QTL Analysis of Yield-related Traits using an Advanced Backcross Population Derived from Common Wild Rice (Oryza rufipogon L)

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Abstract Wild rice (Oryza rufipogon L) is recognized as an important germplasm that has abundant genetic diversity and specific characters. Many of rice breeders attempt to utilize elite wild rice for rice genetic improvement and breeding program to broaden genetic background of cultivating rice. Although There are some reports that wild rice germplasms were successfully applied in rice breeding program, it is definitely depend on what we understand the traits in particular complex traits. In this research the goal was to identify alleles for yield and yield related components. An advanced backcross BC3 population was generated by a cross between recurrent parent named Yuexiangzhan and donor parent named G52–9, a wild rice collected in Gaozhou of Guangdong. This population was evaluated for 10 agronomic traits related to yield and yield related components. Forty three QTLs of ten traits were detected based on phenotypic data in this study. There are thirty eight QTLs of eight traits detected in continuing two years and thereinto nine QTLs consistently detected in two continuing years. It is also confirmed that nine QTLs mapped on the same or adjacent regions conferring the same traits by previous research. Two QTLs, qGYP–2–I and qGYP–3–I mapped on chromosome 2 and 3 can increase the yield of Yuexiangzhan by 40.05% and 49.04%, respectively, contributed from wild rice. Furthermore, the results show that many of detected alleles are likely associated with the trait with wild rice genetic background, which imply that Gaozhou wild rice should be used as excellent gene donor in rice breeding program.

Keywords Wild Rice (Oryza rufipogon L); Yield-related Trait; Quantitative trait locus (QTL); Advanced backcross population; AB-QTL analysis

Background Rice is not only a major food grain in the world but also is a model plant with a small completely sequenced whole genome in cereal crops. Modern rice cultivars have characters of high yielding potentials and narrow genomic backgrounds due to selection of traits related to high yield during the past several decades. Indeed, Enhancing rice yield is still one of the major goals in rice breeding program so as to meet the increasing food demands for the growing populations.

There are abundant genetic diversities formed in the long-term evolutions from wild rice to cultivating rice. Wild rice is an important germplasm donor for rice improvement in rice breeding program. Unfortunately, Specific and useful genes of the wild rice are only few available whereas some of characters derived from wild rice are disappeared or lost in the cultivated rice (Xiao et al., 1998). The study showed that the number of alleles in cultivated rice were about 58.2% derived from of the wild rice among the tested 44 RFLP loci. Further results demonstrated that the number of allele and genetic diversity was obviously decreased and reduced. Some useful alleles even were lost in modern cultivar of rice due to the breeding pressure of the targeted selections (Sun et al., 2001).

Most of agronomical traits are quantitative traits showing normal distributions in phenotype of the
traits. These quantitative traits generally controlled by quantitative trait loci that shortly called as QTL. QTL analysis allows us to identify chromosome loci conferring the complex traits (Wade et al., 2001). In the past decade, there are large of numbers of QTLs in cultivated rice (Oryza sativa) cultivars identified (Xiao et al., 1998; Xiong et al., 1999; Cai et al., 2002). The introgression of novel alleles from wild germplasm is one effective approach for further improving of agronomic traits, which have been successfully used in cultivated rice breeding program as reported in QTL mapping studies (Xiao et al., 1998; Aluko et al., 2004; Moncada et al., 2001; Septiningsih et al., 2003a; Tian et al., 2006). Studies also indicated that wild rice species usually show their agronomic characters inferior to that of cultivated species, whereas these wild rice species definitely contain much more favorable alleles that might be important for cultivated crops (Frey et al., 1983). Although the wild rice germplasms were successfully utilized in rice breeding program to some extent, it becomes much difficult to employ these favorable traits due to lots of linking inferior characters. For example, adverse factors (unfavorable agricultural characters and undesirable linked genes, overrepresentativeness of wild rice genes in balanced population, and negative genetic linkage drag, and so on) have fatal influenced on the utilization of wild rice germplasm, in particular to find favorable genes from wild rice (De Vicente et al., 1993; Eshed et al., 1994).

