

Article

Genetic Dissection of Yield-Related Traits Using an Inter-Subspecific Chromosome Segment Substitution Line Population in Rice

Yongle Xu ^{1,†}, Yue Pan ^{2,†}, Yong Xiang ³, Yue Sun ², Junying Xu ², Haiyang Liu ² , Longwei Yang ², Zhilian Qi ¹, Xinxin Tang ⁴, Famao Liang ⁵, Hui Hu ^{1,*}, Xianjin Qiu ^{2,*}  and Jian Yu ^{6,*}

¹ College of Arts and Sciences, Yangtze University, Jingzhou 434020, China; xuuyuelee@163.com (Y.X.); shuyamino777@163.com (Z.Q.)

² Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education/Key Laboratory of Green and Efficient Production of Crops in the Middle Reaches of the Yangtze River, Ministry of Agriculture and Rural Affairs (Coconstructed by Ministry and Province), Jingzhou 434025, China; panyue0229@126.com (Y.P.); m19267178529@163.com (Y.S.); jyxu@yangtzeu.edu.cn (J.X.); hylu@yangtzeu.edu.cn (H.L.); ylwei1968@126.com (L.Y.)

³ Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China; xiangy1979@hotmail.com

⁴ College of Biology and Agricultural Researches, Huanggang Normal University, Huanggang 438000, China; cyndiwei@163.com

⁵ Yichang Academy of Agricultural Sciences, Yichang 443004, China; liangfamao92@hotmail.com

⁶ College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

* Correspondence: 13476974813@163.com (H.H.); xjqiu216@yangtzeu.edu.cn (X.Q.); laoci@163.com (J.Y.)

† These authors contributed equally to this work.

Abstract

Rice yield is a complex quantitative trait. Although a lot of genes for yield have been cloned, their genetic basis remains unknown. In the present study, a set of chromosome segment substitution line population (CSSL) was developed, derived from the *indica* variety Huanghuazhan as the recipient parent and the *Aus* variety N22 as the donor parent, and a high-density bin map containing 609 bins was constructed by resequencing. The CSSL population comprised 155 families with an average background recovery rate of 93.02%. Nine yield-related traits, including plant height, panicle number, panicle length, primary branch number, spikelet number per panicle, grain number per panicle, seed setting rate, 1000-grain weight, and grain yield per plant, were evaluated across four environments. The results showed significant differences in yield-related traits between the two parents across four environments. All nine traits showed continuous distribution with transgressive segregation. Spikelet number per panicle, grain number per panicle and 1000-grain weight showed strong correlations with each other, whereas panicle number had weak correlations with them. A total of 80 main-effect quantitative trait loci (QTLs) affecting yield-related traits were identified, among which 13 QTLs were repeatedly detected in multiple environments, 45 QTLs were located in 8 pleiotropic QTL regions, and 47 QTLs showed significant interactions with environments. In addition, 260 pairs of epistatic QTLs underlying yield-related traits were identified, of which 2 pairs stably expressed across different environments, and 11 pairs controlled more than two traits. These findings provide a theoretical basis for clarifying the genetic differentiation between *indica* and *Aus* and cloning yield-related genes, and offer valuable gene resources for molecular breeding of high-yield rice varieties.



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1. Introduction

Rice is one of the most important staple crops worldwide, feeding half of the global population [1]. Over the past decades, due to the application of green revolution gene and the utilization of heterosis, rice yield has increased by two times, and even tripled in some regions [2]. However, rice yield has barely increased since the late 20th century [3]. With the rapid growth of the global population, the world's food supply needs to rise by 70% to meet the consumption of staple foods, meat, and biofuel use [4]. Given that the cultivated land area is almost impossible to increase, crop yield per unit area should be greatly increased. Therefore, it is of importance to elucidate the genetic basis of rice yield, identify and clone yield-related genes, mine favorable alleles, and breed new high-yield rice varieties through a molecular breeding strategy.

Grain yield per plant (YD) is determined by three factors, panicle number (PN), grain number per panicle (GN) and 1000-grain weight (KGW). It is also affected by plant height (PH), panicle length (PL), primary branch number (PB), spikelet number per panicle (SN) and seed setting rate (SSR). All of them are typical quantitative traits and controlled by multi-genes and highly affected by environments. In recent years, with the advances in rice functional genomics and development of molecular marker technology, researchers have identified a large number of quantitative trait loci (QTLs) associated with rice yield in many populations, such as F_2 , $F_{2,3}$, Doubled Haploid, Recombinant Inbred Line, Introgression Line, Chromosome Segment Substitution Line (CSSL) populations and germplasm resources [1–9]. Among them, many genes such as *sd1* [10], *MOC1* [11], *Gn1a* [12], *Ghd7* [13], *Ghd7.1* [14], *Ghd8/DTH8* [15,16], *IPA1/OsSPL14* [17,18], *GW2* [19], *GL2/GS2/OsGRF4* [20–22], *GS3* [23], *GW5/GSE5* [24,25], *GS5* [26], *GW8* [27] and *GS9* [28] have been successfully cloned. Studies have revealed that genes related to auxin, cytokinin and gibberellin have significant effects on rice yield [29]. In addition, genes involved in the G protein pathway, MAPK signaling pathway, peptide signaling pathway, ubiquitin-proteasome degradation pathway, as well as several transcription factors, are also involved in the regulation of rice yield. Notably, some genes have pleiotropic effects on multiple yield-related traits. For instance, *IPA1/OsSPL14* [17,18] simultaneously affects PH, PN, PL, SN and YD. *Ghd7* [13], *Ghd7.1* [14] and *Ghd8/DTH8* [15,16] have pleiotropic effects on heading date, PH and SN. *GW2* [19], *GL2/GS2/OsGRF4* [20–22], *GS3* [23], *GW5/GSE5* [24,25], *GS5* [26], *GW8* [27] and *GS9* [28] affect both grain shape and KGW. Moreover, *GW2* [19], *GW8* [27] and *GS9* [28] also affect chalkiness.

Asian cultivated rice is divided into two subspecies, *indica* and *japonica*. They were further classified into five distinct subpopulations, namely *indica*, *temperate japonica*, *tropical japonica*, *Aus* and *aromatic* [30]. Using whole-genome resequencing data of 3010 global accessions, Asian cultivated rice has been further divided into nine subpopulations [31]. Different subspecies/subpopulations harbor distinct favorable alleles. For instance, *japonica* harbors the *Wx^b* allele, resulting in a lower amylose content and better eating quality compared with *indica* [32]. The elite allele of *NRT1.1B* is derived from *indica*, which endows *indica* with a higher nitrogen use efficiency than *japonica* [33]. The superior allele of *GL7*, originating from *tropical japonica*, can increase length to width ratio and reduce chalkiness [34,35]. The narrow-grain allele of *GW8* is from *basmati*, which could improve the grain appearance quality [27]. In addition, due to strong heterosis between *indica* and

japonica, breeders have developed a series of inter-subspecific hybrid rice varieties with over 10% higher yield than intra-subspecific varieties.

Aus is mainly distributed in Southeast Asia and had diverged from *indica* prior to domestication [36]. Compared with *indica*, most *Aus* varieties showed photoperiod insensitivity, blast resistance, drought tolerance, and local environmental adaptation [37]. It harbors numerous elite genes. Ma et al. [38] cloned a seed dormancy gene *qSdn5* using the *Aus* variety N22, and the N22 allele has strong dormancy. Guo et al. [39] isolated a gene *SDR3.1* for seed dormancy from the *Aus* variety Mengjialaxiaoli, with this allele also conferring strong dormancy. Introgression of this allele into the *indica* variety Zhonghui261 led to the development of new germplasm resources with pre-harvest sprouting resistance.

