1	7	SSR7-145	13.15	−0.11	4.74
	2020LS	6	STS6-1	7	SSR7-145	11.58	−0.08	4.26
	2020LS	7	SSR7-145	8	SSR8-417	14.18	−0.08	5.44
	2020LS	7	SSR7-145	8	SSR8-552	11.97	−0.09	4.38
	2020LS	7	SSR7-145	8	SSR8-170	11.71	−0.08	4.30
	2020LS	7	SSR7-145	8	SSR8-390	11.64	−0.08	4.28
	2020LS	7	SSR7-145	9	SSR9-306	11.53	−0.08	4.25
BLWR	2020LS	2	SSR2-19	3	SSR3-120	11.27	−0.04	26.50
	2021YZ	3	SSR3-120	3	SSR3-105	17.13	−0.06	13.14
	2021YZ	3	SSR3-120	8	SSR8-235	16.56	0.04	12.80
	2021YZ	3	SSR3-120	12	STS12-4	16.12	0.04	12.54
	2022YZ	3	SSR3-120	3	SSR3-105	14.62	−0.07	15.37
	2022YZ	3	SSR3-120	6	SSR6-71	12.90	0.04	13.90

Table 3. Cont.

Traits	Environment	Chr.	Marker	Chr.	Marker	LOD	Epistasis (AA)	PVE (%)
BRP	2022YZ	1	SSR1-4	3	STS3-1	13.68	0.17	2.42
	2022YZ	1	SSR1-492	3	STS3-1	13.77	0.17	2.44
	2022YZ	3	STS3-1	3	SSR3-35	14.13	0.24	2.49
	2022YZ	3	STS3-1	4	SSR4-3	13.53	0.19	2.40
	2022YZ	3	STS3-1	6	SSR6-20	13.98	0.21	2.46
	2022YZ	3	STS3-1	9	SSR9-3	13.69	0.17	2.43
	2022YZ	3	STS3-1	11	SSR11-235	13.74	0.17	2.43
BRA	2020LS	5	SSR5-241	7	SSR7-145	11.36	−0.50	8.78
	2020LS	5	STS5-1	7	SSR7-145	11.21	−0.57	8.69
	2020LS	7	SSR7-145	8	SSR8-390	10.09	−0.38	7.95

3.5. Pleiotropic QTLs for Brown Rice Traits

The pleiotropic effects of genes could cause QTL to affect related traits mapped into the same or adjacent interval [25]. Thus, we further compared the pleiotropic QTLs for brown rice traits. A total of sixteen multi-trait QTLs were found on chromosomes 1, 2, 3, 5, 6, 7, 8, and 12, and nine digenic epistatic QTLs were mapped in the same position as main effect QTLs (Figure 3, Tables 3 and 4). Three main effect QTLs were related to BRL, BLWR, and BRP, and 11 epistatic loci were mapped on the marker STS3-1 on chromosome 3. *qBRL2-1*, *qBRW2*, *qBRP2*, *qBRA2*, and *qBRGW2* were anchored in the marker SSR2-24 on chromosome 2. Additionally, at least two QTLs controlling different brown rice traits were mapped on 15 markers (Figure 3).

Table 4. Pleiotropic analysis of QTL contributing to brown rice traits in CSSLs population.

