Identification of QTLs for Heat Tolerance at Flowering Stage Using Chromosome Segment Substitution Lines in Rice

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Research Article

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Abstract

High temperature is a major stress in rice production. Although considerable progresses have been made on investigating heat tolerance (HT) in rice, the genetic basis of HT at heading stage remains largely unknown. In this study, a novel set of chromosome segment substitution lines (CSSLs) consisting of 113 lines derived from a heat-resistant *indica* variety N22 and a heat-sensitive *indica* variety 9311 was developed and used for the analysis of genetic basis of HT. The heat sensitivity index (HSI) calculated based on seed-setting rates under natural and high temperature environments was used to evaluate the influence of HT at rice heading stage. Totally, five QTLs associated with HT were detected based on seed-setting rate (SSR) evaluation; these were named *qSSR6-1*, *qSSR7-1*, *qSSR8-1*, *qSSR9-1* and *qSSR11-1* located on chromosomes 6, 7, 8, 9 and 11, respectively. Heat-tolerant alleles of the QTLs were all derived from N22. Among them, *qSSR9-1* overlapped with QTLs identified previously, while the remaining QTLs were found novel. Especially, *qSSR7-1* explained a high phenotypic variation of 26.35% with a LOD score of 10.75, thus deserved to be further validated. These findings will increase our understanding of the genetic mechanism underlying HT and facilitate the breeding of heat-tolerant rice varieties.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop for over half of the world's population. However, the high frequency of high temperature stress has brought a huge challenge facing rice production in recent years (Zhang 2007). It has been reported that the optimal temperature of rice at seedling stage is 28–32°C, and that of rice at heading stage is 25–35°C (Qiu et al. 2018). When the temperature is below 20°C or higher than 40°C, rice fertilization will be seriously harmed (Hakata et al. 2017). Moreover, high temperature has been found to cause heat stress for rice growth, which led to the reduction of rice yields (Huang et al. 2017). Therefore, it is becoming urgent to breed heat-tolerant rice varieties or identify them from pre-existing germplasms.

Many studies have primarily focused on heat tolerance (HT) in rice at flowering stage or booting stage. For instance, Matsui et al. (2002) found that high temperature induced sterility in rice at flowering. It was also reported that high temperature (> 33.7°C) affected spikelet fertility of rice (Jagadish et al. 2007). Following the increased temperature and the prolonged duration, the seed-setting rate (SSR) gradually decreased (Chen et al. 2017). Additionally, many HT-related quantitative trait loci (QTLs) have been detected on all 12 chromosomes in rice, such as *qHTSF1.2*, *qHTSF2.1*, *qHTSF3.1*, *qHTSF4.1*, *qHTSF6.1* and *qHTSF11.2* (Kobayashi et al. 2013; Ye et al. 2015a; Lafarge et al. 2017; Shanmugavadivel et al. 2017). Especially, one major QTL (*qHTSF4.1*) has been observed in several studies across different genetic backgrounds, and it was validated to increase spikelet fertility under heat stress at flowering (Ye, et al. 2015a and 2015b), indicating that this QTL is a valuable target for enhancing HT in rice at flowering stage. In addition, Zhu et al. (2017) identified 12 QTLs for HT at the booting stage in rice. Among them, one of the major effect QTLs, *qHTB3-3*, was detected on chromosome 3 and finally mapped between the markers RM3525 and 3-M95, approximately 2.8 Mb.

Several rice populations have been used in QTL analysis, such as backcross inbred lines (BILs) (Matsumoto et al. 2017), recombinant inbred lines (RILs) (Gichuhi et al. 2016) and chromosome segment substitution lines (CSSLs) (Qi et al. 2017). Of which, CSSLs are generated by crossing a donor parent with a recipient, then followed by several backcrosses to the recurrent parent. The CSSLs contain the entire genome of the donor parent based on marker assisted selection (MAS). Each line of CSSLs carries only one or a few homozygous fragments of the donor genotype in the genetic background of the recurrent parent, thereby eliminating genetic background noise and allowing the detection of QTL with additive minor effects (Yamamoto et al. 2009; Li et al. 2015). Currently, this strategy has been widely applied in rice as a powerful tool to precisely detect QTLs of important agronomical traits without concerning complex interactions among QTLs (Ando et al. 2008; Zhao et al. 2016; Surapaneni et al. 2017).

In this study, a novel set of CSSLs population was developed by using MAS with the heat-tolerant cultivar N22 as the donor and the heat-sensitive cultivar 9311 (an elite variety with good agronomic traits and available genome sequences) as the receptor parent. Subsequently, this set of population was applied to identify QTLs associated with HT assessed by SSR at heading stage. The results will provide a useful clue to understand the genetic basis of HT in rice and contribute to breeding heat-tolerant rice varieties in the future.