Advanced backcross QTL (AB-QTL) analysis is a known approach to find elite genes in wild rice. AB-QTL analysis has been successfully applied in detecting and transferring QTLs from un-adapted germplasm into advanced breeding lines in various plant species (Xiao et al., 1998; Tanksley et al., 1996; Bernacchi et al., 1998a; Bernacchi et al., 1998b; Xiao et al., 1996).

In this research, we developed an interspecific advanced backcross population and planted at two different locations in two continuous years. The recurrent parent employed is an elite cultivar called ‘Yuexiangzhan’ in Chinese and the donor parent is a wild rice germplasm (O. rufipogon L) deposited in germplasm bank with accession No. G52–9. The objectives of this study were to evaluate the agronomic performance and yield component of the advanced backcross populations based on the analysis of genotypic and phenotypic data. In addition, quantitative trait loci conferring some interesting traits were identified by using generated introgression lines (ILs) and near-isogenic lines (NILs) in these studies.

## 1 Results

### 1.1 Trait phenotypic scores and statistic analysis

We phenotyped ten yield related traits and calculated the values of mean, minimum, maximum, and coefficient of variation listed in Table 1. The results showed that differences in variance for all traits were highly significant (P<0.01 or P<0.001) based on the t-test. The phenotypic analysis of the traits in the 245 backcross progenies showed that the frequency distribution of all tested traits fit approximately normal distribution (histograms not shown). As expected for an interspecific cross, distribution of phenotypic vales in progeny showed bi-directional deflective separation for all traits. Most of the trait values of the 245 backcross families have higher than that of the recurrent parent, Yuexiangzhan. According to the statistical data, there are about 33.20% of the BC1F3 lines with better traits than that of the wild type Yuexiangzhan (BC1F3 data not shown).

### 1.2 Correlations among the yield related traits

Phenotypic correlations were conducted among the evaluated yield-related traits based on the means. The traits with highest significant positive correlation were found between grain number per panicle and grain number per plant (Pearson correlation coefficient, 0.659, P<0.001), Whereas the traits with significant negative correlation were found between 1000–grain weight and Spikelet density (Pearson correlation coefficient, −0.235, P<0.001). All of the Traits with their Pearson correlation coefficient are listed in table two.

### 1.3 SSR marker polymorphisms

Polymorphism is recognizes as a measurement for genetic diversities between the breeding parents. In this study total of 551 SSR markers were used to detect the polymorphism between the parents, which are 162 SSRs to show polymorphism (29.4%). The results show that the rate of polymorphism is much lower than that
Table 1 Statistics of agronomic traits variance of Yuexiangzhan and the BC₁F₁ families

| Trait Abbv. | Unit | Yuexiangzhan O.sativa | BC₁F₁ families |
|-------------|------|-----------------------|----------------|
|             |      | Mean | Min. | Max. | Max. | C.V  |
| 1000–GW**   | g    | 19.1 | 18.53 | 15 | 27.2 | 27.2 | 8.22 |
| GN*         | score | 125  | 129.78 | 38.01 | 209.75 | 209.75 | 22.05 |
| GNP**       | score | 1462.5 | 1772.73 | 460.56 | 5995.4 | 5995.4 | 32.32 |
| SN**        | score | 139.6 | 177.41 | 81.88 | 312.21 | 312.21 | 19.22 |
| SNP**       | score | 1633.3 | 2348.13 | 942.23 | 7745.1 | 7745.1 | 30.22 |
| SSP**       | percent | 89.5 | 75.35 | 47.51 | 91.28 | 91.28 | 10.06 |
| PPN**       | score | 11.7 | 13.22 | 8.5 | 27 | 27 | 20.66 |
| SD**        | cm   | 6.31 | 7.95 | 3.23 | 14.87 | 14.87 | 18.63 |
| GY**        | g    | 2.32 | 2.39 | 0.88 | 4.03 | 4.03 | 23 |
| GYP*        | g    | 27.14 | 31.53 | 11.17 | 83.53 | 83.53 | 29.99 |

