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QTL Mapping of Yield, Yield Components, and Morphological Traits in Rice (*Oryza sativa* L.) Using SSR Marker

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ABSTRACT

The experiment was aimed at identifying QTL (quantitative trait loci) controlling ten traits of yield, yield component and plant morphology of rice based on BC_1F_1 of IR75862-206-2-8-3-B-B-B//IR64 mapping population consisted of 115 plants. It was arranged in Completely Randomized Design with three replicates. Ninety three SSR markers spread across the twelve rice chromosomes were used to map the QTL. These markers were mostly segregated according to Mendel Law except for fourteen markers. There were eleven QTL detected in eight traits, i.e., heading date, flag leaf length, plant height, panicle length, panicle weight, seed set, weight of 100 grains, and grain weight per plant, meaning that one or two QTL were detected in each trait. These QTL were located at chromosome 2, 3, 4, 6, 11, and 12. Some QTL were located at the same chromosome even at the same location indicating the close association of the traits. It also indicated that there were common QTL which were found across genetic background and specific QTL which were found at specific genetic background. Further study was prospective for the molecular marker application in rice improvement.

Key words: QTL, agronomic traits, SSR, rice

INTRODUCTON

Important traits of plant such as yield and yield related traits are mostly quantitative traits which have continuous phenotypic distribution. It implied that many genes with relatively small effect termed as QTL (quantitative trait loci) controlling the traits. The ability of molecular marker technique to dissect quantitative trait into Mendelian loci (Tanksley, 1993) opens the chance to identify and locate QTL. In turn it will be very useful for plant improvement (Septiningsih *et al.*, 2003).

Yield and yield related traits have been the major goal of rice breeding and remain important in the future. Studies on agronomic traits have been done and yielded promising result (Jing-hong *et al*., 2008). Some agronomic traits such as heading date, panicle characteristics, spikelet fertility, grain size as well as the yield have become the focus in some studies (Vergara *et al.*, 1996; Moncada *et al.*, 2001; Septiningsih *et al.*, 2003; Thomson *et al.*, 2003; Cui *et al.*, 2004; Linh *et al.*, 2006; Jing-hong *et al.*, 2008). On the other hand, flag leaf characteristic may significantly contribute to the yield, but less study was done on this particular trait. QTL study of those agronomic traits will be useful for the application of molecular marker in breeding for the particular agronomic traits and yield as well.

Simple Sequence Repeats (SSR) or micro satellite marker is a marker of choise for rice study and is increasingly used in the recent studies. SSR marker is simple and relatively cheap (PCR based marker), accurate (work into the DNA), could be used for high throughput application (Panaud *et al.*, 1995), also is a co-dominant marker that can detect multi allele in a locus (Powell *et al*., 1996). A lot of SSR markers have been developed for rice study. McCouch *et al.* (2002) reported that there were 2240 unique marker loci validated in rice, or approximately one SSR at every 157 kb of rice genome. More SSR could be searched especially after the completion of rice genome sequencing (IRGSP, 2005; Yu *et al*., 2002). Zhang *et al.* (2007) reported the availability of another 52,845 SSR markers.

This study was aimed at identifying QTL controlling yield, yield components, and morphological traits using SSR marker.

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MATERIALS AND METHODS

Plant Material

Selection of parents was conducted in the previous research consisting of elite lines as well as modern and traditional varieties having *indica*, *japonica*, and wild relative genetic background. The materials were tested for at least two seasons and sites in the Philippines. IR75862-206-2-8-3-B-B-B and IR64 were selected as parents. IR75862-206-2-8-3-B-B-B is a new plant type line, derived from cross combination of IR65600-81-5-3-2/*Oryza longistaminata*//2*IR65600-81-5-3-2. IR75862-206-2-8-3-B-B-B has mixed characteristics of *indica* and *japonica* rice sub-species. IR64 is an indica variety and is a popular modern high yielding variety widely adopted by farmers.

Development of mapping population started by single crossing in dry season 2006 (January to June) and continued by backcrossing into IR64 in wet season of 2006 (July to December). Backcross population (BC_1F_1) of IR75862-206-2-8-3-B-B-B//IR64 consisting of 115 individual genotypes was used for mapping population.