Materials And Methods

Plant materials

In this study, two *indica* rice varieties, N22 and 9311, were used to develop CSSLs. The donor parent N22 is internationally recognized as a variety with specific heat resistance but poor agronomic traits, thus it is very difficult to be directly applied in production. The receptor parent 9311 is an elite variety which belongs to the rice genome sequencing project, and has good comprehensive agronomic traits and wide compatibility; however, it is very sensitive to high temperature.

Construction of CSSLs

The F_1 plants derived from a cross between 9311 and N22 were backcrossed to the recurrent parent 9311 to produce 98 BC_1F_1 plants. Then BC_1F_1 generation was preliminary detected to produce BC_2F_1, using 143 simple-sequence-repeat markers distributed across the 12 rice chromosomes. BC_2F_1 plants were then backcrossed twice to 9311, to produce a BC_4F_1 generation. According to the genotype determination, the BC_4F_1 generation was selfed consecutively to generate BC_4F_2, BC_4F_3, and BC_4F_4, and finally a set of CSSLs consisted of 113 lines was developed to nearly cover the entire N22 genome.

DNA isolation and PCR

The DNA was extracted from freshly frozen leaves of individuals by using improved CTAB method (Oliveira et al. 2015). Extracted DNA was stored in ultra-pure water at − 20°C in a refrigerator. Simple-sequence-repeat marker primers were selected from genetic marker maps and public databases, and

synthesized by Sangon Biotech Company (Shanghai, China). DNA amplification was performed using PCR with the following conditions: 94°C for 4 min; 33 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s; and a final cycle of 72°C for 5 min. PCR products were separated in 4% polyacrylamide denaturing gels (PAGE), and bands were visualized using the silver-staining protocol (Panaud et al. 1996).

Measurement of heat tolerance traits

A total of 113 CSSLs and parents were used to evaluate heat resistance in 2014 and 2015 at the experimental farm of Anhui Agricultural University (Hefei, China). Seed-setting rate was taken as the indicator of HT and used for QTL analysis.

For the SSR and heat sensitivity index (HIS) measurement, CSSLs and the two parents were planted under two different conditions when they were grown to four-leaf stage. The plants grown under natural environment condition of the field were considered as the control, while those planted in pots and moved into a greenhouse for high temperature treatment were the treatment. The temperature was set to 38°C ± 2°C and the humidity was 75% ± 5% from 8:00 to 16:00 in the greenhouse, while during the rest periods of a day, the greenhouse films were opened to make sure the growth condition of the greenhouse was the same as that of the natural environment. High temperature treatment was continuously performed until the end of the heading stage, then these plants were moved back to the natural environment until maturity. Finally, five panicles of each line were randomly harvested to calculate the SSR. The mean SSR of the five panicles selected in each pot was investigated as the HSI and used to evaluate HT of the CSSLs and analyze genetic effects of the substitution fragments. SSR and HSI were calculated according to the following formulas:

$$\mathrm{SSR}(\%) = \frac{\mathrm{Thenumberoffullyfilledseeds}}{(\mathrm{Thenumberoffullyfilledseeds} + \mathrm{Thenumberofemptyseeds})} \mathrm{x100}\%$$

$$\mathrm{HSI}(\%) = (\mathrm{Seedsettingrateofcontrol} - \mathrm{Seedsettingrateofheatstress})$$

Statistical analysis and QTL mapping

Statistical analyses were conducted using SPSS20.0 software (SPSS, Chicago, IL, USA). QTL analysis were performed using QTLIciMapping 4.2 (http://qtl-icimapping.software.informer.com/), and recombination values were converted to cent-Morgans (cM) using the Kosambi mapping function. A total of 143 simple-sequence-repeat markers were used in construction of linkage map. IciMapping 4.2 was also used to calculate the phenotypic variance explained (PVE, %) by a QTL and the additive effect. Primer 5.0 software was used to develop simple-sequence-repeat markers for fine mapping. The genomic sequence was obtained from the National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/. RAP-DB (http://rapdb.dna.affrc.go.jp/) was used to carry out gene annotation within specific genomic regions according to the Nipponbare genome sequence. The GGT 2.0 software (http://www.plantbreeding.wur.nl) (Van Berloo 2008) was used to calculate pair-wise r^2 values between 143 markers distributed throughout the genome and analyse genotype of each sample and

med as follows: the abbreviation of the

corresponding trait (e.g., HT for heat tolerance and SSR for seed-setting rate) followed by 1–12 (the rice chromosome on which the corresponding QTL was detected), and a final number indicating the marker interval on one chromosome. For example, *qSSR6-1* indicates that the 1st interval for seed-setting rate that was detected on chromosome 6.