The genetic relationship of *indica/Aus* is closer than that of *indica/japonica*, and they have no reproductive isolation. However, as they belong to two subspecies, they might have some novel genes. *Indica* is widely cultivated in southern China, and its yield is often severely reduced due to higher temperatures during the heading stage. N22 is an elite *Aus* variety with heat tolerance [40]; it is a valuable resource for heat tolerance improvement for *indica*. Although previous studies have identified a large number of QTLs affecting rice yield using multiple genetic populations, there are very few reports on the genetic dissection of yield in *Aus*. In the present study, a set of CSSL population was developed, derived from the *indica* variety Huanghuazhan as the recipient parent and the *Aus* variety N22 as the donor parent [41], and a high-density bin map containing 609 bins was constructed via resequencing. Nine yield-related traits, including PH, PN, PL, PB, SN, GN, SSR, KWG and YD, were evaluated across four environments, and major QTLs and epistatic QTLs for rice yield-related traits were identified. In addition, interactions between major QTLs and environments were also detected. These findings provide a theoretical basis for clarifying the genetic differentiation of rice subspecies and cloning yield-related genes, and offer valuable gene resources for the molecular breeding of high-yield rice varieties.

2. Materials and Methods

2.1. Plant Materials and Development of CSSL Population

In the present study, the *indica* variety Huanghuazhan (HHZ) was used as the recipient parent, and the *Aus* variety N22 was used as the donor parent. HHZ is one of the *indica* conventional varieties with the largest planting area in southern China. It has early maturity, strong lodging resistance, high yield, and superior grain quality [40]. N22 is an *Aus* variety from India with strong heat tolerance and seed dormancy [37,41].

HHZ was crossed with N22, and the F₁ was backcrossed with the recurrent parent HHZ three times, followed by three self-crosses to obtain the BC₃F₃ generation. Eventually, a set of CSSL population consisting of 155 lines was successfully developed.

2.2. Genotyping of the CSSL Population

Young leaves were sampled from all BC₃F₃ plants and the parental lines, and genomic DNA was extracted using the DNeasy mini Kit (Qiagen, Düsseldorf, Germany). Whole-genome resequencing was performed on the Illumina Genome Analyzer IIx (Illumina, Inc., San Diego, CA, USA), with sequencing depth of 25× for HHZ and N22, and 5× for the CSSLs. After obtaining the raw sequencing data, the genomic sequences of the two parents were first aligned to the *Nipponbare* reference genome to identify genome-wide single-nucleotide polymorphisms (SNPs) between them. After that, genomes of the CSSLs were aligned to the same reference genome using the same method and each CSSL was genotyped by comparison with the parental SNPs. A total of 87,299 SNPs were identified between the two parents. Finally, a bin map containing 609 bins was constructed in the CSSLs based on these SNPs following the method described by Xie et al. [42].

2.3. Field Experiment and Trait Measurement

A total of 155 CSSLs and their parents were grown in 2023 and 2024 on the experimental farm of Yangtze University at Jingzhou (30.18° N, 112.15° E). Two sowing dates, middle and late seasons, were set in each year. Thus, they were planted under four environments (represented as 23M, 23L, 24M and 24L). The sowing dates were 7 May and 1 June in 2023, and 5 May and 2 June in 2024, respectively. A randomized complete block design with two replications was adopted for each environment. Each line was planted in three rows with 10 plants per row, at a plant spacing of 20 cm × 20 cm. All field management practices were followed in conventional rice cultivation.

About two weeks after flowering, three plants of each line with consistent growth conditions in the middle row were selected to evaluate PH and PN. After maturing, the main panicles of these three plants were harvested for panicle trait measurement, and these three plants were bulked and harvested to determine YD. The panicle-related traits included PL, PB, SN, GN, SSR and KGW. They were evaluated according to the method description by Hu et al. [9].

2.4. Data Analysis

Descriptive statistics and correlation analysis of phenotypic data were performed using Statistica 5.5 [43]. The GGT32 [44] was used to calculate the background recovery rate and plot the graphical genotypes. Major effect QTL (M-QTL) and epistatic QTL (E-QTL) were identified using the BIP module in IciMapping 4.2 [45], and the analysis of interactions between M-QTLs and environments ($Q \times E$) was conducted in the MET module. The inclusive composite interval mapping method was used for all analyses, with the logarithm of odds (LOD) thresholds set at 2.5, 5.0 and 2.5 for M-QTL, E-QTL identification, and $Q \times E$ analysis, respectively.

3. Results

3.1. Development of CSSL Population and Analysis of Genotypes

HHZ was used as the female parent and N22 as the male parent for crossing. A total of 15 F_1 plants were grown and backcrossed with HHZ. At the BC_1F_1 generation, 37 individuals were planted, and they were backcrossed with HHZ. A total of 127 plants were grown at BC_2F_1 generation, and all of them were backcrossed with HHZ. At the BC_3F_1 generation, 203 plants were planted. About 10 seeds were harvested from each plant, and all harvested seeds were bulked. A total of 297 individual plants were grown at BC_3F_2 generation, and the same seed harvesting and bulking protocol was conducted. At the BC_3F_3 generation, 252 plants were grown, and all were harvested separately. Meanwhile, leaf samples were collected from each plant for DNA extraction, and the genotype was identified via whole-genome resequencing. CSSL with fewer than 10 introgressed segments were selected. Ultimately, a set of CSSL population consisting of 155 lines was successfully developed.

The genome of the CSSL population was 373.25 Mb, and they were divided into 609 bins based on the 87,299 SNPs between the two parents. Chromosome 5 had the most bins (106), while chromosome 9 had the fewest (only 2). The average bin length was 612.88 kb, ranging from 11 bp on chromosome 8 to 14.44 Mb on chromosome 9. The average background recovery rate of CSSLs was 93.02%, with the highest and lowest of 99.78% and 80.97%, respectively (Figure 1). The graphical genotypes of the CSSL are shown in Supplementary Figure S1.

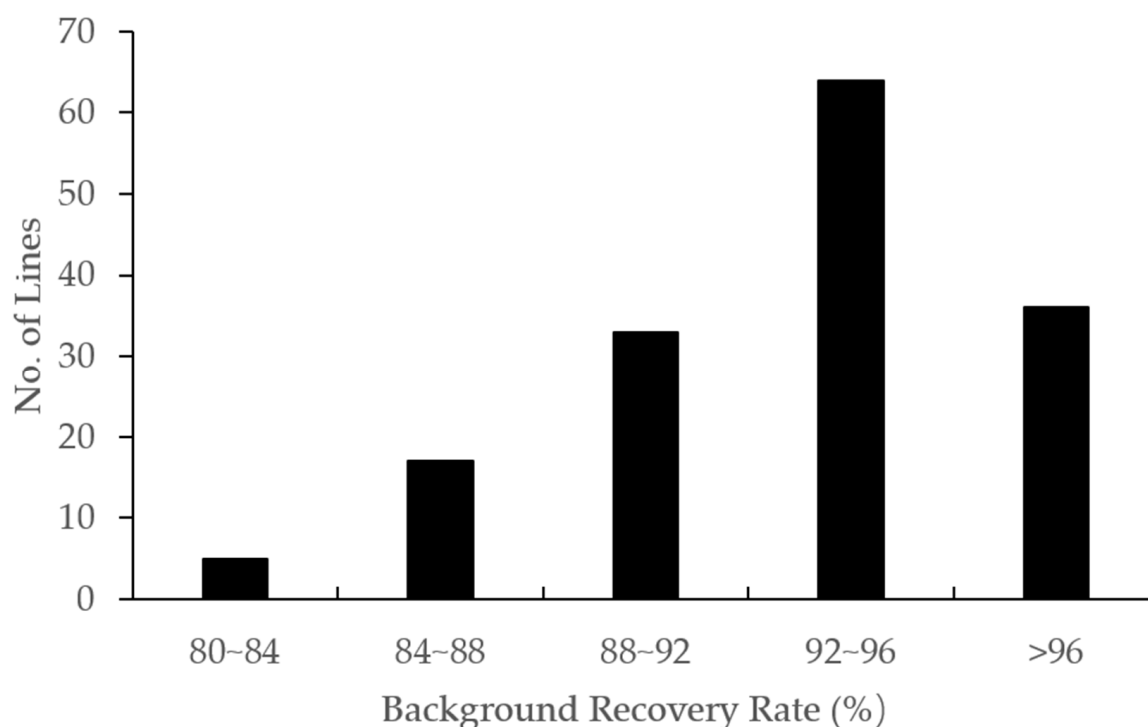


Figure 1. Background recovery rate of the CSSL population derived from *indica* HHZ and *Aus* N22.