Field Observation

The backcross population was planted in dry season 2007 (January – July, agronomic trait observation was included) at IRRI Experimental Station. Each 15 plants of parents and F_1 population were included as check. The seed was sown individually and transplanted at 21 days after sowing into field as single seedling per hill with the space of 20 cm x 20 cm. At the maximum tillering stage, the plant was splitted into three identical clones and transplanted according to Completely Randomized design of three replicates (triplicate) at the prepared field with the space of 20 cm x 20 cm. Purple rice (rice plant having purple color in a whole plant) was planted as two rows border around the nursery. Fertilization was done by considering the need of the plant based on site specific nutrient management. Fertilizers were applied four times. Basal aplication was 50 kg/ha of complete fertilizer (14 N 14P 14K), second one was 30 kg N/ha (as Urea) three weeks after transplanting, the third was 40 kg N/ha (as Urea) five weeks after transplanting, and the fourth was 20 kg N/ha (as Urea) at nine weeks after transplanting.

Observation was done on ten agronomic traits, i.e., heading date (number of days required from sowing to the panicles emergence from the leaf sheath of the plant), flag leaf length (the length from the base to the tip of randomly five flag leaves per plant; mm), flag leaf width (the width of five selected flag leaves measured at the middle of the leaf; mm); plant height (the length of stem measured from the soil surface to the tip of highest panicle without any consideration about the awn; cm); panicle length (the length from the base of panicle to the tip of the last grain or awn of the grain; two panicles were measured from each plant; mm); panicle weight (weight of both panicles from each plants from base of panicle to the tip of the last grain or awn of the grain after removing the flag leaf; mg); number of total grain per panicle (total from number of filled and unfilled grain of each panicle); seed set (percent of filled grain out of the total grain per panicle); grain weight per plants (weight of clean filled grain of each plant); and weight of 100 grains (weight of 100 randomly chosen, clean and filled grains; g).

SSR Analysis

Molecular works were done at GAMMA (Gene Array and Molecular Marker Arrangement) Laboratory of IRRI during the year of 2007. A set of 527 SSR markers were tested to find polymorphic marker between the parents (McCouch *et al.*, 2002). The sequences of each of the primer pairs were downloaded from http://www.gramene.org/microsat/ssr.html. Among the 143 polymorhic SSR markers, 93 were selected, i.e. those that were robust and relatively well distributed in the rice genome, and used for QTL mapping. There were 12, 10, 9, 7, 9, 8, 7, 7, 7, 4, 7, and 6 markers used at chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, respectively.

Molecular works for parental survey (selection of polymorphic markers between parent) as well as for the mapping population (BC_1F_1 of IR75862-206-2-8-3-B-B-B//IR64) was done as follows. DNA was extracted from 35 day-old leaves using modified CTAB method (Murray and Thompson 1980). PCR reaction was conducted in 10 µl reaction solution consisting of 50 ng of template DNA, 0.25 µM of each forward and reverse primer, 100 µM of each dNTPs, 1 X reaction buffer (20 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin) and 0.5 unit of Taq DNA polymerase. The profile of PCR reaction is 94^{o}C for 5 minutes continued by 35 cycles of denaturizing in 94 ^{o}C for one minutes, annealing temperature at 55 ^{o}C or 61 ^{o}C for one minute, and extension at 72 ^{o}C for two minutes, after 35 cycles then final product extension at 72 ^{o}C for five minutes. PCR product was kept in 4 ^{o}C until further use. Eight percent of polyacrilamide gel electrophoresis was conducted in 1 x TBE buffer at 100 Volt for two to four hours depending on the product size. Cyber safe^{TM} (invitrogen) staining was done for 30 minutes and DNA visualization was then conducted under UV light using Gel Documentation System.

Data Analysis

Chi square (χ^2) test formulated as $\{(O-E)^2/E\}$at α=5% was applied to detect the deviation of marker segregation from Mendel Law. O means the observed proportion of alleles and E means the predicted value of the proportion.

Frequency distribution of phenotypic data of each traits and QTL mapping including Single Marker Regression (SMR), Simple Interval Mapping (SIM), and Composite Interval Mapping (CIM) was analyzed using QGene version 4.0 (Nelson, 1997). LOD value of 3 or higher than the threshold value based on 1000 times permutation (Moncada *et al.*, 2001) was used to select the putative QTL.

