Genetic dissection of main and epistatic effects of QTL based on augmented triple test cross design

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Abstract

The use of heterosis has considerably increased the productivity of many crops; however, the biological mechanism underpinning the technique remains elusive. The North Carolina design III (NCIII) and the triple test cross (TTC) are powerful and popular genetic mating design that can be used to decipher the genetic basis of heterosis. However, when using the NCIII design with the present quantitative trait locus (QTL) mapping method, if epistasis exists, the estimated additive or dominant effects are confounded with epistatic effects. Here, we propose a two-step approach to dissect all genetic effects of QTL and digenic interactions on a whole genome without sacrificing statistical power based on an augmented TTC (aTTC) design. Because the aTTC design has more transformation combinations than do the NCIII and TTC designs, it greatly enriches the QTL mapping for studying heterosis. When the basic population comprises recombinant inbred lines (RIL), we can use the same materials in the NCIII design for aTTC-design QTL mapping with transformation combination Z₁, Z₂, and Z₄ to obtain genetic effect of QTL and digenic interactions. Compared with RIL-based TTC design, RIL-based aTTC design saves time, money, and labor for basic population crossed with F₁. Several Monte Carlo simulation studies were carried out to confirm the proposed approach; the present genetic parameters could be identified with high statistical power, precision, and calculation speed, even at small sample size or low heritability. Additionally, two elite rice hybrid datasets for nine agronomic traits were estimated for real data analysis. We dissected the genetic effects and calculated the dominance degree of each QTL and digenic interaction. Real mapping results suggested that the dominance degree in Z₂ that mainly characterize heterosis showed overdominance and dominance for QTL and digenic interactions. Dominance and overdominance were the major genetic foundations of heterosis in rice.
Introduction

Heterosis, or hybrid vigor, describes the superior performance of heterozygous hybrid plants over their homoygous parental inbred lines [1–3]. The development of heterotic crops, especially those for hybrid rice and maize, is one of the most important applications of genetics in agriculture [4–5], but the molecular basis underlying heterosis remains elusive.

Indeed, much of our knowledge regarding heterosis derives from classical genetic studies on maize, during which the fundamental hypotheses for heterosis were defined, with the main competing hypotheses including dominance, overdominance, and epistasis [1,5–6]. The dominance hypothesis explains heterosis by the complementing action of superior dominant alleles from both parental inbred lines at multiple loci over the corresponding unfavorable alleles leading to the improved vigor of hybrid plants [1,5,7–8]. The overdominance hypothesis attributes heterosis to allelic interactions at one or multiple loci in hybrids that result in superior traits compared to the homozygous parental inbred lines [1,9–10]. In addition, the epistasis hypothesis considers epistatic interactions between non-allelic genes at two or more loci as the main factor for the superior phenotypic expression of a trait in hybrids [1,10–12].

To decipher the genetic basis of heterosis, NCIII [13] and TTC [14] are powerful genetic mating designs widely used in maize [12,15–19], rice [12,8,20–26], and Arabidopsis thaliana [27–30]. Rice is the staple food for a large segment of the world’s population. The success of hybrid rice breeding [31], together with its relatively small genome size [32], saturated molecular linkage maps [33], and rapid advances in genome sequencing [34–35], have provided a novel opportunity for dissecting the genetic basis of heterosis.

In Xiao et al.’s study [8], based on the NCIII design, 194 F$_2$ RIL were backcrossed to their parental lines to develop the mapping population, and 37 QTL were detected for 12 quantitative traits by single-point analysis [one-way analysis of variance (ANOVA)] and an interval mapping method. In one of the two BC$_1$F$_2$ populations, 82% of the detected heterozygotes were superior to the respective homozygotes; therefore, Xiao et al. concluded that dominance complementation was the major genetic basis of heterosis in rice. On the other hand, Li et al. [21] and Luo et al. [22] investigated five interrelated mapping populations by an interval mapping method in which 254 F$_1$ RIL were selected as the base population; two BC$_1$F$_2$ populations were derived from the NCIII design and two test cross populations were obtained by test crossing the RIL with two testers (Zhong 413 and IR64). The results suggested that epistasis and overdominance, rather than dominance, were the major genetic bases of heterosis in rice. Yu et al. [20] also pointed out that epistasis played a major role as the genetic basis of heterosis.