3.2. Yield-Related Trait Performances of Parents and CSSL Population

The parents showed significant differences in almost all yield-related traits across the four environments (Table 1). Under four environments, N22 had significantly higher PH (on average 149.9 cm for N22, while 107.26 cm for HHZ) and lower PB (on average 8.6 for N22, while 13.18 for HHZ) than HHZ. Under two or three environments, N22 had more PN, higher SSR and YD (averagely 14.65, 93.62% and 52.05 g for N22 while 10.52, 82.66% and 25.83 g for HHZ respectively) but lower PL, SN, GN and KGW (averagely 22.93 cm, 140.58, 131.13, 20.73 g for N22 while 26.25 cm, 202.80, 181.45 and 25.27 g for HHZ respectively) than HHZ. All traits of the CSSL population were similar to HHZ, and showed continuous distributions with transgressive variations, indicating that they were controlled by multi-genes. Of the nine yield-related traits, the coefficients of variation (CV) for PL, PB, SSR, and KGW were less than 10% in most environments, suggesting low variation for these traits. The CVs for PH, PN, SN and GN ranged from 10% to 30% in most environments, indicating moderate to high variations. The CV for YD exceeded 30%, showing extremely high variation. In addition, genotype showed significant effects on all traits, environments had significant effects on most traits except SN and SSR, while genotype $\times$ environment had significant effects on PH, PN, PL, PB, SN, GN, SSR, and YD (Table 2).

Table 1. Performance of nine yield-related traits of the two parents and the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments.

Trait	Env	HHZ	N22	CSSL Population						
				Mean	SD	Max	Min	Kurtosis	Skewness	CV (%)
PH (cm)	23M	106.33 $\pm$ 5.23	154.67 $\pm$ 4.39 **	114.68	11.86	71.00	155.00	3.11	0.95	10.34
	23L	96.97 $\pm$ 7.22	133.91 $\pm$ 9.21 **	108.14	12.67	86.33	158.00	2.97	1.60	11.71
	24M	114.67 $\pm$ 4.99	148.67 $\pm$ 8.22 **	121.29	15.59	99.67	186.33	4.38	2.13	12.85
	24L	111.07 $\pm$ 2.88	162.33 $\pm$ 5.09 **	122.79	33.29	98.67	492.67	100.72	9.20	27.11

Table 1. Cont.

Trait	Env	HHZ	N22	CSSL Population						
				Mean	SD	Max	Min	Kurtosis	Skewness	CV (%)
PN	23M	7.23 ± 0.22	13.00 ± 0.22 **	9.66	2.00	5.00	15.33	−0.30	0.13	20.74
	23L	10.33 ± 0.27	11.67 ± 0.53	12.41	3.31	6.33	26.00	1.03	0.90	26.67
	24M	12.00 ± 0.34	15.28 ± 0.55 **	10.60	2.43	4.67	18.00	0.22	0.38	22.91
	24L	12.33 ± 0.47	15.67 ± 1.06 *	11.10	2.20	6.33	17.67	−0.01	0.48	19.78
PL (cm)	23M	25.67 ± 1.01	22.73 ± 0.88 *	27.29	1.83	21.33	32.67	0.36	−0.16	6.72
	23L	23.67 ± 1.00	23.67 ± 0.62	26.56	6.45	21.90	30.10	119.23	10.50	24.28
	24M	26.83 ± 1.22	23.13 ± 1.37 **	27.58	1.82	21.57	34.53	0.97	0.10	6.59
	24L	24.20 ± 1.22	24.87 ± 1.69	26.02	1.59	14.00	29.83	20.39	−2.57	6.13
PB	23M	14.00 ± 1.36	8.33 ± 1.00 **	12.33	1.27	8.33	18.33	1.79	0.32	10.30
	23L	12.33 ± 1.64	9.03 ± 1.53 *	12.61	1.17	9.33	18.00	2.05	0.65	9.27
	24M	13.04 ± 1.29	8.06 ± 1.00 **	12.27	0.89	10.00	15.00	0.56	0.14	7.24
	24L	13.33 ± 1.05	9.33 ± 1.00 **	12.88	1.13	10.67	17.33	0.94	0.57	8.77
SN	23M	182.67 ± 5.37	129.67 ± 6.20 **	216.94	46.77	97.67	390.67	0.58	0.43	21.56
	23L	243.67 ± 8.24	164.00 ± 10.24 **	247.97	54.25	120.67	406.33	−0.03	0.13	21.88
	24M	182.07 ± 4.29	128.07 ± 5.13 **	222.06	43.22	112.00	312.33	−0.67	−0.09	19.46
	24L	215.67 ± 5.08	201.00 ± 8.23	183.30	36.51	111.33	363.33	3.29	1.12	19.92
GN	23M	156.35 ± 3.39	123.58 ± 7.22 **	176.81	41.52	61.67	335.00	0.84	0.47	23.48
	23L	222.33 ± 7.46	148.33 ± 5.08 **	201.56	45.00	91.00	330.33	−0.04	0.02	22.33
	24M	165.67 ± 5.38	121.47 ± 4.24 **	180.88	41.44	27.33	273.00	0.35	−0.53	22.91
	24L	172.33 ± 7.04	185.67 ± 9.99	231.73	42.61	151.33	426.00	2.18	0.98	18.39
SSR (%)	23M	85.40 ± 2.47	94.86 ± 4.21 *	81.54	7.84	44.18	94.96	2.72	−1.25	9.61
	23L	91.24 ± 4.26	90.45 ± 3.07	81.57	8.04	54.26	96.18	−0.07	−0.65	9.86
	24M	90.66 ± 0.49	94.53 ± 1.22	81.84	12.28	14.59	95.00	8.79	−2.69	15.00
	24L	79.91 ± 3.05	92.37 ± 2.00 **	79.38	1.41	13.99	23.62	1.25	0.31	7.27
KGW (g)	23M	24.57 ± 1.03	20.87 ± 0.69 **	23.05	2.43	17.65	29.00	51.90	5.06	10.55
	23L	19.01 ± 1.26	19.42 ± 1.05	20.57	1.55	16.30	28.36	2.90	1.00	7.52
	24M	25.96 ± 1.27	20.58 ± 0.89 **	19.42	1.26	16.98	25.03	2.21	1.01	6.49
	24L	18.11 ± 1.02	18.63 ± 0.87	19.16	6.29	62.61	90.02	−0.27	−0.66	7.94
YD (g)	23M	27.44 ± 6.22	53.00 ± 5.84 **	49.95	18.66	12.90	103.79	0.09	0.57	37.36
	23L	44.25 ± 5.07	47.45 ± 3.29	49.35	12.80	23.56	99.62	1.48	0.94	25.95
	24M	27.50 ± 5.22	51.33 ± 7.81 **	24.92	8.49	5.83	52.67	0.76	0.56	34.08
	24L	22.55 ± 4.28	51.83 ± 7.23 **	24.72	7.14	8.00	46.50	0.56	0.68	28.88

* and ** represent significant level of $p < 0.05$ and 0.01 , respectively; CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; Env, environment; 23M, middle season in 2023; 23L, late season in 2023; 24M, middle season in 2024; 24L, late season in 2024; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

Table 2. Analysis of variance for yield-related traits of the two parents and the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments.