Chr.	Marker	Position (cm)	QTLs
1	STS1-12	132.60	<i>qBRL1-1</i> (2020LS), <i>qBRP1</i> (2022YZ)
1	SSR1-104	167.85	<i>qBRL1-2</i> (2022YZ), <i>qBLWR1-2</i> (2020LS, 2021YZ, 2022YZ)
2	SSR2-24	92.20	<i>qBRL2-1</i> (2022YZ), <i>qBRW2</i> (2022YZ), <i>qBRP2</i> (2020LS, 2021YZ, 2022YZ), <i>qBRA2</i> (2020LS, 2021YZ, 2022YZ), <i>qBRGW2</i> (2020LS)
3	SSR3-120	41.63	<i>qBRW3</i> (2021YZ, 2022YZ), <i>qBLWR3-2</i> (2020LS, 2021YZ, 2022YZ), <i>qBRT3</i> (2021YZ)
3	STS3-1	113.84	<i>qBRL3-2</i> (2020LS, 2022YZ), <i>qBLWR3-3</i> (2021YZ), <i>qBRP3-1</i> (2020LS, 2022YZ)
3	SSR3-337	132.32	<i>qBRL3-3</i> (2022YZ), <i>qBRP3-2</i> (2022YZ), <i>qBWC3</i> (2021YZ)
3	SSR3-36	147.67	<i>qBRL3-4</i> (2021YZ), <i>qBRP3-3</i> (2021YZ), <i>qBRA3-2</i> (2021YZ, 2022YZ)
5	SSR5-1	9.60	<i>qBRL5-1</i> (2022YZ), <i>qBRP5</i> (2022YZ)
6	SSR6-111	6.25	<i>qBRT6</i> (2020LS), <i>qBRGW6</i> (2020LS)
6	SSR6-71	38.71	<i>qBTV6-2</i> (2021YZ), <i>qBWC6</i> (2021YZ)
7	SSR7-145	20.13	<i>qBRW7</i> (2020LS), <i>qBRP7</i> (2020LS), <i>qBRA7</i> (2020LS), <i>qBWC7-1</i> (2020LS)
7	SSR7-76	56.11	<i>qBLWR7-1</i> (2020LS), <i>qBWC7-2</i> (2022YZ)
7	SSR7-174	99.43	<i>qBLWR7-2</i> (2021YZ), <i>qBRT7</i> (2020LS, 2021YZ), <i>qBTV7</i> (2020LS, 2021YZ, 2022YZ)
8	SSR8-417	90.25	<i>qBRW8</i> (2020LS, 2021YZ), <i>qBRA8</i> (2020LS, 2021YZ), <i>qBRGW8</i> (2020LS)
8	SSR8-390	111.97	<i>qBRL8</i> (2020LS, 2022YZ), <i>qBRP8</i> (2020LS, 2021YZ, 2022YZ)
12	SSR12-107	66.43	<i>qBRW12</i> (2022YZ), <i>qBRP12</i> (2020LS), <i>qBRA12</i> (2020LS, 2022YZ)

3.6. Comparative Genetic Analysis for QTLs and Grain Size Traits

There was a significant correlation between brown rice traits and grain size traits (Figure S1). For example, the correlation between brown rice width and grain width reached 0.97, 0.96, and 0.92 in the three environments. Thus, we integrated the QTL mapping for grain size genes in this study. Five grain size genes, namely, *qLG3/OsLG3b* [26,27], *GS5* [28], *GW6a* [29], *GLW7* [30], and *GW7/GL7/SLG7* [31–33], were integrated with brown rice trait QTLs (Table S1).

4. Discussion

Grain yield and quality are the two main goals of rice breeding. As an important part of the rice grain, brown rice is directly affected by grain yield and quality. For example,

enhancing brown rice recovery can significantly increase rice yield, and improving the taste value of brown rice can significantly improve the rice quality. In particular, brown rice contains a lot of nutrients, which makes it beneficial to human health. Therefore, the study of brown rice traits is becoming more and more important. In this study, the CSSLs population was developed against the genetic background of Koshihikari, a premium short-grain with a good-quality cultivar, with substituted chromosomal fragments from Nona Bokra, a well-known salt-tolerant cultivar [18]. We evaluated ten brown rice traits of the CSSLs, which enabled us to identify relevant QTLs.

In the present study, a total of sixty-one QTLs were identified for all brown rice traits except for BRR, including eleven QTLs for BRL, five QTLs for BRW, nine QTLs for BLWR, five QTLs for BRT, nine QTLs for BRP, six QTLs for BRA, three QTLs for BRGW, seven QTLs for BTV, and six QTLs for BWC (Table 2). Among those QTLs, thirteen QTLs were identified under two environments, and seven QTLs were identified under three environments, indicating that a potential gene controlling those brown rice traits might exist. Correlation analysis showed that brown rice size traits were significantly correlated with grain size traits, indicating the same genetic mechanism might exist between brown rice size traits and grain size traits. (Figure S1). Therefore, we compared the reported QTLs for grain size and weight with the QTLs for brown rice size and weight mapped in this study. Thirty-three out of forty-eight QTLs related to BRL, BRW, BRT, BLWR, BRP, BRA, and BRGW were mapped on the same position or close to the QTLs that we previously identified for grain size and weight. For BRL, 10 out of 11 QTLs (*qBRL1-1*, *qBRL1-2*, *qBRL2-1*, *qBRL2-2*, *qBRL3-1*, *qBRL3-2*, *qBRL3-3*, *qBRL3-4*, *qBRL5-2*, and *qBRL8*) were in the same intervals or near to previously reported intervals [20,34–38]. The QTLs *qBRL3-2* and *qBRL8*, located on chromosome 3 and 8, were detected under two environments, indicating that a potential gene controlling BRL might exist in this region. The novel QTL *qBRL5-1* was detected on chromosome 5 only in Yangzhou 2022. Similarly, for BRL, four out of five QTLs (*qBRW2*, *qBRW3*, *qBRW7*, and *qBRW8*) were in the same intervals or near to previously reported ones [14,35,39,40]. The QTLs *qBRW3* and *qBRW8*, located on chromosome 3 and 8, were detected under two environments and explain 19.48% and 13.54% and 9.10% and 9.35% of the phenotypic variation, respectively. For BLW, BRT, BRP, BRA, and BRGW, six out of nine, one out of five, seven out of nine, three out of six, and two out of three QTLs were located in the same regions or close to previously reported regions, respectively [20,39–41]. The QTLs *qBLWR1-2*, *qBLWR3-2*, *qBRP2*, *qBRP8*, and *qBRA2*, located on chromosome 1, 3, 2, 8, and 2, were detected in all tested environments, indicating that a potential gene controlling those traits might exist in those regions. BTV and BWC are important agronomic traits associated with grain quality. In our study, seven and six QTLs were found for BTV and BWC, respectively (Figure 3, Table 2). In particular, *qBTV5* and *qBTV7* were detected under the three environments, indicating a stable QTL was found and is worthy of further cloning. In addition, among the QTLs, five of those intervals contained the identified grain size and weight genes that are involved in *qLGY3/OsLG3b* [15,16], *GS5* [27], *GW6a* [29], *GLW7* [35], and *GW7/GL7/SLG7* [32–34] on chromosome 3, 5, 6, 7, and 7, respectively (Table S1). *qLGY3/OsLG3b* encodes a MADS-domain transcription factor, which is close to the marker SSR3-288 on chromosome 3 [26,27]. *GS5* encodes a putative serine carboxypeptidase, which is integrated with *qBRL5-1* and *qBRP5* near to the marker SSR5-1 on chromosome 5 [27]. *GW6a* encodes a new-type GNAT-like protein, which is located adjacent to *qBTV6-4* on chromosome 6 [29]. *GLW7* encodes the plant-specific transcription factor OsSPL13, which is associated with *qBWC7-3* on chromosome 7 [30]. In addition, *GW7/GL7/SLG7* encodes a TONNEAU1-recruiting motif protein, which is co-localized with *qBLWR7-2*, *qBRT7*, and *qBTV7* on chromosome 6 [31–33].