Hua et al. [23] investigated the genetic components conditioning the heterosis of yield and yield component traits in an elite rice hybrid using an immortalized F$_2$ population with modified composite interval mapping (CIM) and two-way ANOVA methods and found that heterotic effects at the single-locus level and a dominance × dominance interaction at the two-locus level could adequately explain the genetic basis of heterosis. In our previous study [24] based on the NCIII design, two recombinant inbred populations were backcrossed to their respective parents to develop mapping populations (L$_1$ and L$_2$) in which main-effect QTL were detected by the CIM method and epistatic QTL were detected by the mixed linear approach in the RIL population and summation (L$_1$ + L$_2$) and subtraction (L$_1$ – L$_2$) data of two backcross populations. The research demonstrated that heterosis was attributable to the orchestrated outcome of partial-to-complete dominance, overdominance, and epistasis. In addition, based on an ultra-high-density single nucleotide polymorphism bin map constructed with population sequencing, the immortalized F$_2$ population in Hua et al. [23] was reanalyzed by Zhou et al. [26] with an h test in one-locus effects detection and two-way ANOVA in two-locus interactions for the whole genome. The results suggested that relative contributions of the genetic
components varied with traits; single-locus dominance had relatively small contributions in all of the traits and the cumulative effects of these different components may adequately explain the genetic basis of heterosis. This conclusion was consistent with our previous study [24].

In summary, most of the mapping populations above derive from the NCIII design, and QTL mapping methods usually employ ANOVA, interval mapping, or the CIM method in one-locus effects detection and two-locus interactions. However, the estimated additive and dominant effects are confounded with epistatic effect if epistasis is present. Kao and Zeng [36] pointed out that a two-way ANOVA-exploiting genetic marker and trait phenotype data from an F2-segregating population was, in principle, inappropriate for testing for pairwise epistasis, even though this approach has been widely used in analyses of such data sets [20–21,23]. In addition, only one variable was involved in the model at one time, which was not able to capture all types of genetic effects, especially epistatic effects, simultaneously on the whole genome. In 2008, Garcia et al. [25] developed a multiple-interval mapping model for the NCIII design that provided a platform to simultaneously estimate the number, genomic positions, augmented additive and dominance effects, and digenic interactions \( (aa + dd \text{ and } ad + da) \) of QTL. This method was used to reanalyze the datasets by Stuber et al. [12], who found that additive \( \times \) additive effect \( (aa) \) epistatic effects of QTL could be the main cause for the heterosis in rice. After this, He et al. [37] proposed a method for mapping epistatic QTL associated with heterosis using the RIL-based NCIII design by a series of simulation studies; however, main or epistatic effects were mixed measured as augment effects.

In 1988, Liu [38] proposed an aTTC design based on the TTC design. In TTC design, base populations are backcrossed to \( P_1 \), \( P_2 \) and \( F_1 \) to get \( L_{i1} \), \( L_{i2} \), and \( L_{i3} \), \( i = 1 \ldots n \), whereas in aTTC design, base populations are simultaneously self-mated to get \( L_{4i} \). The aTTC design provided several ways to detect epistasis by detecting a variance component. However, there was no report based on aTTC design for QTL mapping on a Mendelian factor level.

In this paper, under aTTC design, based on four data sets \( (L_{11}, L_{21}, L_{31}, \text{ and } L_{41}) \), we developed six data set transformations [38]: 
\[
\begin{align*}
Z_{11} &= L_{11} + L_{21}, \\
Z_{21} &= L_{11} - L_{21}, \\
Z_{31} &= L_{11} + L_{21} - 2L_{31}, \\
Z_{41} &= L_{11} + L_{21} - L_{41}, \\
Z_{51} &= L_{11} + L_{21} + L_{31}, \text{ and } Z_{61} = 2L_{31} - L_{41}.
\end{align*}
\]
By employing \( Z_{11}, Z_{21}, \text{ and } Z_{31} \), He and Zhang [39] provided a complete solution for dissecting main and epistatic effects in the F2-based TTC design through a simulation study. Our study utilized different data set combinations \( (Z_1, Z_2, \text{ and } Z_4), (Z_1, Z_2, \text{ and } Z_5), \text{ and } (Z_1, Z_2, \text{ and } Z_6) \), respectively, to provide a two-step approach for estimating, in an unambiguous and unbiased manner, all the main and, especially, epistatic effects of QTL; this method also fits for many types of base populations such as RIL, F2, and Double Haploid (DH). Here, we will take the first combination \( (Z_1, Z_2, \text{ and } Z_4) \) of the RIL-based aTTC design as an instance for QTL mapping to dissect genetic effects. The other combinations \( (Z_1, Z_2, \text{ and } Z_5) \) and \( (Z_1, Z_2, \text{ and } Z_6) \) listed above can also be used to estimate genetic effects. A series of Monte Carlo simulation studies were carried out to confirm the proposed approach. We further applied the proposed method to real data analysis.