	PH	PN	PL	PB	SN	GN	SSR	KGW	YD
Genotype	888.02 **	9.89 **	10.42 **	2.12 **	4448.94 **	3010.77 **	95.47 **	6.55 **	245.45 **
Environment	17,425.78 **	390.19 **	124.86 **	12.37 **	241.36	29,978.67 **	34.29	916.49 **	88,340.05 **
Genotype × Environment	384.79 **	6.2 *	7.43 *	0.88 *	1730.02 *	1251.86 *	78.3 *	2.8	174.18 **
Error	224.57	3.71	5.2	0.71	1291.62	641.51	65.74	4.11	119.35

* and ** represent significant level of $p < 0.05$ and 0.01 , respectively; CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

As shown in Table 3, YD was significantly positively correlated with SN across all environments, and with GN under three environments. It was also significantly positively correlated with PN in two environments, and with KGW only in the middle season of 2024. Among three yield components, SN was significantly positively correlated with GN across all environments, and both of them were significantly negatively correlated with KGW in all environments. PN was significantly negatively correlated with SN and GN,

and significantly positively correlated with KGW in only one environment. The results suggested that the correlations among SN, GN and KGW were stronger, whereas the correlation between the PN and them was weaker in the CSSL population.

Table 3. Correlation analysis of nine yield-related traits of the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments.

	Env	PH	PN	PL	PB	SN	GN	SSR	KGW
PN	23M	0.17 *							
	23L	−0.07							
	24M	0.04							
	24L	−0.18 *							
PL	23M	0.48 **	−0.09						
	23L	0.10	0						
	24M	0.45 **	0.17 *						
	24L	0.17 *	0.02						
PB	23M	0.30 **	−0.03	0.53 **					
	23L	0.20 *	−0.09	0.16 *					
	24M	0.35 **	−0.10	0.43 **					
	24L	−0.03	−0.04	−0.05					
SN	23M	0.40 **	−0.04	0.68 **	0.62 **				
	23L	0.36 **	−0.02	0.11	0.55 **				
	24M	−0.12	0.12	0.45 **	0.32 **				
	24L	0.19 *	−0.18 *	0.23 **	0.31 **				
GN	23M	0.32 **	−0.03	0.57 **	0.54 **	0.90 **			
	23L	0.35 **	−0.02	0.13	0.50 **	0.93 **			
	24M	0.41 **	0.06	0.64 **	0.59 **	0.72 **			
	24L	0.28 **	−0.23 **	0.33 **	0.32 **	0.91 **			
SSR	23M	−0.10	0	−0.10	−0.05	0	0.42 **		
	23L	0.04	0	0.05	−0.01	−0.01	0.35 **		
	24M	−0.66 **	0.08	−0.12	−0.24 **	0.56 **	0.16 *		
	24L	−0.16 *	0.06	−0.18 *	0.06	0.36 **	−0.05		
KGW	23M	−0.06	−0.04	−0.18 **	−0.18 **	−0.44 **	−0.39 **	−0.03	
	23L	0.01	0.10	0.01	−0.23 **	−0.40 **	−0.39 **	−0.03	
	24M	−0.07	0.18 *	−0.05	−0.19 *	−0.27 **	−0.37 **	0.04	
	24L	0.12	0.04	0.06	−0.26 **	−0.44 **	−0.45 **	−0.05	
YD	23M	0.07	0.09	0.30 **	0.24 **	0.21 **	0.24 **	0.10	0.05
	23L	0.17 *	0.79 **	0.02	0.13	0.31 **	0.33 **	0.10	−0.05
	24M	−0.06	0.76	0.34 **	0.11	0.50 **	0.30 **	0.33 **	0.17 *
	24L	0.04	0.56 **	0.19 *	0.19 *	0.23 **	0.14	0.26 **	0.11

* and ** represent significant level of $p < 0.05$ and 0.01 , respectively; CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; Env, environment; 23M, middle season in 2023; 23L, late season in 2023; 24M, middle season in 2024; 24L, late season in 2024; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

In addition, PH and PL were significantly positively correlated with PB, SN and GN, while significantly negatively correlated with SSR under multiple environments. PL and PB were significantly positively correlated with YD, while PB was significantly negatively correlated with KGW.

3.3. M-QTL Identification for Yield-Related Traits

A total of 80 M-QTLs for yield-related traits were identified in the CSSL population across four environments (Table 4), including 8, 5, 12, 14, 9, 6, 12, 12, and 2 QTLs for PH, PN, PL, PB, SN, GN, SSR, KGW and YD respectively. They were located on 11 chromosomes except chromosome 9 and explained 0.02–65.84% of phenotypic variations. N22 alleles

reduced phenotypic values at 32 QTLs while increasing phenotypic values at the remaining 47 QTLs. At *qPB1.3*, the N22 allele decreased PB in 23L while increasing it in 24M.

Table 4. M-QTLs affecting yield-related traits in the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments.

Traits	QTL	Env	Chr	Physical Position (Mb)	LOD	R ² (%)	A
PH	<i>qPH1.1</i>	24L	1	7.97–9.35	39.75	3.68	6.95
	<i>qPH1.2</i>	24M	1	21.79–26.78	3.14	1.45	10.13
	<i>qPH1.3</i>	24M	1	32.17–35.63	44.58	44.63	7.95
		24L	1	32.17–35.63	5.45	0.54	3.83
	<i>qPH1.4</i>	23M	1	38.28–39.00	17.54	41.07	11.82
		23L	1	38.28–39.00	22.3	48.35	14.7
		24M	1	38.28–39.00	40.35	34.38	17.52
		24L	1	38.28–39.00	21.15	2.02	26.88
	<i>qPH1.5</i>	24M	1	42.22–42.40	5.87	2.81	−4.04
	<i>qPH2</i>	24L	2	4.01–20.32	6.23	0.55	5.33
	<i>qPH6</i>	24L	6	19.40–27.55	20.37	1.11	−5.37
	<i>qPH11</i>	23M	11	26.11–29.02	3.2	8.82	0.6
PN	<i>qPN1.1</i>	24M	1	32.17–35.63	3.81	10.68	5.14
	<i>qPN1.2</i>	24L	1	38.37–39.28	3.29	9.46	1.05
	<i>qPN2</i>	23L	2	0.77–4.01	3.23	9.09	−2.42
	<i>qPN5.1</i>	23L	5	4.60–4.87	5.59	16.1	−1.76
	<i>qPN5.2</i>	24M	5	15.99–16.88	2.73	7.13	−0.86
PL	<i>qPL1.1</i>	24L	1	23.67–32.17	7.05	1.64	5.02
	<i>qPL1.2</i>	24M	1	32.17–35.63	48.86	65.84	1.38
	<i>qPL1.3</i>	23L	1	40.48–40.82	6.97	0.06	−0.86
	<i>qPL2.1</i>	24L	2	6.81–16.27	8.08	1.64	0.5
	<i>qPL2.2</i>	23L	2	19.77–22.52	59	26.48	2.97
	<i>qPL3</i>	24M	3	15.69–16.26	3.99	2.47	0.96
	<i>qPL4</i>	23L	4	2.52–6.64	13.19	11.62	0.29
		24M	4	2.52–6.64	3.76	2.15	1.04
	<i>qPL6</i>	24L	6	19.40–27.55	8.24	1.64	0.5
	<i>qPL8</i>	23L	8	3.39–4.50	3.3	0.02	0.97
	<i>qPL11</i>	24L	11	21.26–22.43	4.4	0.91	1.33
	<i>qPL12.1</i>	24M	12	18.73–19.01	3.96	2.27	0.58
	<i>qPL12.2</i>	24M	12	27.05–27.11	3.12	1.78	0.59
PB	<i>qPB1.1</i>	24L	1	5.12–6.08	3.86	7.18	0.41
	<i>qPB1.2</i>	23M	1	7.49–7.97	3.42	7.86	0.44
		24M	1	7.49–7.97	8.48	5.06	0.42
	<i>qPB1.3</i>	23M	1	32.17–35.63	2.75	8.26	−1.05
		24M	1	32.17–35.63	49.61	60.12	0.67
	<i>qPB1.4</i>	24M	1	37.31–37.47	7.56	4.45	−0.51
	<i>qPB2</i>	24L	2	23.98–24.57	3.01	5.45	−0.66
	<i>qPB3.1</i>	23M	3	4.23–5.03	3.25	7.45	−0.67
	<i>qPB3.2</i>	24M	3	14.83–14.85	4.15	2.32	0.31
	<i>qPB4</i>	23L	4	17.19–19.01	3.77	9.46	0.58
	<i>qPB5</i>	23L	5	23.49–23.60	4.76	10.14	0.38
	<i>qPB7</i>	24L	7	8.91–14.96	3.24	5.93	0.6
	<i>qPB8.1</i>	24M	8	3.39–4.50	5.13	3.09	−0.55
		24L	8	3.39–4.50	9.54	19.76	−1.18
	<i>qPB8.2</i>	23L	8	21.17–21.97	2.89	6.06	0.35
	<i>qPB10</i>	24M	10	1.30–2.20	2.95	1.66	0.35
	<i>qPB12</i>	24M	12	0–2.45	4.53	2.6	0.58
SN	<i>qSN1.1</i>	23M	1	6.24–7.26	4.29	13.91	19.07
		24M	1	6.24–7.26	13.82	23.04	29.46

Table 4. Cont.