RESULTS AND DISCUSSION

Markers Segregation and Traits Frequency Distribution

Based on the χ^2 test, there were 14 SSR markers, out of the 93 markers, significantly deviated from the Mendel Law showing segregation distortion and skewed to one of the possible genetic constitution (IR64 or heterozygous). RM508, RM204, RM276, RM7193, RM3827 of chromosome 6 and RM248 of chromosome 7, RM3428 and RM5349 of chromosome 11 skewed to IR64 constitution. RM1869 of chromosome 4, RM5432 and RM332 of chromosome 8, RM288 of chromosome 10, RM1240 of chromosome 11, and RM3226 of chromosome 12 skewed to heterozygous constitution.

Skewness might be due to the sterility or incompatibility gene(s) that disturb the viability of gamete to the progeny. It may due to the wide genetic background of parents. Association of the sterility or incompatibility genes with the marker tested caused the distortion of the marker segregation. Segregation distortion was reported in some crosses such as BC_2F_2 of IR64/*O rufipogon* (Septiningsih *et al.*, 2003), BC_2F_2 of Jefferson/*O. rufipogon* (Thomson *et al.*, 2003), DH population of IR64/Azuceha (Huang *et al.*, 1997), Nipponbare/Kasalath (Harushima *et al.*, 1996), F_2 population of Taichung 65/*O. glaberrima* (Doi *et al.*, 1998), and RIL of LH422/9024 (Xiao *et al.*, 1998).

The frequency distribution of the observed traits along with the predicted value of the parents and F_1 is illustrated in Figure 1. It was seem that the traits were divergently distributed and the frequency mostly accumulated near to IR64 as recurrent parent.

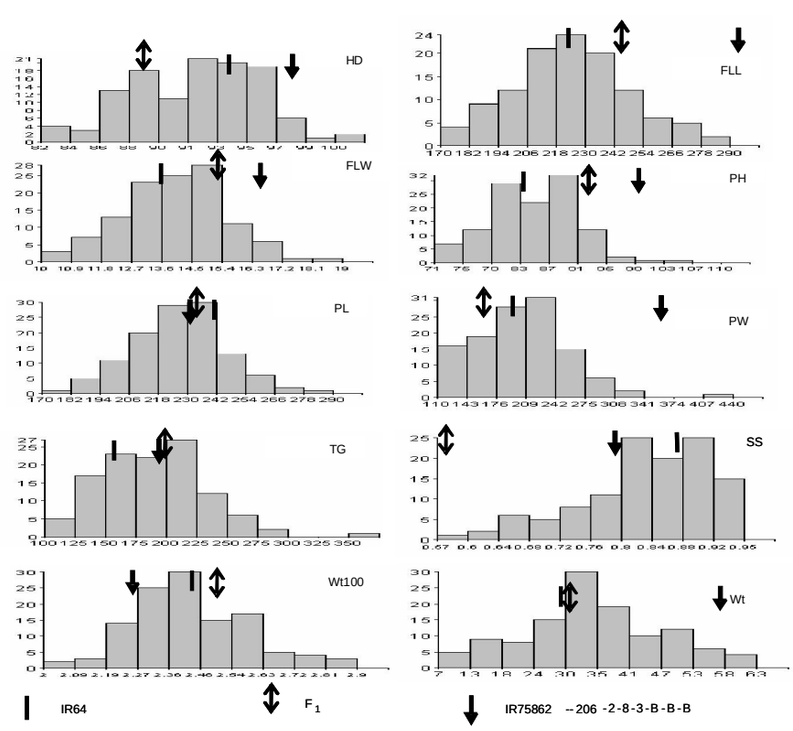

Figure 1. Frequency distribution of some agronomic traits at the BC_1F_1 population

Note: The code refers to Table 1, FLL=flag leaf length (mm), TG=total grain per panicle (grains)

QTL Analysis

Eleven putative QTL (then termed as QTL) controlling eight agronomic traits were detected (Table 1) located at chromosome 2, 3, 4, 6, 11, and 12 (Figure 2). No of QTL of two traits, i.e., flag leaf length and total number of grain per panicle was detected in this study.