Results

Polymorphic simple-sequence-repeat markers identification

In total, 356 simple-sequence-repeat markers distributed throughout the 12 rice chromosomes were applied to analyze polymorphisms between 9311 and N22, among which 143 (40.17 %) were polymorphic between these two parents (Table 1, Fig. 1). The average distance between two adjacent markers on the rice linkage map was 11.3 cM. The number of polymorphic marker in each chromosome ranged from 9 to 18. The polymorphism ratio of chromosome 3 was as high as 52.94% and that of chromosome 12 was as low as 32.14%. Additionally, the polymorphism ratio of other chromosomes ranged from 33.33–48.39%. Subsequently, these polymorphic simple-sequence-repeat markers were used to develop CSSLs.

Table 1
Distribution of polymorphic markers on the 12 chromosomes

Chromosome	Number of polymorphic marker	Number of total marker	Polymorphic rate
Chr.1	11	33	33.33%
Chr.2	15	31	48.39%
Chr.3	18	34	52.94%
Chr.4	12	31	38.71%
Chr.5	11	28	39.29%
Chr.6	14	33	42.42%
Chr.7	12	31	38.71%
Chr.8	11	29	37.93%
Chr.9	10	23	43.48%
Chr.10	9	25	36.00%
Chr.11	11	30	36.67%
Chr.12	9	28	32.14%
Average	11.9	29.7	40.17%
Total	143	356	

CSSLs development and marker-assisted selection (MAS)

CSSLs were schematically developed according to the procedure of Fig. 2. Following the initial cross between 9311 and N22, 9311 was selected as the recurrent parent to backcross with the hybrid. A total of 98, 110, and 121 individuals from BC_1F_1, BC_2F_1, and BC_3F_1 populations were genotyped, respectively, using the 143 polymorphic simple-sequence-repeat markers. From each backcross generation, the optimal individuals that have minimal number of donor segments were selected, to make sure that all the selected lines can nearly cover the whole genome of the donor parent. In winter 2011, a total of 129 BC_4F_1 individuals were obtained and planted for self-fertilization to produce BC_4F_2. After screening of 2,580 BC_4F_2, 179 individuals were selected and then planted for self-fertilization to produce BC_4F_3. Similarly, 127 out of 3,580 BC_4F_3 individuals were selected and self-fertilized to produce BC_4F_4. Finally, a set of CSSLs including a total of 113 lines (BC_4F_4) was developed to nearly cover the entire N22 genome.

Distribution of substituted segments on chromosomes in CSSLs

There were 166 homozygous chromosome segments and 9 heterozygous segments introgressed in the 113 CSSLs, on average, each CSSL contained 1.6 introgressed chromosome segments (Table 2, Fig. 3). Moreover, the distribution of introgressed chromosome segments was uneven among the 12 chromosomes. Chromosome 3 had the most introgressed segments (20), while chromosome 1, 11 and 12 had the fewest segments (11). Among the 113 CSSLs, the length of the 175 introgressed chromosome segments ranged from 0.5 cM to 59 cM, with an average of 15.3 cM (Table 2, Fig. S1). Specifically, 36.6% of the substituted segments were smaller than 10 cM, 34.3% were from 10 to 20 cM, 20% ranged from 20 to 30 cM, and 12.1% were over 30 cM (Fig. S1).

Table 2
Substitution of N22 segments in CSSLs

Chromosome	Number of lines	Number of segments	Total segment length (cM)	Average segment length (cM)
Chr.1	9	11	167.7	15.2
Chr.2	14	19	370.0	19.5
Chr.3	14	20	238.5	11.9
Chr.4	11	14	233.6	16.7
Chr.5	7	17	226.4	13.3
Chr.6	10	14	187.5	13.4
Chr.7	9	17	276.9	16.3
Chr.8	11	16	269.7	16.9
Chr.9	8	12	187.8	15.7
Chr.10	6	13	178.2	13.7
Chr.11	7	11	181.8	16.5
Chr.12	7	11	164.8	15.0
Total	113	175	2682.9	15.3[a]

Note: [a] Average length of all introgression segments

Phenotypic performance of SSR under natural and high temperature at heading stage

Seed-setting rates under natural and high temperature at heading stage were used to calculate the HSI to assess HT. If HSI was low, HT would be good and vice versa. As shown in Table 3, the SSRs of 9311 in natural environment (F1, 78.84% and 92.88%) were significantly higher than that in high temperature

environment (E2, 3.14% and 11.03%), and the HSI values of the parental varieties 9311 were 75.70% and 82.85%, respectively. Similarly, the SSRs of N22 in natural environment (E1, 97.48% and 97.52%) were also higher than that in high temperature environment (E2, 50.21% and 61.89%), and the HSI values of the parental varieties 9311 were 47.27% and 35.63%, respectively. The results showed that the HT of N22 was better than that of 9311, indicating that cultivar N22 was more tolerant to heat stress than cultivar 9311. In natural environment, the SSRs of CSSLs ranged from 35.37 to 85.21% with a mean of 71.38% in 2014, and from 68.76 to 94.69% with a mean of 89.6% in 2015, respectively. In high temperature environment, however, the SSRs of CSSLs ranged from 1.03 to 36.52% with a mean of 8.56% in 2014, and ranged from 2.1 to 52.39% with a mean of 18.19% in 2015, respectively. Thus, the mean HSI of the CSSLs was 62.82% in 2014 and 71.41% in 2015. The CSSL population segregation for the SSR of rice was distributed continuously in the two environments (Fig. S2). These results strongly indicated that high temperature at heading stage significantly hindered rice production.