Traits	QTL	Env	Chr	Physical Position (Mb)	LOD	R ² (%)	A
	<i>qSN1.2</i>	24M	1	32.17–35.63	11.5	18.52	13.02
	<i>qSN1.3</i>	24L	1	37.45–37.69	7	14.41	−25.92
	<i>qSN1.4</i>	24M	1	39.99–40.48	3.87	5.59	−15.93
	<i>qSN1.5</i>	23L	1	40.82–41.61	5.35	14.44	−24.88
	<i>qSN1.6</i>	23M	1	42.22–42.40	3.34	10.77	−16.72
	<i>qSN3</i>	24L	3	6.16–6.43	4.73	14.15	−5.55
	<i>qSN6</i>	24M	6	17.62–27.45	2.68	3.77	5.87
	<i>qSN8</i>	24L	8	0.18–0.48	2.64	5.07	12.53
GN	<i>qGN1.1</i>	23M	1	6.24–7.26	3.4	7.63	14.98
		24L	1	6.24–7.26	7.35	12.77	19.53
	<i>qGN1.2</i>	23M	1	32.17–35.63	4.45	13.79	−4.44
		24M	1	32.17–35.63	7.15	8.22	−9.14
		24L	1	32.17–35.63	4.25	6.72	−18.02
	<i>qGN1.3</i>	24M	1	38.28–39.00	12.94	15.86	3.34
	<i>qGN1.4</i>	23L	1	40.82–41.61	5.9	13.05	−22.76
	<i>qGN3</i>	24L	3	6.16–6.43	6.05	14.7	−5.55
	<i>qGN6</i>	24M	6	17.62–27.45	2.52	2.67	5.2
SSR	<i>qSSR1.1</i>	24M	1	7.97–9.35	4.55	6.99	11.23
	<i>qSSR1.2</i>	24M	1	32.17–35.63	11.74	7.32	32.89
	<i>qSSR1.3</i>	24M	1	38.37–39.28	13.33	8.46	9.33
		24L	1	38.37–39.28	5.73	11.07	3.6
	<i>qSSR1.4</i>	23M	1	42.40–42.92	20.95	9.5	7.83
	<i>qSSR2.1</i>	23M	2	4.01–20.32	3.99	3.3	11.7
		23L	2	4.01–20.32	3.89	6.06	6.63
		24M	2	4.01–20.32	6.98	7.52	12.12
	<i>qSSR2.2</i>	23L	2	29.63–31.20	2.67	2.75	7.29
	<i>qSSR3.1</i>	24M	3	3.54–4.23	5.1	6.97	12.32
	<i>qSSR3.2</i>	23M	3	10.08–10.12	3.49	2.44	6.18
	<i>qSSR5</i>	24L	5	22.65–23.49	3.26	6.22	2.02
	<i>qSSR6</i>	24M	6	19.40–27.55	5.95	6.57	14.33
	<i>qSSR8</i>	24M	8	28.27–28.35	11.59	10.11	15.01
	<i>qSSR12</i>	24L	12	18.91–19.46	5.27	10.33	3.15
KGW	<i>qKGW1.1</i>	24M	1	6.24–7.26	4.66	2.45	−0.59
		24L	1	6.24–7.26	6.56	16.83	−0.68
	<i>qKGW1.2</i>	24M	1	12.30–23.00	5.79	4.6	−2.17
	<i>qKGW1.3</i>	24M	1	32.17–35.63	34.5	29.91	0.96
	<i>qKGW2.1</i>	24M	2	4.61–5.62	5.97	5.09	−1.79
	<i>qKGW2.2</i>	23L	2	6.05–6.38	5.75	10.52	−1.77
	<i>qKGW3</i>	24M	3	6.16–6.43	5.63	4.09	−2.53
	<i>qKGW4</i>	23L	4	33.47–34.91	2.9	6.13	−1.64
		24L	4	33.47–34.91	2.55	7.61	−1.6
	<i>qKGW5</i>	24M	5	0.83–18.48	4.85	5.53	−1.49
	<i>qKGW6</i>	24M	6	19.40–27.55	5.65	3.82	−3.42
	<i>qKGW7</i>	23L	7	0–2.40	2.66	4.5	−0.58
	<i>qKGW8</i>	24L	8	23.21–28.32	3.54	8.76	−1.01
	<i>qKGW12</i>	23L	12	23.90–23.92	2.77	5.35	−0.57
YD	<i>qYD1</i>	23L	1	32.17–35.63	2.72	6.43	−8.74
	<i>qYD2</i>	23L	2	4.01–4.61	3.65	8.62	−2.62

CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; Env, environment; 23M, middle season in 2023; 23L, late season in 2023; 24M, middle season in 2024; 24L, late season in 2024; Chr, chromosome; R², phenotypic variation explained by QTL; A, additive effect; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

Two QTLs affecting PH were repeatedly detected across multiple environments. Among them, *qPH1.3* was consistently identified in both 24M and 24L, while *qPH1.4* was stably expressed across all four environments. They explained that 0.54–48.35% of phenotypic variations under different environments, and N22 alleles at both loci increased PH.

qPL4 for PL was identified in both 23L and 24M, with 11.62% and 2.15% of phenotypic variations explained, respectively. The N22 allele increased PL in both environments.

Three QTLs controlling PB were repeatedly detected in two environments. Among them, *qPB1.2* and *qPB1.3* were stably expressed in 23M and 24M, while *qPB8.1* was repeatedly detected in the 24M and 24L. They explained up to 60.13% of the variations. The N22 allele increased PB at *qPB1.2*, but decreased it at *qPB1.3*. Notably, the N22 allele at *qPB1.3* decreased PB in 23M, while increasing it in the 24M.

qSN1.1 affected SN in 23M and 24M, with 13.91% and 23.04% of phenotypic variations explained, respectively. The N22 allele increased SN under both environments.

Two QTLs for GN on chromosome 1 were repeatedly detected across multiple environments. Of them, *qGN1.1* was stably expressed in 23M and 24L, with an average phenotypic variation explained of 10.20%, and the N22 allele increased GN. *qGN1.2* was repeatedly identified in 23M, 24M, and 24L, explaining at least 6.72% of phenotypic variations, and the N22 allele decreased GN.

qSSR1.3 and *qSSR2.1* were repeatedly identified for SSR across multiple environments. *qSSR1.3* was repeatedly detected in the 24M and 24L, while *qSSR2.1* was stably expressed in 23M, 23L and 24M. The phenotypic variations explained by these two QTLs ranged from 3.30% to 11.07%, and N22 alleles increased SSR at both QTLs.

qKGW1.1 and *qKGW4* were repeatedly detected to affect KGW under multiple environments. Among them, *qKGW1.1* was identified in 24M and 24L, while *qKGW4* was stably expressed in 23L and 24L. These two QTLs averagely explained 8.26% of the phenotypic variations, and N22 alleles reduced KGW.

3.4. Pleiotropic QTLs for Yield-Related Traits

Of the 80 detected M-QTLs, 45 QTLs (56.25%) were located in eight pleiotropic QTL regions (Table 4; Figure 2).