Abbreviation of trait: 1000–GW: 1000–grain weight, GN: grain number per panicle, GNP: grain number per panicle, SNP: spikelet number per panicle, SSP: seed set percentage, PPN: productive panicle number, SD: spikelet density, GY: grain yield per panicle, GYP: grain yield per plant; Marked with one asterisk * means significance at P<0.01 level; two asterisk** means significance at P<0.001 level

Table 2 Pearson correlation coefficients among 10 traits in BC₁F₁ population

| Trait | 1000–GW | GN | GNP | SN | SNP | SSP | PPN | SD | GY |
|-------|---------|----|-----|----|-----|-----|-----|----|----|
|       |         |    |     |    |     |     |     |    |    |
| GN   | −0.088  |    |     |    |     |     |     |    |    |
| GNP  | −0.085  | 0.659*** | 0.543*** |    |     |     |     |    |    |
| SN   | −0.148* | 0.855*** | 0.536*** | −0.184**  | 0.546*** | 0.235  |    |    |
| SNP  | −0.128* | 0.565*** | 0.954*** | 0.588*** |    |     |     |    |    |
| SSP  | 0.051   | 0.458*** | 0.348*** | 0.006   | 0.064 | 0.235  |    |    |
| PPN  | −0.116  | −0.106 | 0.536*** | −0.184**  | 0.546*** | 0.235  |    |    |
| SD   | −0.235*** | 0.817*** | 0.435*** | 0.913*** | 0.466*** | 0.031 | −0.232*** |    |
| GY   | 0.243*** | 0.936*** | 0.615*** | 0.795*** | 0.504*** | 0.487*** | −0.128* | 0.696*** |    |
| GYP  | 0.133*  | 0.689*** | 0.888*** | 0.538*** | 0.813*** | 0.419*** | 0.564*** | 0.418*** | 0.726*** |

Abbreviations of the trait listed in this table are the same as table one
Marked with one asterisk * means significance at P<0.05 level; two asterisk** means significance at P<0.01 level; three asterisk*** means significance at P<0.001 level

Markers from mentioned above polymorphic SSRs were employed to genotype the BC₁F₁ population.

1.4 QTLs for yield and yield-related traits

Total of 43 QTLs were identified by using the QTL approach of composite interval mapping (CIM). The numbers of QTLs were from two to ten detected in different traits, as well as the phenotypic variance ranged from 3.74% to 40.51% among the detected QTLs. The 43 QTLs were mapped on the ten chromosomes except chromosomes 8 and 9. Some
QTLs are clustered in several chromosomal regions. The detail information about the location, explained variation (R²), and additive effect of QTL detected for yield and yield-related traits are listed in Table 3, Table 4 and Figure 1. Some yield traits were selected to be analyzed in detail as follows.

**Thousand grain weight (1000–GWt)**

Ten QTLs were detected for the trait of 1000–GWt on chromosomes 3, 4, 5, 6 and 11, explaining the phenotypic variance from 3.77% to 28.67%. The loci of qGWt–6–1 and qGWt–6–2 for the 1000–GWt are reduced from the parent of Yuexiangzhan, whereas the other eight loci for the 1000–GWt are increased by the wild rice allele. The loci of qGWt–5–1, qGWt–5–2 and qGWt–11–1 explained the largest phenotypic variance (28.67%, 26.29% and 24.28%, respectively) the increase of the 1000–GWt (2.49 g, 2.08 g and 5.56 g, respectively) contributed by the parent of Yuexiangzhan. Two QTLs, qGWt–4–1 and qGWt–11–2, were detected in continuing two years.