### Materials and methods

#### Genetic design

In aTTC design, F2 populations or their offspring (BC, DH, or RIL) derived from the hybridization of two pure lines \( (P_1 \text{ and } P_2) \) and were selected as the base population. On one hand, \( n \) individuals in the base population were crossed to three testers \( (P_1, P_2, \text{ and } F_1) \) to get \( L_{11}, L_{21}, \text{ and } L_{31} \), respectively \( (i = 1, 2 \ldots n) \); on the other hand, the \( n \) individuals in the base population were self-mated to get \( L_{4i} \). Therefore, \( 4n \) aTTC lines \( (L_{11}, L_{21}, L_{31} \text{ and } L_{41}) \) can be obtained and used for the detection of epistasis.
All \( 4nm \) families, each with \( m \) replications, were planted. Molecular marker information was observed from all of the \( n \) base population lines and the testers \( P_1, P_2 \) and \( F_1 \), whereas quantitative traits were measured for all \( 4nm \) aTTC progeny. The phenotypic observations were denoted by \( y_{ij} \), where \( t = 1, 2, 3 \), and 4 for \( L_{1i}, L_{2i}, L_{3i} \) and \( L_{4i} \) respectively; \( j = 1, 2, \ldots m \). The family means were denoted by \( L_i = \frac{\sum_j y_{ij}}{m} \).

The genetic expectations of six data set transformations, \( Z_{1i}, Z_{2i}, Z_{3i}, Z_{4i}, Z_{5i}, \) and \( Z_{6i} \), were obtained from \( L_{1i}, L_{2i}, L_{3i}, L_{4i}, Z_{1i} = L_{1i} + L_{2i}, Z_{2i} = L_{1i} - L_{2i}, Z_{3i} = L_{1i} + L_{2i} - 2L_{3i}, Z_{4i} = L_{1i} + L_{2i} - 4L_{4i}, Z_{5i} = L_{1i} + L_{2i} + L_{3i} + L_{4i} \) and \( Z_{6i} = 2L_{3i} - L_{4i} \). Two main metrics were adopted for the \( 4m \) aTTC lines: the \( F_\infty \) and \( F_2 \) metrics \([36,40]\); their genetic expectations are listed in S1 Supporting Information.

**Genetic models for mapping heterotic QTL in RIL-based aTTC design**

The derivation of the expected genetic values of \( Z_{1i}, Z_{2i}, Z_{3i}, Z_{4i}, Z_{5i}, \) and \( Z_{6i} \) under both the \( F_\infty \) and \( F_2 \) metric models is presented in S3 Supporting Information under the assumption that the quantitative trait was determined by two QTL with digenic epistasis and arbitrary linkage. The genetic effect symbols adopted in this research were described by Kao and Zeng \([36]\). He et al. \([37]\) simulated and estimated main and epistatic QTL in the RIL-based NCIII design under both the \( F_\infty \) and \( F_2 \) metrics and found that QTL mapping results under the \( F_\infty \) metric were superior to the \( F_2 \) metric; therefore, this paper simulated QTL under the \( F_\infty \) metric models.

**QTL mapping models in the RIL-based aTTC design under the \( F_\infty \) metric model.** The phenotypic values of \( Z_{1i} \) and \( Z_{2i} \) in the RIL-based aTTC design are the same as the RIL-based NCIII design. Details can be found in the publication by He et al. \([37]\). According to the genetic expectations of \( Z_{1i} \) under the \( F_\infty \) metric model (Table A5 in S1 Supporting Information), the phenotypic value of \( Z_{1i} \) can be described as

\[
Z_{1i} = 2\mu + x_{a_1}a_1 + d_1 + x_{a_2}a_2 + d_2 + x_{a_1a_2}i_{a_1a_2} + x_{d_1d_2}i_{d_1d_2} + x_{d_1d_2}i_{d_1d_2} + c_{1i},
\]