Three pleiotropic QTLs containing six QTLs were identified in the 6.24–9.35 Mb region on chromosome 1. The region of 6.24–7.26 Mb affected SN, GN and KGW, the region of 7.49–7.97 Mb controlled PB, and the region of 7.97–9.35 Mb affected PH and SSR. These QTLs explained 3.68–23.04% of the phenotypic variations. The N22 allele increased phenotypic values at all QTLs except *qKGW1.1*.

The 32.17–42.40 Mb region on chromosome 1, identifying 18 QTLs, contained four adjacent pleiotropic QTLs. Among them, the 32.17–35.63 Mb region controlled all nine yield-related traits, the 38.28–39.28 Mb region showed pleiotropic effects on PH, PN, GN and SSR, the 40.82–41.61 Mb region affected both SN and GN, and the 42.22–42.92 Mb region controlled PH, SN and SSR. They explained at least 0.54% of the phenotypic variations. N22 allele decreased phenotypic values at *qPH1.5*, *qSN1.5*, *qSN1.6*, *qGN1.2*, *qGN1.4* and *qYD1*, while increased phenotypic values at the rest QTLs.

CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; 23M, middle season in 2023; 23L, late season in 2023; 24M, middle season in 2024; 24L, late season in 2024; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

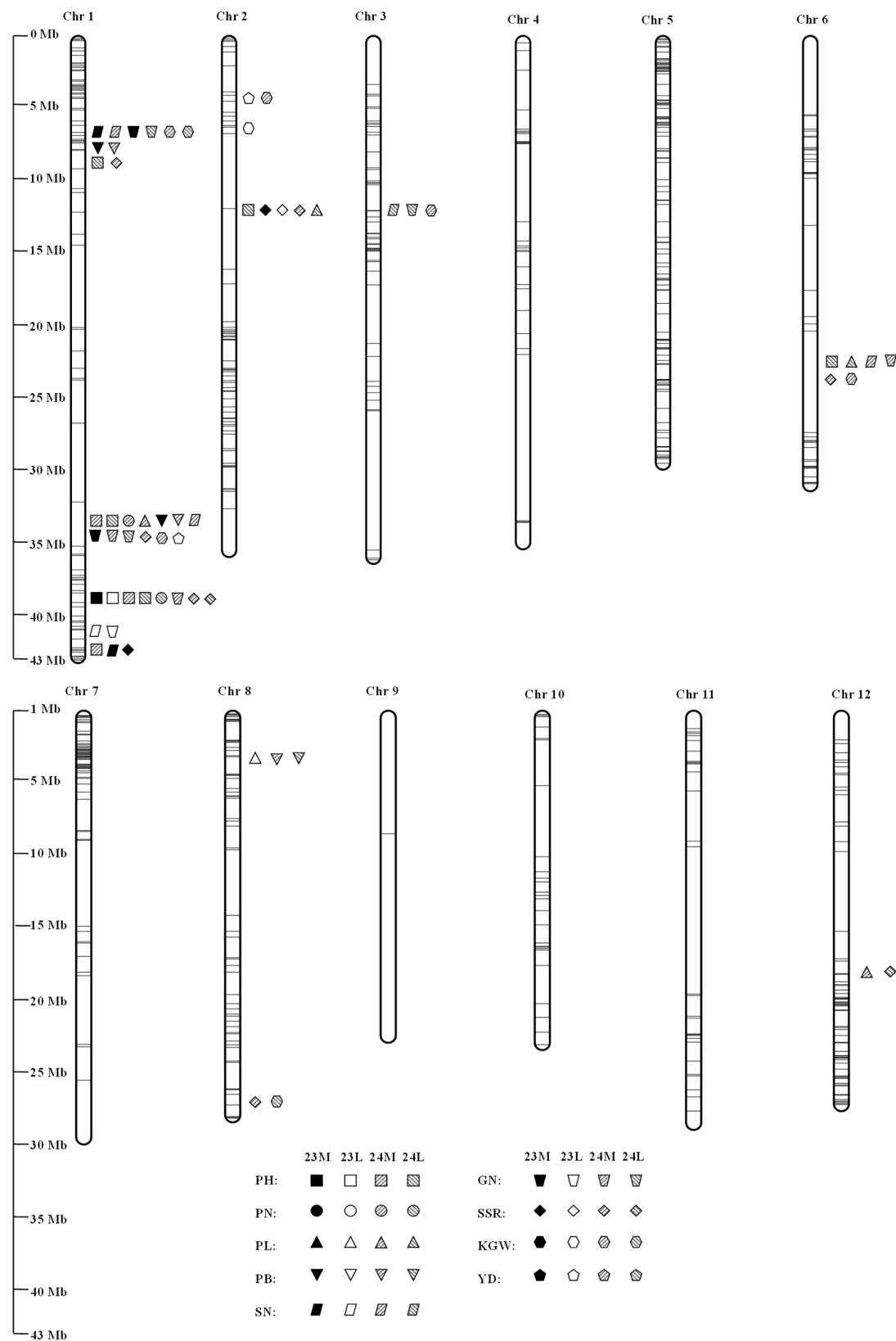


Figure 2. Pleiotropic QTLs detected in the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments (23M, 23L, 24M and 24L, respectively).

Six QTLs in the region of 4.01–20.32 Mb on chromosome 2 simultaneously affected PH, PL, SSR, KGW and YD. They explained 5.41% of the average phenotypic variation. N22 allele increased PH, PL and SSR, while decreasing KGW and YD.

Three QTLs in the 6.16–6.43 Mb region on chromosome 3 simultaneously affected SN, GN and KGW. It explained up to 14.70% of the phenotypic variations. The HHZ allele increased all of SN, GN and KGW.

Six QTLs in the 17.62–27.55 Mb region on chromosome 6 controlled all yield-related traits except PN, PB and YD. This region explained 1.11–6.57% of the phenotypic variations. N22 allele decreased PH and KGW, while increasing the rest traits.

Two QTLs affecting both PL and PB were identified in the region of 3.39–4.50 Mb on chromosome 8. The N22 allele simultaneously increased both traits, with phenotypic variations explained ranging from 0.02% to 19.76%.

qSSR8 and *qKGW8*, simultaneously controlling SSR and KGW, were co-mapped in the 28.27–28.35 Mb region on chromosome 8. This region increased both traits, and explained 10.11% and 8.76% of the phenotypic variations respectively.

Both of *qPL12.1* and *qSSR12*, which simultaneously affected PL and SSR, were identified in the region of 18.73–19.46 Mb on chromosome 12. It increased both PL and SSR, explaining 2.27% and 10.33% of the phenotypic variations, respectively.

3.5. E-QTL Underlying Yield-Related Traits

A total of 260 pairs of E-QTLs affecting yield-related traits were identified (Supplementary Table S1), 65, 51, 5, 3, 30, and 106 E-QTL pairs for PH, PL, SN, GN, SSR and KGW respectively. Among them, four pairs occurred between two M-QTLs, 50 pairs between one M-QTL and one locus, and the rest between two loci without main-effect. A total of 88 pairs decreased phenotypic values, while 172 pairs increased them.

Two pairs, between 6.24 and 7.26 Mb on chromosome 1 and 0.99–1.57 Mb on chromosome 7 and 0–2.45 Mb on chromosome 12, reduced KGW under 23M and 24M, explaining 0.89–1.25% of the phenotypic variations.