**Grain number per panicle (GNP)**

Five putative QTLs were found for GN on chromosomes 2, 10 and 11. These single QTLs explained the phenotypic variance from 6.07 to 21.51% with LODs ranging from 2.40 to 3.61. The loci for GN, qGN–2–1, qGN–11–1 and qGN–11–2, were increased by wild rice alleles with the number of 22.72, 18.77 and 18.81, respectively.

**Grain number per plant (GNP)**

Three putative QTLs were detected on chromosomes 1 and 2. Alleles from wild rice at all three QTLs (qGNP–1–1, qGNP–2–1 and qGNP–2–2) increased the number of GNP. The most significant loci for with the trait of GNP were qGNP–2–2, which explained 33.44% of the phenotypic variance.

**Spikelet number per panicle (SNP)**

Six putative QTLs for SN were mapped on chromosome 2, 4, 6, 7, 10 and 11. These single QTLs explained the phenotypic variance from 3.74% to 31.01%. Two loci (qSN–6–1 and qSN–7–1) caused a decrease in SN, whereas the locus of qSN–7–1 was detected in continuing two years.

**Spikelet number per plant (SNP)**

Two putative QTLs (qSNP–2–1 and qSNP–2–1) were identified for SNP on chromosome 2, explaining 16.67% and 31.15% phenotypic variance. Alleles from wild rice at these loci increased the number of SNP (819.87 and 1073.85).

**Seed set percentage (SSP)**

Three putative QTLs for SSP were found on chromosomes 10, 11 and 12 that can explain 4.76% and 28.81% phenotypic variance. All of three loci negative affected on SSP from the wild rice allele. The locus, qSSP–10–1, was detected in continuing two years.

**Productive panicle number (PPN)**

The loci for PPN was mapped on chromosomes 1, 2, 3 and 7. Alleles from wild rice at four QTLs (qPPN–1–1, qPPN–2–1, qPPN–3–1, qPPN–7–1) increased the number of PPN. The most significant QTL associated with PPN was the allele, qPPN–3–1, which explained 40.51% phenotypic variance. The locus, qPPN–7–1, was detected in continuing two years.

**Spikelet density (SD)**

Four QTLs associated with SD mapped on chromosomes 4, 5, 6 and 10, and the explained phenotypic variance ranged from 3.87% to 22.39%. The allele from wild rice at qSD–5–1 has the negative effect for SD. One of QTLs, qSD–6–1, was detected in continuing two years.

**Grain yield per panicle (GY)**

Three QTLs were identified for GY on chromosomes 2, 4 and 10 with R² ranged from 5.49% to 15.65%. Alleles from wild rice at two QTLs (qGY–4–1 and qGY–10–1) increased GY by 0.56 g and 0.38 g.

**Grain yield per plant (GYP)**

Three putative QTLs were identified for GYP on chromosomes 1, 2 and 3, which explained the phenotypic variance from 3.29 to 24.04%. The strongest positive effect was detected at qGYP–3–1 for GYP on chromosome 3, which showed 13.31 of the additive effect.

2 Discussions

Wild rice G52–9 is a germplasm of common wild rice collected from Gaozhou wild rice reserve in Gaozhou city of Guangdong province in China, where is the transitional region from southern subtropics to north subtropics. Gaozhou wild rice reserve is the largest wild rice population in Guangdong Province covering about
Table 3 QTL for yield and yield-related traits detected in the BC$_3$F$_1$ and BC$_3$F$_3$ populations