SMR, SIM, and CIM analyses were run to determine the QTL. The methods mostly gave the same result, however CIM analysis was the best analysis to conclude the results, because CIM could remove the background effect from neighboring markers and locate the QTL more precisely.

Table 1. QTL of some agronomic traits of the backcross mapping population

No	Trait	QTL Name	Chr	Position (cM)	Marker	Donor	Additive	LOD	R^2
1	HD	*hd-vb12.1*	12	16	RM8216-RM3472	IR64	-3.811	3.362*	0.126
2	FLW	*fll-vb4.1*	4	104	RM317-RM3836	IR75862-	1.632	7.489**	0.259
3	PH	*ph-vb4.1*	4	128	RM3333	IR75862-	4.296	3.413*	0.128
4	PL	*pl-vb11.1*	11	48	RM3701-RM3428	IR64	-13.615	3.073*	0.116
5	PL	*pl-vb12.1*	12	62	RM101-RM1246	IR75862-	15.794	3.528*	0.132
6	PW	*pw-vb4.1*	4	104	RM317-RM3836	IR75862-	0.39	3.321*	0.125
7	SS	*ss-vb6.1*	6	34	RM276	IR64	-0.105	9.944**	0.328
8	SS	*ss-vb6.2*	6	68	RM8226-RM7193	IR64	-0.073	4.195**	0.155
9	Wt100	*wt100-vb2.1*	2	108	RM5430-RM526	IR75862-	0.141	5.24**	0.189
10	Wt100	*wt100-vb3.1*	3	68	RM1324-RM6931	IR64	-0.142	5.36**	0.193
11	Wt	*wt-vb11.1*	11	62	RM3428-RM5349	IR64	-9.884	4.005**	0.148

Note : HD=heading date(days), FLW=Flag Leaf Width (mm), PH=Plant Height (cm), PL=Panicle length (mm), PW=Panicle Weight (g), SS=Seed Set, Wt100=Weight of 100 grains (g), Wt =Weight of grain per plant (g), Chr=chromosome, LOD=Log of Odd

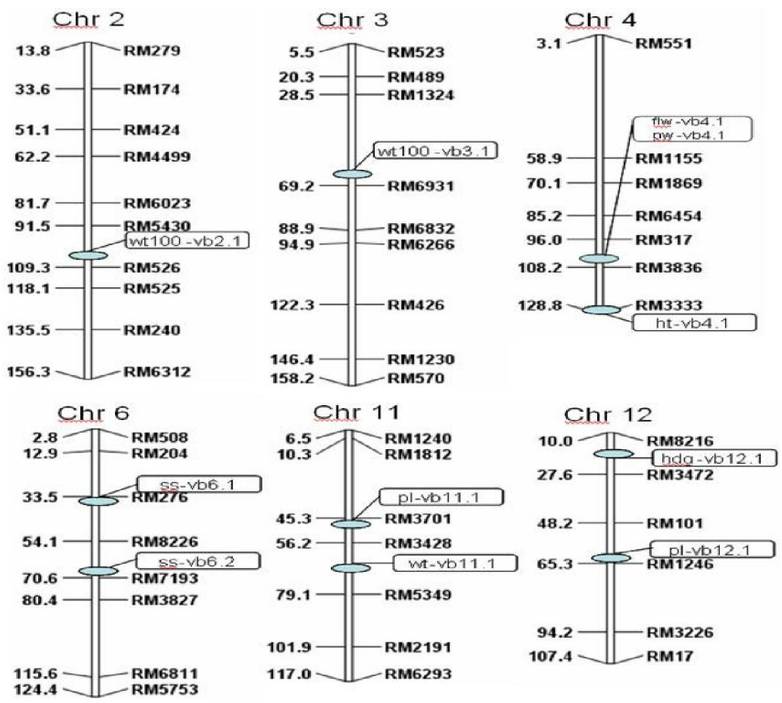

Figure 2. Distribution of the detected QTL among the SSRs applied in the chromosomes
Note: QTL name refers to Table 1

QTL of Heading Date

QTL of *hd-vb12.1* (Table 1) associated with heading dates was detected at chromosome 12 at the position of 16 cM (between RM8216 and RM3472). It contributed to 12.6% of variation and was contributed by IR64 with additive effect of 3.811 day.