One pair between 0 and 23.01 Mb on chromosome 9 and 23.90–23.92 Mb on chromosome 12 was found for decreasing PH and increasing PL. One pair between 3.54 and 4.23 Mb on chromosome 3 and 17.31–20.97 Mb on chromosome 5 affected both PH and SSR. One pair between 12.92 and 13.74 Mb on chromosome 3 and 16.02–29.70 Mb on chromosome 7 was found for both PH and KGW. One pair between 22.52 and 23.07 Mb on chromosome 2 and 0.83–18.48 Mb on chromosome 5 had pleiotropic effects on PH, SN and SSR. One pair between 0.83 and 18.48 Mb on chromosome 5 and 0–23.01 Mb on chromosome 9 was identified for PL and KGW. Two pairs, between 0.83 and 18.48 Mb on chromosome 5 and 2.73–2.85 Mb on chromosome 7, and between 0.83 and 18.48 Mb on chromosome 5 and 19.99–20.02 Mb on chromosome 12, were found for SN and SSR. One pair between 4.39 and 5.12 Mb on chromosome 1 and 0.83–18.48 Mb on chromosome 5 simultaneously affected SN and KGW. Three pairs, between 0.17 and 23.33 Mb on chromosome 3 and 9.67–9.91 Mb on chromosome 6, between 0.83 and 18.48 Mb on chromosome 5 and 19.40–27.55 Mb on chromosome 6, and between 0.17 and 23.33 Mb on chromosome 8, were identified for both SSR and KGW. These E-QTLs explained 0.50% to 6.03% of phenotypic variations.

3.6. M-QTL $\times$ Environment Interaction Analysis

In the present study, 6, 2, 5, 9, 6, 4, 7, 7, and 1 Q $\times$ E were detected for PH, PN, PL, PB, SN, GN, SSR, KGW and YD, respectively (Table 5), with an average phenotypic variation explained of 7.90%. Among them, 20 interactions decreased phenotypic values across three environments, 16 interactions increased phenotypic values under two environments, and 11 interactions increased phenotypic values in three environments.

Table 5. Interaction between M-QTL and environment for yield-related traits in the CSSL population derived from *indica* HHZ and *Aus* N22.

Traits	M-QTL	LOD	R ² (%)	AE1	AE2	AE3	AE4
PH	<i>qPH1.1</i>	16.71	18.48	−15.05	−14.89	−12.30	42.24
	<i>qPH1.2</i>	3.13	0.29	−6.12	−1.22	7.61	−0.26
	<i>qPH1.3</i>	27.64	9.85	−31.26	−34.93	68.94	−2.74
	<i>qPH1.4</i>	32.09	5.05	3.99	4.40	−12.61	4.22
	<i>qPH1.5</i>	4.44	0.23	0.71	0.45	−2.13	0.97
	<i>qPH11</i>	2.52	0.47	−1.13	−2.28	1.43	1.98
PN	<i>qPN1.1</i>	5.95	4.95	0.45	−1.87	4.86	−3.45
	<i>qPN5.2</i>	2.69	2.42	0.30	0.46	−0.58	−0.18
PL	<i>qPL1.2</i>	18.71	1.93	−4.80	−3.66	12.14	−3.68
	<i>qPL1.3</i>	2.85	0.08	0.24	−0.44	0.21	−0.01
	<i>qPL3</i>	2.68	0.05	−0.16	−0.34	0.55	−0.06
	<i>qPL8</i>	2.55	0.07	−0.16	0.72	−0.37	−0.19
	<i>qPL12.2</i>	2.80	0.06	−0.10	−0.12	0.46	−0.25
PB	<i>qPB1.2</i>	6.07	3.72	0.20	−0.14	0.18	−0.24
	<i>qPB1.3</i>	33.10	44.06	−1.89	−2.51	5.66	−1.25
	<i>qPB1.4</i>	6.64	3.81	−0.19	0.20	−0.30	0.29
	<i>qPB2</i>	2.68	2.95	0.34	0.14	0.01	−0.49
	<i>qPB3.2</i>	2.96	1.23	−0.04	−0.18	0.12	0.10
	<i>qPB5</i>	2.91	3.10	0.02	0.24	−0.16	−0.11
	<i>qPB7</i>	4.33	5.07	−0.49	−0.06	−0.01	0.56
	<i>qPB8.1</i>	3.87	3.74	0.19	0.30	0.06	−0.55
	<i>qPB10</i>	2.81	1.42	0.08	−0.11	0.23	−0.19
SN	<i>qSN1.1</i>	10.48	10.78	7.43	−13.31	17.66	−11.78
	<i>qSN1.2</i>	12.74	11.32	−43.79	−70.69	115.87	−1.38
	<i>qSN1.3</i>	4.45	3.40	2.72	6.98	6.74	−16.45
	<i>qSN1.4</i>	3.15	2.43	4.34	4.99	−11.60	2.28
	<i>qSN1.5</i>	3.05	6.02	5.08	−18.04	6.60	6.35
	<i>qSN1.6</i>	2.63	3.47	−12.56	2.14	5.58	4.84
GN	<i>qGN1.1</i>	12.20	13.35	−6.73	−8.42	23.06	−7.91
	<i>qGN1.2</i>	9.88	7.66	−45.77	−15.73	59.18	2.32
	<i>qGN1.3</i>	10.69	11.32	−11.99	−7.17	25.75	−6.59
	<i>qGN1.4</i>	2.69	5.81	1.59	−14.95	7.86	5.50
SSR	<i>qSSR1.2</i>	9.61	18.88	−11.18	−5.76	26.39	−9.45
	<i>qSSR1.3</i>	9.64	23.08	−2.79	−3.99	6.15	0.63
	<i>qSSR1.4</i>	12.20	26.89	5.51	−2.12	−1.26	−2.13
	<i>qSSR3.2</i>	3.22	4.34	3.65	−2.49	−0.05	−1.11
	<i>qSSR5</i>	2.91	2.92	−1.72	0.58	0.31	0.84
	<i>qSSR8</i>	8.45	19.93	−3.31	−5.73	10.83	−1.79
	<i>qSSR12</i>	4.17	3.27	−1.34	−0.10	−0.65	2.09
KGW	<i>qKGW1.1</i>	4.49	4.42	−0.36	0.35	0.35	−0.34
	<i>qKGW1.2</i>	2.90	1.09	0.47	0.45	−0.82	−0.11
	<i>qKGW1.3</i>	30.02	16.83	−2.07	−2.22	6.72	−2.43
	<i>qKGW2.1</i>	4.14	1.57	0.38	0.49	−0.81	−0.06
	<i>qKGW2.2</i>	5.93	2.62	0.22	−1.07	1.04	−0.19
	<i>qKGW4</i>	3.11	0.45	−0.39	−0.19	0.70	−0.11
	<i>qKGW8</i>	3.60	1.19	0.23	−0.15	0.46	−0.53
YD	<i>qYD1</i>	4.63	1.43	−0.70	−7.28	11.13	−3.15

CSSL, chromosome segment substitution line; HHZ, Huanghuazhan; M-QTL, M-QTL listed in Table 4; R², phenotypic variation explained by QTL; AE1~AE4, M-QTL × environment interaction in 23M, 23L, 24M and 24L respectively; PH, plant height; PN, panicle number; PL, panicle length; PB, primary branch number; SN, spikelet number per panicle; GN, grain number per panicle; SSR, seed setting rate; KGW, 1000-grain weight; YD, grain yield per plant.