Table 3
Phenotype data of seed-setting rate of rice under natural and high temperature for CSSLs and parents (9311 and N22) across two environments

Year	Materials	Environment [a]	SSR (%)			HSI (%)			*P*-value [b]
			Mean	Max	Min	Mean	Max	Min	
2014	9311	E1	78.84	-	-	75.70	-	-	0.0001**
		E2	3.14	-	-				
	N22	E1	97.48	-		47.27	-	-	0.0054**
		E2	50.21	-	-				
	CSSLs	E1	71.38	85.21	35.37	62.82	48.69	34.34	-
		E2	8.56	36.52	1.03				
2015	9311	E1	93.88	-	-	82.85	-	-	0.0001**
		E2	11.03	-	-				
	N22	E1	97.52	-	-	35.63	-	-	0.0076**
		E2	61.89	-	-				
	CSSLs	E1	89.60	94.69	68.76	71.41	42.30	66.66	-
		E2	18.19	52.39	2.10				

Note: [a] E1 indicates natural environment, while E2 indicates high temperature environment; [b] ** means the significance levels of 0.01 between 9311 and N22.

QTL mapping for the SSR under high temperature at heading stage

In total, five QTLs (*qSSR6-1*, *qSSR7-1*, *qSSR8-1*, *qSSR9-1*, and *qSSR11-1*), referred to the HSI under high temperature at heading stage were detected and mapped on chromosomes 6, 7, 8, 9 and 11, respectively (Table 4, Fig. 4). The LOD values ranged from 2.81 to 10.75, and the PVE ranged from 5.83 to 26.35%. Among them, *qSSR7-1* (RM248) had a high PVE of 26.35% with a LOD score of 10.75, therefore was considered as a major QTL for HSI under high temperature. *qSSR11-1* (RM224) had a medium PVE of 14.21%, while the remaining three had low PVEs which ranged from 5.83–7.66%. The additive effects of these QTLs ranged from − 8.17 to -17.37, indicating that these QTLs contributed by N22 had synergistic effect on the HSI of rice.

Table 4
QTLs for seed-setting rate of rice under high temperature detected in CSSLs population

QTLs	Chromosome	Marker	LOD value	PVE (%)	Additive effect
qSSR6-1	6	RM528	2.81	5.83	-8.17
qSSR7-1	7	RM248	10.75	26.35	-17.37
qSSR8-1	8	RM284	2.95	6.11	-9.12
qSSR9-1	9	RM242	3.63	7.66	-11.36
qSSR11-1	11	RM224	6.37	14.21	-13.90

Discussion

High temperature is one of the major environmental factors influencing rice growth and productivity. Song et al. (2011) showed that 45°C high temperature resulted in suppressed root and stem growth. In addition, high temperature caused low SSR and reduced yield especially during flowering period. One of the main reasons is that heat stress can cause bad anther dehiscence, which resulted in low pollen germination of stigma and reduced pollen production and spikelet fertility (Prasad et al. 2006; Rang et al. 2011). Therefore, spikelet fertility and SSR were commonly used as indicators of HT in rice (Yeet al. 2015a; Zhao et al. 2016; Zhu et al. 2017). In this study, SSR was selected for HT evaluation and QTL mapping at rice heading stage.

We identified five detected QTLs (*qSSR6-1*, *qSSR7-1*, *qSSR8-1*, *qSSR9-1* and *qSSR11-1*) associated with HT (Fig. 1, Table 4). Among them, *qSSR9-1* was identified on chromosome 9 and accounted for 7.66% of the phenotypic variations. Similarly, Shanmugavadivel et al. (2017) also identified a QTL (*qSTIPSS9.1*) for HT of rice on chromosome 9 through using a 5K SNP array. It is worthy to note that two HT QTLs, *qHt9a* (RM108-RM242) and *qHt9a* (RM242-RM566), which shared the same location of *qSSR9-1*, were previously reported using a set of RILs (Chen et al. 2008), indicating that *qSSR9-1* is a major QTL for the HT of rice. *qSSR8-1* located on chromosome 8 and accounted for 6.11% of the phenotypic variations. Two QTLs, *qDFT8* and *qHT-8*, were also previously identified for HT in rice and located on chromosome 8, which explained 31.10% and 51.67% of the phenotypic variation, respectively (Zhao et al. 2016; Liu et al.