where \( \mu \) is the mean genotypic value of the four homozygotes in the RIL population; \( a_k \) and \( d_k \) are additive and dominance effects of the \( k \)th QTL (\( k = 1, 2 \)); \( i_{a_1}, i_{a_2}, i_{a_1a_2}, i_{d_1}, i_{d_2}, \) and \( i_{d_1d_2} \) are additive \( \times \) additive, additive \( \times \) dominance, dominance \( \times \) additive, and dominance \( \times \) dominance interactions between two QTL, respectively; \( x_{a_1}, x_{a_2}, x_{a_1a_2}, x_{d_1}, x_{d_2}, \) and \( x_{d_1d_2} \) are dummy variables and are determined by the genotype of the \( i \)th RIL line (Table A5 in S1 Supporting Information); and \( c_{1i} \) is the residual error with an \( N(0, \sigma^2) \) distribution. According to Table A5 in S1 Supporting Information, \( x_{a_1a_2} = x_{d_1d_2} \) and \( x_{a_1d_2} = -x_{d_1a_2} = \frac{1}{2}(x_{a_1} - x_{a_2}) \), model (1) can be reduced to

\[
Z_{1i} = \mu_{z_1} + x_{a_1}a_1^* + x_{a_2}a_2^* + x_{i_{a_1a_2}}i_{a_1a_2} + c_{1i},
\]

where \( \mu_{z_1} = 2\mu + d_1 + d_2, a_1^* = a_1 + \frac{1}{2}(i_{a_1a_2} - i_{a_1d_2}), a_2^* = a_2 + \frac{1}{2}(i_{a_1d_2} - i_{a_2d_2}), i_{a_1a_2} = i_{a_1d_2} + i_{d_1d_2}, \) and \( x_{i_{a_1a_2}} = x_{i_{a_1d_2}} = x_{i_{d_1d_2}} = x_{i_{a_1a_2}} = x_{i_{d_1d_2}} \). If the quantitative trait was controlled by \( q \) QTL, model (2) should be extended to

\[
Z_{1i} = \mu_{z_1} + \sum_{k=1}^{q} x_{a_k}a_k^* + \sum_{k=1}^{q-1} \sum_{l=k+1}^{q} x_{i_{kl}}i_{kl} + c_{1i},
\]

where the model mean \( \mu_{z_1} = 2\mu + \sum_{k=1}^{q} d_k; a_k^* = a_k + \frac{1}{2}\sum_{l=1}^{q} (i_{a_k} - i_{a_l}) \) is the augmented additive effect of QTL \( k; i_{kl} = i_{a_k} + i_{d_k} \) is the augmented epistatic effect between QTL \( k \) and \( l \).
Table 1. Coefficients of genetic parameters for the RIL based aTTC $Z_{1i}$, $Z_{2i}$ and $Z_{4i}$ data under the $F_2$ metric model.

| Genotype of Marker | $Z_{1i}$ | $Z_{2i}$ | $Z_{4i}$ |
|-------------------|----------|----------|----------|
|                   | $x_{a_i}$| $u_{a_i}$| $w_{a_i}$|
| $MM, MM, MM$      | 1        | 1        | 1        |
| $Mm, Mm, mm$      | 1        | -1       | 0        |
| $mM, mM, MM$      | -1       | 1        | 1        |

where the model mean

$$Z_{2i} = a_i + u_{d_i}d_{i} + a_2 + u_{d_j}d_{j} + u_{a_j}u_{a_j} + u_{d_i}u_{d_j}d_{i}d_{j} + e_{2i}, \quad (4)$$

Similarly, the phenotypic value of $Z_{2i}$ can be described as

$$Z_{2i} = \mu_{2i} + u_{d_i}d_{i}^{*} + u_{d_j}d_{j}^{*} + u_{a_j}u_{a_j}^{*} + e_{2i}, \quad (5)$$

where $\mu_{2i} = a_i + a_2, d_{i}^{*} = d_i - \frac{1}{2}(i_{a_2}a_2 - i_{a_2}d_2), d_{j}^{*} = d_j - \frac{1}{2}(i_{a_2}a_2 - i_{a_2}d_2), \tilde{i}_{12} = i_{a_j}d_j + i_{d_j}a_j,$

and $\mu_{2i} = u_{a_j}u_{a_j} = u_{d_j}d_j.$ If the quantitative trait controlled by 4 QTL, model (5) should be extended to

$$Z_{2i} = \mu_{2i} + \sum_{k=1}^{q} u_{d_k}d_{k}^{*} + \sum_{k=1}^{q-1} \sum_{l=k+1}^{q} u_{a_k}u_{a_l}^{*} + e_{2i}, \quad (6)$$