| QTL          | Chr. | Marker Interval          | LOD score | Additive effect | R$^2$ (%) | LOD score | Additive effect | R$^2$ (%) |
|--------------|------|--------------------------|-----------|-----------------|-----------|-----------|-----------------|-----------|
| **Spikelet number per panicle** |      |                          |           |                 |           |           |                 |           |
| qSN–2–1      | 2    | RM110–RM211              | 2.63      | 22.93           | 6.11      |           |                 |           |
| qSN–4–1      | 4    | RM518–RM1223             |           |                 |           | 3.08      | 20.05           | 18.17     |
| qSN–6–1      | 6    | RM1161–RM3               | 2.23      | −17.49          | 3.74      |           |                 |           |
| qSN–7–1      | 7    | RM82–RM1134              | 2.58      | −17.40          | 5.60      |           |                 |           |
| qSN–10–1     | 10   | RM467–RM5629             |           |                 |           | 3.59      | 33.10           | 31.01     |
| qSN–11–1     | 11   | RM332–RM3701             |           |                 |           | 2.98      | 21.79           | 25.21     |
| **Grain number per panicle**    |      |                          |           |                 |           |           |                 |           |
| qGN–2–1      | 2    | RM110–RM211              | 3.10      | 22.72           | 8.36      |           |                 |           |
| qGN–10–1     | 10   | RM7492–RM222             | 2.73      | −20.53          | 6.07      |           |                 |           |
| qGN–10–2     | 10   | RM222–RM4915             | 3.14      | −22.59          | 9.94      |           |                 |           |
| qGN–11–1     | 11   | RM332–RM3701             |           |                 |           | 2.56      | 18.77           | 21.51     |
| qGN–11–2     | 11   | RM287–RM209              |           |                 |           | 2.40      | 18.81           | 8.14      |
| **1000–grain weight**            |      |                          |           |                 |           |           |                 |           |
| qGWt–3–1     | 3    | RM175–RM517              | 3.42      | 2.43            | 15.10     |           |                 |           |
| qGWt–4–1     | 4    | RM401–RM518              | 4.50      | 1.36            | 10.7      |           |                 |           |
| qGWt–4–2     | 4    | RM1223–RM2441            |           |                 |           | 2.68      | 0.58            | 6.33      |
| qGWt–5–1     | 5    | RM509–RM440              | 11.73     | 2.49            | 28.67     |           |                 |           |
| qGWt–5–2     | 5    | RM440–RM534              | 10.06     | 2.08            | 26.29     |           |                 |           |
| qGWt–6–1     | 6    | RM5814–RM1031            | 2.20      | −0.72           | 3.96      |           |                 |           |
| qGWt–6–2     | 6    | RM1031–RM494             | 2.37      | −0.78           | 3.77      |           |                 |           |
| qGWt–11–1    | 11   | RM332–RM3701             | 8.27      | 5.56            | 24.28     |           |                 |           |
| qGWt–11–2    | 11   | RM287–RM209              | 3.31      | 1.22            | 8.56      | 3.24      | 15.1            | 15.18     |
| **Spikelet density**             |      |                          |           |                 |           |           |                 |           |
| qSD–4–1      | 4    | RM518–RM1223             |           |                 |           | 6.50      | 0.98            | 22.23     |
| qSD–5–1      | 5    | RM289–RM509              | 2.04      | −0.74           | 4.25      |           |                 |           |
| qSD–6–1      | 6    | RM510–RM253              | 2.30      | 0.64            | 3.87      | 4.35      | 0.80            | 19.82     |
| qSD–10–1     | 10   | RM467–RM5629             |           |                 |           | 3.30      | 0.56            | 22.39     |

Gaozhou wild rice is known as the excellent characters and favorable traits that are worth mining and utilizing. We are the first research group to construct an advanced backcross population using Gaozhou wild rice for genetics and breeding studies in China.

Although overall agronomic characteristics of wild rice is inferior to that of the cultivated rice, our results demonstrated that wild rice alleles had a significant effect on yield and yield-related components in the 15 hectares. Gaozhou wild rice is known as the excellent characters and favorable traits that are worth mining and utilizing. We are the first research group to construct an advanced backcross population using Gaozhou wild rice for genetics and breeding studies in China.
background of Yuexiangzhan, which contributed up to 74.42%. This would be higher percentage than that reported in previous studies. In previous studies, the beneficial alleles from the same donor of wild rice accounted for 33%, 51%, 56% and 53% by Septiningsih et al. (2003a), Xiao et al. (1998), Moncada et al. (2001), and Thomson et al. (2003), respectively. And Lee et al. (2005) reported 20.6% of QTLs identified with desirable agronomic traits. The higher percentage obtained in this study might imply that the Gao52-9 present more favorable alleles compared with other wild rice (O. rufipogon) used in previous studies.