2017); however, they were distinctly different from *qSSR8-1* according to their mapping results, suggesting that *qSSR8-1* might be a novel QTL for HT. *qSSR7-1* was found to be a major QTL located on chromosome 7, which explained up to 26.35% of the phenotypic variance. According to Zhao et al. (2016), $qPSL^{ht}7$, which was associated with spikelet fertility under high temperature in "Sasanishiki"/"Habataki" CSSLs population across three environments, located adjacent to *qSSR7-1* on chromosome 7 and explained 79.0% of the phenotypic variation. Therefore, *qSSR7-1* might also be a new QTL. *qSSR6-1* and *qSSR11-1* explained 5.83% and 14.21% of the phenotypic variation, respectively. On the basis of data obtained from the QTL Annotation Rice Online Database [Q-TARO, http://qtaro.abr.affrc.go.jp/] and comparison with previously reported QTLs, the two minor QTLs (*qSSR6-1*, *qSSR11-1*) detected in this study might be new.

Since extremely high temperatures caused significant loss in rice production, breeding heat-tolerant rice varieties or identifying heat-tolerant rice varieties from pre-existing germplasm has become a big concern to rice breeders. Ishimaru et al. (2010) utilized the Early-Morning Flowering (EMF) trait from *O. glaberrima* and screened heat-tolerant rice from improved and traditional rice varieties. Compared with EMF trait screen, the progeny selection of heat resistant plants in a traditional crossing program requires high labor and economic costs. Therefore, the MAS breeding has become more and more essential for breeders to breed heat-tolerant rice varieties (Zhao et al. 2016). In recent years, many putative QTLs for HT have been identified in rice; however, the QTLs of stable effect still remain rare. Hence, we designed this research to explore and further confirm useful QTLs associated with HT of rice in heading stages. Of the five QTLs identified in this study, *qSSR9-1* is consistently identified with previous studies (Chen et al. 2008; Ye et al. 2015a) and can be used for rice HT improvement by MAS, while the others are novel and need to be further confirmed. These findings would contribute to better understanding of the genetic basis of HT in rice and accelerating the process of breeding heat-tolerant rice varieties.

Abbreviations

BILs, backcross inbred lines; *Chr,* chromosome*; cM,* centiMorgan*; CSSLs*, chromosome segment substitution lines; *GGT*, graphical genotypes; *HT,* heat tolerance; *HSI, heat sensitivity index; LOD*, logarithm of odds; *MAS*, marker-assisted selection; *NCBI*, National Center for Biotechnology Information; *PCR*, polymerase chain reaction; *PVE*, phenotypic variance explained; *QTL*, quantitative trait locus; *QTLs*, quantitative trait loci; *RAP-DB*, Rice Annotation Project Database; *RILs*, recombinant inbred lines; *SSR*, seed-setting rate.

Declarations

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Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable

Availability of data and materials

All data supporting the conclusions of this article are provided within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NTL and CQQ conceived and designed the experiments. NTL and SSJ, performed the experiments and analyzed the data. CMY were responsible for material plant and field management. NTL wrote the manuscript. CQQ revised the manuscript. All authors read and approved the manuscript.