4. Discussion

In the present study, a total of 80 M-QTLs for rice yield-related traits were identified using a set of CSSL population derived from the *indica* variety HHZ as the recipient parent and the *Aus* variety N22 as donor parents. Of these, 56.25% of the QTLs were located in eight pleiotropic QTL regions. Most of these QTL regions were mapped in the same or adjacent regions with reported cloned yield-related genes/QTLs. For example, the region of 6.24–9.35 Mb on chromosome 1 (*qPH1.1*, *qPB1.2*, *qSN1.1*, *qGN1.1*, *qSSR1.1* and *qKGW1.1*) was located in the adjacent region of *Gn1a* [12], *qPPG1-2*, *qSSR1* [46] and *qKw1.2* [47], and co-mapped with *TN1a* [48]; the region of 32.17–42.40 Mb (*qPH1.3*, *qPH1.4*, *qPH1.5*, *qPN1.1*, *qPN1.2*, *qPL1.2*, *qPB1.3*, *qSN1.2*, *qSN1.5*, *qSN1.6*, *qGN1.2*, *qGN1.3*, *qGN1.4*, *qSSR1.2*, *qSSR1.3*, *qSSR1.4*, *qKGW1.3* and *qYD1*) was located in the same region of *sd1* [10], *OsPH1* [49], *qGpp1*, *qKw1.2*, *qYd1* [47], and near *RGN1a/qGNP1.3* [50]. The region of 4.01–20.32 Mb on chromosome 2 (*qPH2*, *qPL2.1*, *qSSR2.1*, *qKGW2.1*, *qKGW2.2* and *qYD2*) was mapped in the same region of *GW2* [19] and in the adjacent region of *GL2/GS2/OsGRF4* [20–22]. The 6.16–6.43 Mb region on chromosome 3 (*qSN3*, *qGN3* and *qKGW3*) was identified near *gn3* [51]. The 17.62–27.55 Mb region on chromosome 6 (*qPH6*, *qPL6*, *qSN6*, *qGN6*, *qSSR6* and *qKGW6*) was mapped in the same region of *TGW6* [52], *MOC1* [11], *GW6a* [53] and *GL6* [54] and near *DEP3* [55]. The 3.39–4.50 Mb region on chromosome 8 (*qPL8* and *qPB8.1*) was mapped in the same region of *Ghd8/DTH8* [15,16], and the region of 28.27–28.35 Mb (*qSSR8* and *qKGW8*) was mapped in the same region of *IPA1/OsSPL14* [17,18]. In addition, *qSSR2.2*, *qPL3*, *qKGW5* and *qPB7* was mapped in the same region of *qGY2-1* [56], *GS3* [23], *GW5/GSE5* [24,25], *GS5* [26] and *Ghd7* [13], respectively; *qPL2.2*, *qSSR3.2*, *qPB4*, *qKGW4*, *qKGW7*, *qKGW12* and *qPL12.2* were mapped in the adjacent region of *qPL2.2* [7], *pss3* [51], *GIF1* [57], *NAL1* [58], *BISEII* [43], *TGW12a* [59] and *PINOID* [60], respectively. In the future, map-based cloning and transgenic verification would be used to determine whether these QTLs are identical or linked with the genes/QTLs reported in previous studies. Furthermore, there were also 25 M-QTLs newly identified in the present study, which may be specific to *Aus*. They would provide a theoretical basis for dissecting rice subspecies differentiation, and identifying elite alleles in germplasm resources will provide valuable gene resources for molecular breeding for high-yield rice varieties.

Of the two parents used in this study, N22 exhibited higher PH, PN, SSR, and YD, while HHZ had longer PL, more PB, SN, GN, and higher KGW. Besides the QTLs in the region of 32.17–42.92 Mb from N22, the N22 alleles at *qPH1.1*, *qPH1.2*, *qPH2*, and *qPH11* increased PH, at *qPN1.1* and *qPN1.2* enhanced PN, and at all 12 QTLs raised SSR, and these alleles from N22 were novel alleles. Moreover, HHZ alleles at *qPL1.3* increased PL, at *qPB1.4*, *qPB2*, *qPB3.1*, and *qPB8.1* enhanced PB, at *qSN1.3*, *qSN1.4*, *qSN1.5*, *qSN1.6*, and *qSN3* were associated with higher SN, at *qGN1.2*, *qGN1.4*, and *qGN3* increased SN, at most QTLs except *qKGW1.3* enhanced KGW, and these alleles from HHZ were novel alleles. In addition, HHZ alleles at 6 QTLs increased PH, PN, and YD, and N22 alleles at 28 QTLs enhanced PL, PB, SN, GN, and KGW. In total, besides the QTLs in the region of 32.17–42.92 Mb for PH, N22 and HHZ contained 46 and 31 novel alleles, respectively. The N22 allele was more predominant. However, HHZ is an elite *indica* variety in southern China and has strong lodging resistance and superior grain quality. Further, we could introgress the N22 novel alleles into HHZ background and increase its yield.

Rice yield-related traits are typical quantitative traits controlled by multiple genes and largely affected by the environment. Xing et al. [4] identified 25 M-QTLs for yield-related traits under two environments, of which 10 M-QTLs were repeatedly detected in both years. Wei et al. [8] identified 68 QTLs for yield-related traits using the same population under low-nitrogen and normal-nitrogen conditions over two years, with only 35.29% of the QTLs stably expressed in both years. Xu et al. [5] detected 19 yield QTLs using an *indica-japonica*

recombinant inbred line population, of which only five were stably expressed in two years. Jiang et al. [6] detected 33 QTLs for panicle traits in F_2 and $F_{2:3}$ populations derived from the super rice “Chaoyou 1000”, with only 2 QTLs stably expressed in both populations. In this study, of the 80 M-QTLs for yield-related traits, only 13 QTLs (16.25%) were repeatedly detected under multiple environments, and 47 QTLs (58.75%) interacted with environments, suggesting that the environment had a large effect on the expression of M-QTLs. Moreover, only 2 of 260 E-QTLs were stably expressed across different environments, suggesting that E-QTLs were more sensitive to environmental effects, consistent with the report of Qiu et al. [43] on rice appearance quality traits. Breeders should use M-QTLs that are stably expressed across different environments and show minimal or no interaction with the environment in high-yield breeding programs to ensure varieties restore high yield across diverse environments.

Nowadays, increasing rice yield via breeding intra-subspecific varieties has become increasingly difficult. Therefore, breeders have proposed a new strategy of utilizing inter-subspecific heterosis. In the present study, a set of CSSL population was developed using an elite *indica* variety HHZ as the recipient parent and the *Aus* variety N22 as the donor parent. A total of 80 M-QTLs affecting rice yield-related traits were identified, of which 45 QTLs (56.25%) were located in eight pleiotropic QTL regions. By introgressing single or pyramiding a few pleiotropic QTLs into elite varieties via a backcross breeding strategy, breeders can simultaneously improve multiple yield-related traits. Compared with pyramiding multiple genes to increase yield, this method not only reduces labor and money but also decreases the risk of failure of genetic improvement [7]. Therefore, they are genetic resources with high value. In addition, 13 M-QTLs stably expressed across different environments can significantly enhance the yield stability across diverse environments. In the future, fine mapping and cloning of these important QTLs and mining favorable alleles in germplasm resources would provide valuable genes for high-yield breeding. Moreover, of the eight pleiotropic QTL regions, the region of 6.24–9.35 Mb on chromosome 1 simultaneously affected PH, PB, SN, GN, SSR, and KGW, and most QTLs were identified in two environments. N22 alleles increased most traits except KGW. It was the first priority to improve the yield-related trait. The second priority was the region of 17.62–27.55 Mb on chromosome 6. This region controlled PH, PL, SN, GN, SSR, and KGW. The N22 allele was associated with increased PL, SN, GN, and SSR, and decreased PH and KGW. The third priority was the region of 18.73–19.46 Mb for PL and SSR. The N22 allele could increase both traits. We could improve multiple yield-related traits across multiple environments by pyramiding these regions and other QTLs for KGW, which saves large amounts of labor and money. More importantly, the former two regions would help breeders improve the heat tolerance of *indica* varieties. Thus, they are the most important gene resources for yield improvement.

In addition, six high-yield CSSLs were screened in this study, which showed higher traits such as PN and GN than HHZ [41]. They can be directly cultivated as new high-yield varieties or used as elite germplasm resources for breeding new high-yield varieties.

5. Conclusions

In this study, a set of CSSL population was developed, derived from the *indica* variety HHZ and the *Aus* variety N22. A total of 80 M-QTLs and 260 E-QTLs for rice yield-related traits were identified across four environments. Among them, 56.25% of the M-QTLs were located in eight pleiotropic QTL regions, 13 QTLs were stably expressed across different environments, and 47 QTLs showed interactions with environment interactions. These findings provide a theoretical basis for clarifying the genetic differentiation of rice sub-

species and cloning yield-related genes and offer valuable gene resources for the molecular breeding of high-yield rice varieties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16050580/s1>, Figure S1: Graphical genotypes of CSSL population derived from *indica* HHZ and *Aus* N22; Table S1: E-QTLs affecting yield-related traits in the CSSL population derived from *indica* HHZ and *Aus* N22 across four environments.

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