It is very important that the QTLs detected at different research groups in different time are comparative and accreditable. QTLs detected in this research can be compared with previous QTL studies using different parents. Nine QTLs conferring the same traits were mapped on the same or adjacent regions that were similar as previous reported studies. Three QTLs (qGWt-5-1, qGWt-5-2, qGWt-11-1) of these nine accreditable QTLs have a large effect, which could predict that three mentioned QTLs be major genes. qGWt-5-1 and qGWt-11-1 were mapped in the same

| QTL          | Chr. | Marker Interval | BC$_2$F$_1$ families in Guangzhou 2007 | BC$_2$F$_3$ families in Guangzhou 2008 |
|--------------|------|----------------|----------------------------------------|----------------------------------------|
|              |      |                | LOD score | Additive effect | $R^2$ (%) | LOD score | Additive effect | $R^2$ (%) |
| **Productive panicle number** |      |                |           |                |          |           |                |          |
| qPPN-1-1     | 1    | RM84–RM490     | 2.40      | 1.89           | 7.90     |           |                |          |
| qPPN-2-1     | 2    | RM341–RM106    | 5.70      | 9.35           | 28.89    |           |                |          |
| qPPN-3-1     | 3    | RM282–RM49     |           |                |          | 10.34     | 10.78          | 40.51    |
| qPPN-7-1     | 7    | RM82–RM1134    | 2.77      | 1.28           | 4.63     | 3.93      | 0.98           | 15.48    |
| **Seed set percentage** |      |                |           |                |          |           |                |          |
| qSSP-10-1    | 10   | RM222–RM4915   | 2.85      | -4.86          | 4.76     | 2.77      | -1.06          | 10.97    |
| qSSP-11-1    | 11   | RM202–RM287    |           |                |          | 4.50      | -8.82          | 23.80    |
| qSSP-12-1    | 12   | RM6288–RM6296  | 2.95      | -5.08          | 5.58     |           |                |          |
| **Grain yield per panicle** |      |                |           |                |          |           |                |          |
| qGY-2-1      | 2    | RM110–RM211    | 3.66      | -0.49          | 10.01    | 2.38      | -0.48          | 8.40     |
| qGY-4-1      | 4    | RM348–RM349    |           |                |          | 2.70      | 0.56           | 15.65    |
| qGY-10-1     | 10   | RM7492–RM222   | 2.63      | 0.38           | 5.49     |           |                |          |
| **Grain yield per plant** |      |                |           |                |          |           |                |          |
| qGYP-1-1     | 1    | RM581–RM24     | 2.02      | 3.68           | 3.29     | 3.29      | 4.19           | 19.63    |
| qGYP-2-1     | 2    | RM110–RM211    | 5.29      | 10.87          | 16.34    |           |                |          |
| qGYP-3-1     | 3    | RM282–RM49     |           |                |          | 9.92      | 13.31          | 24.04    |
| **Grain number per plant** |      |                |           |                |          |           |                |          |
| qGNP-1-1     | 1    | RM84–RM490     | 2.16      | 330.01         | 5.32     |           |                |          |
| qGNP-2-1     | 2    | RM110–RM211    | 4.86      | 684.72         | 17.23    |           |                |          |
| qGNP-2-2     | 2    | RM341–RM106    | 4.43      | 837.64         | 33.44    |           |                |          |
| **Spikelet number per plant** |      |                |           |                |          |           |                |          |
| qSNP-2-1     | 2    | RM110–RM211    | 5.12      | 819.87         | 16.67    |           |                |          |
| qSNP-2-2     | 2    | RM341–RM106    | 8.51      | 1073.85        | 31.15    |           |                |          |

Table 4 QTL for yield and yield-related traits detected in the BC$_2$F$_1$ and BC$_2$F$_3$ populations
Figure 1 Distribution of the putative quantitative trait loci (QTLs) mapped in this study

Note: QTLs with italic letter only detected in 2007. QTLs with overstriking detected in 2008. QTLs with italic letter and underline detected in 2 experiments.
region as that reported by Lu et al. (1996) and Moncada et al. (2001).