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Figures

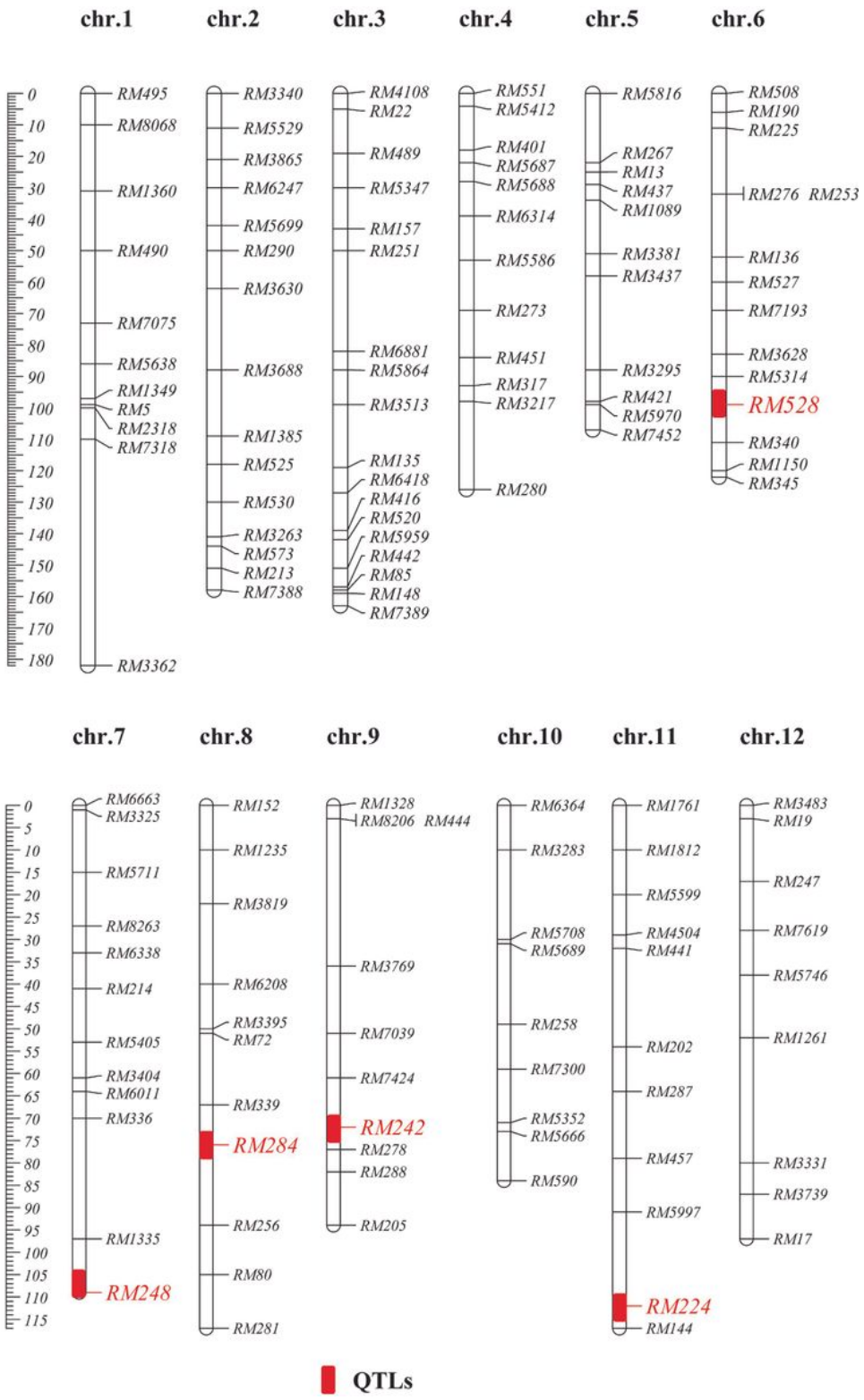

Figure 1

Genetic location of 143 polymorphic simple-sequence-repeat markers and distribution of QTLs for HT traits using simple-sequence-repeat. Molecular markers are shown to the right of chromosomes, and genetic locations (cM) of each marker shown to the left of chromosomes. Red rectangles indicate the QTLs for for HT.