Digenic epistasis interaction has been demonstrated as an important factor in the genetic basis for grain yield and quality traits [25]. In our study, thirty digenic epistatic QTLs for five brown rice traits were detected under three environments, which indicated that digenic epistatic interaction also played an important role in determining brown rice traits (Table 3). Especially for BLWR, the digenic epistatic QTLs explained the phenotypic

variation that ranged from 12.54 to 26.50%, which exceeded most of the main effect QTLs of BLWR. Moreover, the number of digenic epistatic QTLs for BRL are more than those of main effect QTLs. These results remind us to consider the epistatic effects for these traits during gene pyramiding in rice breeding.

The pleiotropic QTL is especially useful for rice breeding, because it can select a multi-trait through a single genomic interval [42]. Among the sixty-one QTLs identified in our study, forty-four QTLs formed sixteen clusters, including nine QTLs that control five brown rice traits (BRL, BRW, BRP, BRA, and BRGW), which were mapped on the marker SSR2-24 on chromosome 2. Four QTLs that control four brown rice traits (BRW, BRP, BRA, and BWC) were mapped on the marker SSR7-145 on chromosome 7. Another remaining thirty-one QTLs controlling two or three brown rice traits were mapped on the marker 14 (Figure 3). These QTL clusters may be useful in improving brown rice traits by marker-assisted selection (MAS).

CSSLs are a powerful tool for the mapping and cloning of quantitative traits. They are also an important material for breeding [43]. In our study, a wide phenotypic variation and transgressive segregants of the brown rice traits in the CSSLs population were observed in the tested environments (Figure 1). Transgressive segregation is a valuable genetic mechanism for breeders to improve brown rice traits. In addition, correlation analysis displayed the high correlation between brown rice size and weight traits, whereas there was no significant correlation between brown rice size and weight traits with BRR, BWC, and BTV. It is suggested that we could simultaneously improve the brown rice size and weight traits and the taste value of brown rice in breeding.

5. Conclusions

In this study, a wide phenotypic variation of the brown rice traits in the Koshihikari (the recipient parent) and Nona Bokra (the donor parent) CSSLs populations was observed. We identified 61 QTLs for nine brown rice traits under three environments, of which twenty-eight QTLs were novel. In addition, five of the intervals contained the identified grain size and weight genes that are involved in *qLGY3/OsLG3b*, *GS5*, *GW6a*, *GLW7*, and *GW7/GL7/SLG7*. Moreover, thirty pairs of digenic epistatic QTLs and sixteen multi-trait QTLs for brown rice traits were detected under the three environments. The results of this study will provide useful information for further improving brown rice traits in rice.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agriculture13050928/s1>, Figure S1: Trait correlations for brown rice traits and grain size traits in three environments; Table S1: Known grain size genes close to brown rice trait QTLs detected in this study.

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