Pleiotropic effect is very common phenomenon in plant biology that explained as one chromosomal region conferring two or more traits. Six cases of wild rice QTL studies using O. rufipogon as the donor parent and different cultivar as the recurrent parent have been reported that the pleiotropic effect exists (Xiong et al., 1999; Cai et al., 2002; Thomson et al., 2003; Lee et al., 2005). QTL Clustering of six traits were found in the region of RM110–RM211 on chromosome 2. The correlations among those traits were high significance. As expected, enhancing rice yielding capacity would be depend on the increase of yield related components.

3 Materials and Methods

3.1 Descriptions of parents used in this research

Yuexiangzhan, a launched indica cultivar developed by Institute of Rice of Guangdong Academy of Agricultural Sciences in South China, was used as a recurrent parent. Yuexiangzhan has high harvest index (up to more than 0.6), low ratio of sheath to blade length, and high productive tiller capability. The wild rice accession G52–9 (O. rufipogon L) collected from Gaozhou, a county of Guangdong province in China, was used as a donor parent. G52–9 is evergreen rice around the year without obvious winter dormancy, but only once heading a year with small panicles, low seed setting and brown kernels. G52–9 is recognized as elite wild rice germplasm with some desirable traits, such as strong resistant to diseases and insects as well as high tolerant to low temperature and low soil fertility.

3.2 Procedures for developing mapping population

An advanced backcross procedure as described by Tian (Tian, et al. 2006) was employed to develop the mapping population in this research. An individual plant of G52–9 was used as the pollen donor crossing with Yuexiangzhan to generate F1 plants. F1 plants were backcrossed continuing three times by recurrent Yuexiangzhan until a BC2F1 population brought out. All of the individuals derived from BC3F1 were then selfing continuing three generations up to BC3F3. Total of 245 BC3F3 progenies were genotyped with SSR markers. Hundred and twenty lines randomly selected from the BC3F3 population were analyzed for detecting QTLs based on the results of BC3F3 genotyping data, and for developing introgression lines.

3.3 Agronomic traits and phenotypic evaluation

We conducted the field trials for measuring and phenotyping the agronomic traits at three different locations in Guangdong and Hainan in 2007 and 2008. The 2060 BC3F2 and 2060 BC3F3 lines multiple derived from the 206 BC3F1 lines selected from 245 BC3F1 were used for field trials. The 245 BC3F1, 2060 BC3F3 lines and the recurrent parent, Yuexiangzhan, were grown at Dafeng Experimental Station of Guangdong Academy of Agricultural Sciences in August of 2007 and 2008, respectively. The 2060 BC3F2 was nursed at Hainan Winter Nursing Experimental Station in Sanya in the winter of 2007.

The protocols for the field trials were followed by the local rice production management instructions. Each line with 50 individuals transplanted by 5 rows and 10 individuals each row with a uniform 10 cm×30 cm space. A randomized complete block design with two replications was employed in this field trial.