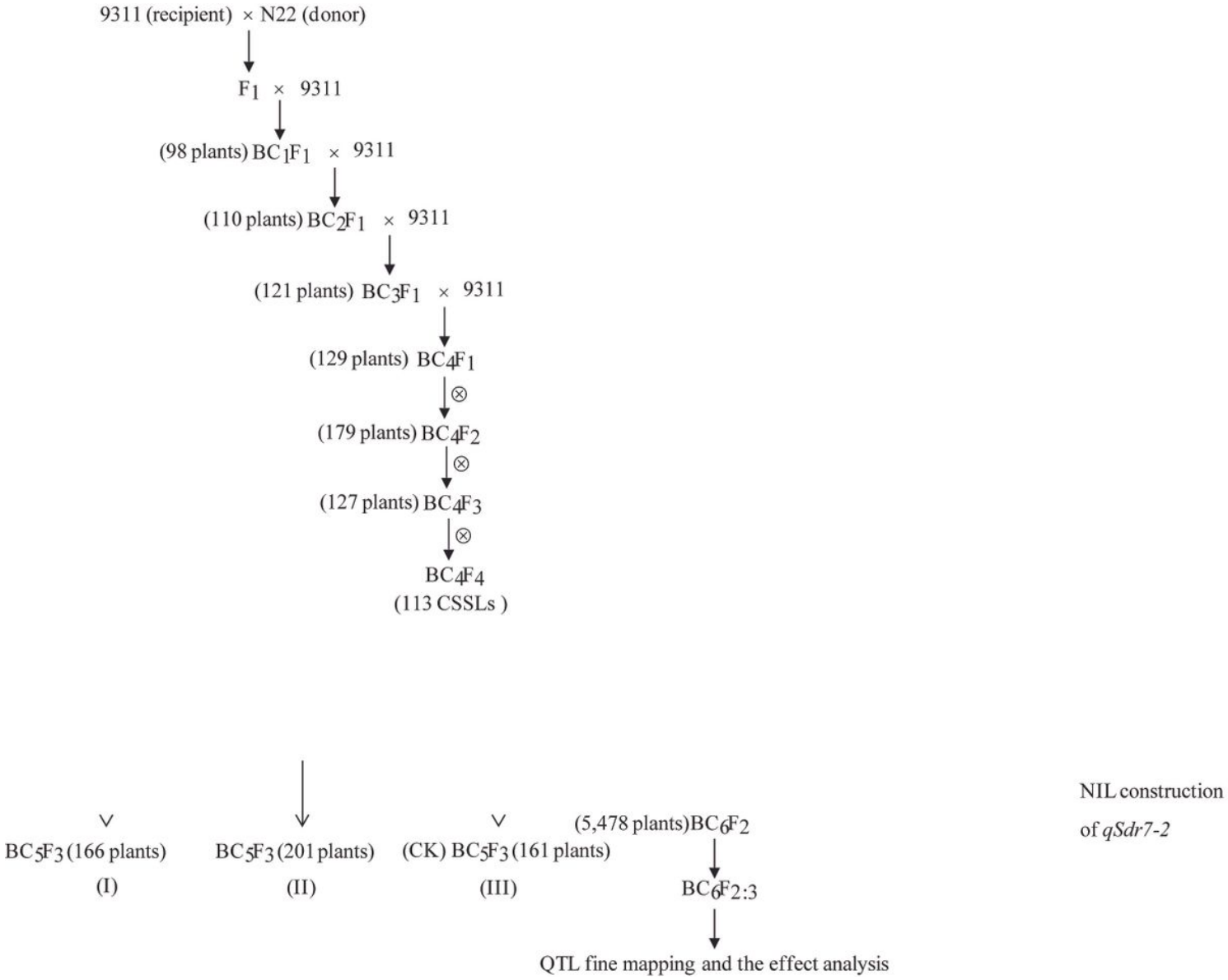

Figure 2

Schematic of the development of CSSLs carrying N22 chromosome segments in 9311 genetic background.