Moncada *et al.* (2001) using *Oryza rufipogon* genetic background reported four QTL controlling heading date, located at chromosome 2, 3, and 7 explaining 6-14% of variation. On the other hand, Septiningsih *et al.* (2003) using population from IR64/*O. rufipogon* reported six QTL controlling heading date, located at chromosome 2, 7, 11, and 12 explaining 4-12% of variation. Linh *et al.* (2006) using population from *O. sativa/O. minuta* reported two QTL controlling heading date or days to heading located at chromosome 6 and 9 explaining a total of 43.2% of variation. Cui *et al.* (2004) using Zenshan97/ Minghui63 (*O. sativa* L. ssp. *indica*) reported six QTL controlling heading date located at chromosome 6, 7, 10, and 11 explaining 59.69% variation. Those studies reported different number and location of QTL detected indicating that different mapping populations or genetic backgrond showed different genetic control of the trait.

QTL of Flag Leaf Width

There was one QTL detected associated with flag leaf width, i.e., *fll-vb4.1* (Table 1) at 104 cM of chromosome 4 (RM317-RM3836) explaining 25.9% variation. The additive effect was 1.632 mm contributed by IR75862-206-2-8-3-B-B-B. Yue *et al*. (2006) reported that QTL controlling flag leaf characteristics was also found in chromosome 4. It indicates that chromosome 4 is important for development of flag leaf characteristic.

QTL of Plant Height

QTL of *ph-vb4.1* (Table 1) associated with plant height was detected at 128 cM of chromosome 4 close to RM3333. It explained 12.8% of variation contributed by IR75862-206-2-8-3-B-B-B with additive effect of 4.296 cm.

Moncada *et al.* (2001) reported six QTL controlling plant height located at chromosome 1, 2, 4, and 5 explaining 6–21% of variation. On the other hand, Septiningsih *et al.* (2003) reported five QTL controlling plant height located in chromosome 1, 4, 6, 10, and 11 explaining 6–55% of variation. Cui *et al.* (2004) reported four QTL controlling plant height at maturity located at chromosome 3, 4, and 6 explaining 39.76% of variation. You *et al*. (2006) reported 17 QTL controlling plant height located spreadly at all of the 12 rice chromosomes explaining from 5-23% of variation. All the above mentioned study obtained QTL at chromosome 4 for the trait. It indicated that there were many QTL controlling plant height. Some QTL may be detected in some genetic background and some may be found only in specific genetic background.

QTL of Panicle Length

Two QTL were detected to be associated with panicle length, i.e., *pl-vb11.1* and *pl-vb12.1*. The *pl-vb11.1* located at 48 cM of chromosome 11 closely linked to RM3333 contributing 11.6% of variation. The donor for the favorable allele was IR64 with the additive effect of 13.62 cm. The *pl-vb12.1* located at 62 cM of chromosome 12 between RM101 and RM1246. It was contributed by IR75862-206-2-8-3-B-B-B with the additive effect of 15.79 cm. Both parents had contribution to the length of panicle indicating the existence of multi loci and variation mechanism controlling the trait.

Septiningsih *et al.* (2003) reported five QTL controlling panicle length located at chromosome 1, 3, 9, and 10 explaining 5 to 25% of variation. The QTL were different from the results in this study indicating that there were different mechanisms in controlling panicle length.

QTL for Panicle Weight

The only QTL detected for panicle weight was *pw-vb4.1*. It was located in 104 cM of chromosome 4 between RM317 and RM3836. It could explain 12.5% of variation with additive value of 0.39 g and was contributed by IR75862-206-2-8-3-B-B-B.

QTL for Seed Set

Two QTL were detected to be associated with seed set, i.e., *tg-vb6.1* and *tg-vb6.2*. Both were located at chromosome 6 and both having IR64 as donor. The first was located at 34 cM linked to RM276 which explained 32.8% of variation with additive effect of 0.1% of seed set. The second was located at 68 cM between RM8226 and RM7193 which explained 15.5% of variation with additive effect of 0.07% of seed set.

Moncada *et al.* (2001) reported two QTL controlling the level of sterility located at chromosome 10 explaining around 13% of variation. It was contributed by *O rufipogon* which increased the level of sterility. Septinigsih *et al.* (2003) reported three QTL for the traits located at chromosome 1, 2, and 11 explaining 6 to 10% of variation.