Twenty plants random sampled in each trial plot at harvest stage for trait measure and evaluation. BC3F1 families and selected 120 lines in BC3F3 were phenotyped for yield and yield-related traits, whereas the recurrent parent used to be as reference. The evaluated traits were included as follows:

(1) 1000–grain weight (1000–GW) – the weight of 1000 fully filled grains averaged by five different panicle in gram, (2) grain number per panicle (GN) - the mean of five panicles, (3) grain number per plant (GNP) - the number of filled grain per panicle by productive panicle number, (4) spikelet number per panicle (SN) – the number of spikelets (including both filled and empty ones) averaged from five panicles in each plant, (5) spikelet number per plant (SNP)-total number of spikelets (including filled and empty ones) in each plant calculated as the average number of spikelets per panicle by the number of productive tillers, (6) seed setting percentage (SSP)- ratio of grain number per plant to spikelet number per plant, (7) productive panicle number (PPN)-total number of
panicles whose filled number are more than five, (8) spikelet density (SD)- the mean value of grain number per centimeter in five spikelets. (9) grain yield per panicle (GY)-weight of filled grains per panicle, and (10) grain yield per plant (GYP)-weight of filled grains per plant.

3.4 Marker genotype analyses
Gnomic DNA was extracted from fresh leaves (one month age seedling) sampled from ten plants each line. The collected leaves were bulked by using the protocol of Li et al (2006). SSR primers were synthesized based on public SSR information (Chen et al., 1997; Temnykh et al., 2000). A volume of 10 μl reaction mixture consists of 2 ng/μl of template DNA, 1 μmol/L primers, 1 μl of 10 mmol/L dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (PH 9.0), 1.5 mmol/L MgCl2 and 0.75 unit Taq polymerase. PCR Amplification was performed with the following steps: predenaturing at 94°C for 5min, followed by 35 cycles of 94°C for 30 s, 55°C for 1min, and 72°C for 2 min, and last step is 8 min at 72°C. The amplified products were separated on 6% polyacrylamide denaturing gels. Linkage maps were constructed by using 117 SSR markers and the order and distance of the markers for each group was determined based on two published SSR maps (Temnykh et al., 2001; McCouch et al., 2002).

3.5 Statistical analysis
Phenotypic data were statistically analyzed by using recognized SAS version (SAS 8.2) (Cary et al., 1992). The normal distribution of phenotypic data was verified by the shapiro-wilk test at level of α=0.01. Some traits need to log conversion or square-root transformation for their normal distribution. Pearson correlation coefficient was calculated among quantitative phenotypic traits. Linkage map was constructed with Kosambi Function by using MAPMARKER (Ver.3.3) (Lander et al., 1987). Linkage groups were assigned based on the rice maps previously developed by Temnykh et al. (2000). Composite interval mapping (CIM) was employed to detect QTL LOD peaks (>2.0). by using QTL Cartographer (Ver.2.5), (Wang et al., 2007) The parameter settings for CIM were model 6; forward and backward stepwise regression with threshold of P<0.05 to select cofactors; window-size 2 cM walking speed along chromosomes; We used a reset likelihood ratio (LR) threshold of 9.22 to detect significant QTLs. Probability of a QTL locus was represented with a LR score where LR= -2 ln (L1/L0) and where L0 represents the probability of an association between the marker and the trait and L1 represents the alternate hypothesis of no association. LOD and LR are related by the formula LOD=0.2172LR. The positions of the significant QTL were given for the maximum LR value within the region under analysis. The phenotypic variance controlled by a given QTL was determined by its determination coefficient (R²), while the phenotypic variance controlled by all the markers in the regression model was represented by a second determination coefficient (TR²) as defined by the software program (Blair et al., 2006).

3.6 QTL nomenclature
QTLs were named by following the instruction of McCouch et al., (1997). Two or three letters abbreviated from the trait name position behind italic q letter, then follow hyphen and arabic figure of rice chromosome code where the QTL is found and add additional figure for the site number at the same locus. For example, qSN-6-2 stands for QTL of the seed number trait mapped on chromosome 6 that is the second site at this locus.

Authors’ contributions
ZBJ and YYQ carried out the trait phenotyping, analyzed the QTL data and drafted the manuscript. YC and DIP worked on the trait phenotyping, helped with the analyses and wrote substantial parts of the paper. ZLF and JYC obtained and analyzed the QTL data and was involved in the writing. CL conceived the overall study, performed the experiment designs and took part to the data analysis and to the writing. All authors read and approved the final manuscript.

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