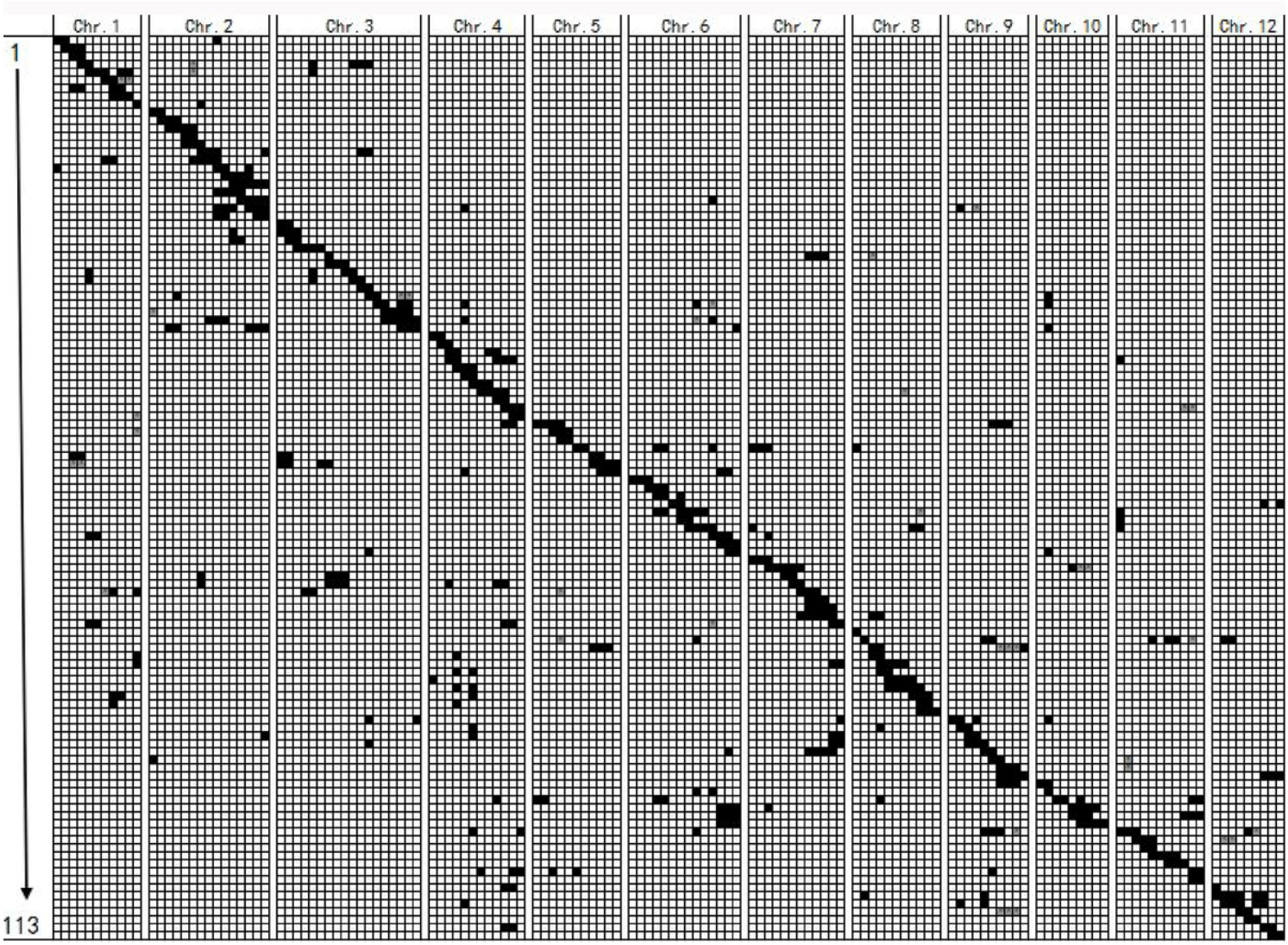

Figure 3

Graphic of genotypes of the 113 CSSLs. Black regions indicated homozygous segments, while gray regions indicate heterozygous segments. Regions with a white background represent 9311 background. The horizontal axis indicates marker segments on chromosome 1 to 12, and the vertical axis indicates CSSLs.

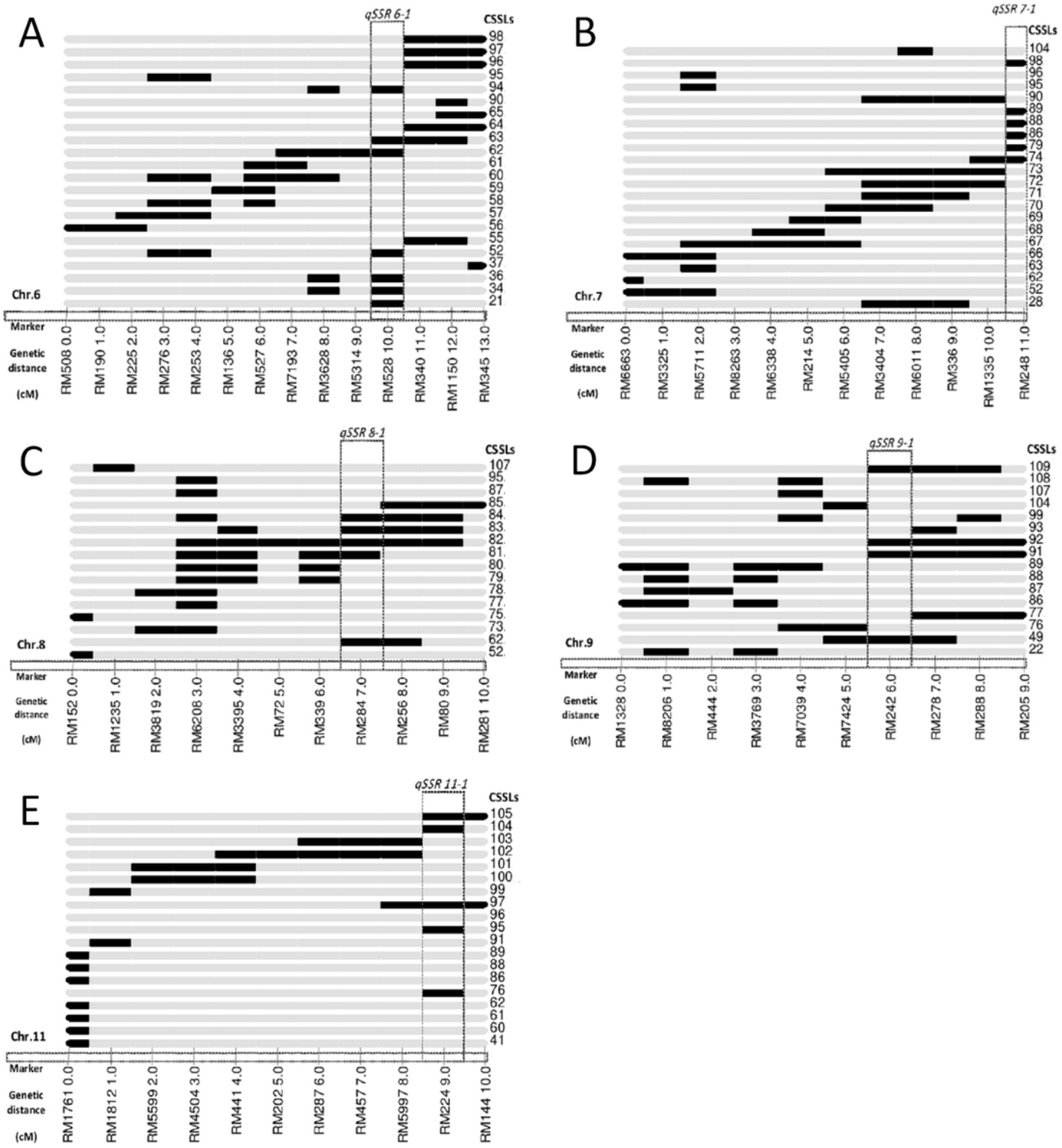

Figure 4

Substitution mapping of qSSR6-1(A), qSSR7-1(B), qSSR8-1(C), qSSR9-1(D) and qSSR11-1 (E) for SSR on rice chromosomes.

Supplementary Files

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