QTL for Weight of 100 Grains

There were two QTL detected for weight of 100 grains, i.e., *wt100-vb2.1* and *wt100-vb3.1*. The first one was contributed by IR75862-206-2-8-3-B-B-B located

in 108 cM of chromosome 2 (between RM5430 and RM 526) having additive effect of 0.14 g and explained 18.9% of variation. The second one was contributed by IR64 located at 68 cM of chromosome 3 (between RM1324 and RM6931) which explained 19.3% of variation.

Moncada *et al.* (2001) reported five QTL associated with 100 grain weight all derived from *O rufipogon* located at chromosome 1, 3, and 11 explaining 5 – 22% of variation. The QTL at chromosome 1 might be close to the QTL found in this study (wt100-vs1.1 at 66 cM). Septiningsih *et al.* (2003) reported five QTL controlling grain weight located at chromosome 1, 2, 3, and 7 explaining 4 to 11% of variation. Those study detected QTL in chromosome 3. The use of different markers across the study make it difficult to check if it may have been the same QTL detected.

QTL for Grain Weight per Plant

QTL for grain weight per plant, i.e., *wt-vb11.1* could be detected. It was located at chromosome 11 at 62 cM (between RM3428 and RM5349) which was contributed by IR64 with additive effect of 9.884 g. It could explain 14.8% of variation.

Moncada *et al.* (2001) reported two QTL associated with yield per plant located at chromosome 1 and 11. Both were contributed by *O rufipogon* for increasing the yield which explained 7 – 14% of variation. On the other hand, Septiningsih *et al.* (2003) reported three QTL associated with yield per plant. Two was at chromosome 1 and one at chromosome 2. They contributed to 3 – 6% of variation.

In general, the number of QTL detected in this study was relatively lower compared to those of other studies such as average of 3.5 QTL per traits (Moncada *et al.*, 2001) and 3.8 QTL per traits (Septiningsih *et al.*, 2003). It might due to the size of the population. Thomson *et al.* (2003) used 258 BC_2F_2 families, Septiningsih *et al.* (2003) used 400 BC_2F_2 families, and Moncada *et al.* (2001) used 274 BC_2F_2 families to map QTL of agronomic traits. The larger the mapping population, the more likely to allow detection of more QTL with smaller effect (Haley and Anderson, 1997). Few number of individual in the mapping population caused downward bias in the number of QTL involved in a trait. This caused of the power of the QTL significance tests was reduced (Holland, 2005). Small size of mapping population faced more of type II error (missing true QTL) than type I error (getting false QTL). Simultaneously, small size of the population over estimated the effect of the QTL (Openshaw and Frascaroli, 1998).

The type of population also determined the result of QTL detected. BC_1F_1 had no chance for planting into plot or row. Replication could be done by carefully splitting the plant into some identical clones. Environmental effect on the single sample at the replication should also be considered to be related to the few QTL detected.

Some QTL associated with different traits were found at the same chromosome indicating the close association of the traits. It was noticed that *fll-vb4.1* associated with flag leaf length and *pw-vb4.1* associated with panicle weight were located at the close position at chromosome 4. It might be due to the QTL was close each other or even it was a pleiotropic QTL.

The result of each trait explained above showed that comparative study with other mapping population resulted some QTL controlling specified traits found at the same location across mapping population. It indicated that the particular region might have common QTL and that particular region was considered to be important for the improvement of certain traits. This QTL might work for wide genetic background. On the other hand, there were QTL found only in a specific genetic background. It might be important if the QTL had big effect and stable across environment. Therefore, it could be used for plant improvement at the specific genetic background.

The results indicated that the QTL found in this study was reliable and confirmed some previous QTL studies. Further study to find more QTL and dissect more detail the promising QTL is important for the application of molecular marker technique for plant improvement.

CONCLUSIONS

1. Mostly the markers segregated according to Mendel Law, unless in some regions of the genome.
2. There were 11 QTL associated with eight yield, yield component, and morphological traits. They were located at chromosome 2, 3, 4, 6, 11, and 12.
3. Comparative study with previous mapping researches found QTL that were existed across genetic background as well as QTL that were existed only at specific genetic background.

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