where the model mean

$$Z_{3i} = \mu_{3i} + \sum_{k=1}^{q} d_{k}^{*} + \frac{1}{2} \sum_{i+j=k} (i_{a_k}a_k - i_{d_k}d_k)$$

is the augmented dominance effect of QTL $k, \tilde{d}_{2i} = i_{a_k}a_k + i_{d_k}d_k,$ is the augmented epistatic effect between QTL $k$ and $l.$ Coefficients $u_{d_k}$, $u_{a_k}$, are determined by genotypes of the $k$th and $l$th QTL for the $i$th RIL line, as shown in Table 1.

Similarly, the phenotypic value of $Z_{4i}$ can be described as

$$Z_{4i} = \mu_{4i} + \sum_{k=1}^{q} a_k + v_{a_j}d_j + \sum_{k=1}^{q} v_{d_k}d_{k}^{*} + e_{4i}, \quad (7)$$

where $\mu_{4i} = r_{i_{a_j}a_j}, r$ is the recombination fraction between two QTL; dummy variables $v_{a_j}d_j, v_{d_k}d_{k}^{*},$ and $v_{a_j}d_j$ are determined by the genotype of the $i$th RIL line (Table A7 in S1 Supporting Information), $e_{4i}$ is the residual error with an $N(0, \sigma^2_4)$ distribution. Genetic effects $\tilde{i}_{a_j}d_j, \tilde{i}_{a_j}d_j, \tilde{i}_{a_j}d_j$ can be estimated directly.

In the same way, the phenotypic value of $Z_{4i}$ can be described as

$$Z_{4i} = u + d_i + d_j + w_{a_j}d_j + w_{a_j}d_j + w_{d_j}d_j + e_{4i}, \quad (8)$$

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Coefficients $x_{a_i}$ and $x_{a_i}$ are determined by genotypes of the $k$th and $l$th QTL for the $i$th RIL line, as shown in Table 1.
where $w_{i,aj}$, $w_{i,dj}$, and $w_{i,daj}$ are determined by the genotype of the $i$th RIL line (Table A8 in S1 Supporting Information), $e_i$ is the residual error with an $N(0, \sigma_i^2)$ distribution. According to Table A8 in S1 Supporting Information, there are $w_{i,aj} = -w_{i,daj}$ and $w_{i,daj} = 1 - w_{i,dj}$. Therefore, model (8) can be reduced to

$$Z_i = \mu_i + \sum_{k=1}^{q} w_{i,k} \bar{t}_{i,k} + \sum_{k=q+1}^{q} w_{i,k} \bar{t}_{i,k} + e_i,$$

where $\mu_i = \mu + d_i + d_2 + i_{d,aj} - i_{d,dj}$, $\bar{t}_{i,k} = \bar{t}_{i,k} - \bar{t}_{i,dj}$ and $w_{i,dj} = w_{i,daj}$. If the quantitative trait was controlled by $q$ QTL, model (9) can be extended to

$$Z_i = \mu_i + \sum_{k=1}^{q} w_{i,k} \bar{t}_{i,k} + \sum_{k=q+1}^{q} w_{i,k} \bar{t}_{i,k} + e_i,$$

where the model mean $\mu_i = \mu + \sum_{k=1}^{q} d_k + \sum_{k=q+1}^{q} \bar{t}_{i,k} = \bar{t}_{i,k} - \bar{t}_{i,dj}$ is the augmented epistatic effect between QTL $k$ and $l$. Coefficients $w_{i,k}$ and $w_{i,l}$ are determined by genotypes of the $k$th and $l$th QTL for the $i$th RIL line, as shown in Table 1.

In the same way, the phenotypic value of $Z_{hi}$ can be described as

$$Z_{hi} = 3\mu + s_{a1,a1} + \frac{3}{2}d_1 + s_{a2,a2} + \frac{3}{2}d_2 + s_{a1d1,a1d1} + s_{a2d2,a2d2} + s_{a1d1,a2d2} + s_{a2d2,a1d1} + e_{hi},$$

where $s_{a1,a1}$, $s_{a2,a2}$, $s_{a1d1,a1d1}$, $s_{a2d2,a2d2}$, and $s_{a1d1,a2d2}$ are determined by the genotype of the $i$th RIL line (Table A9 in S1 Supporting Information), $e_{hi}$ is the residual error with an $N(0, \sigma_i^2)$ distribution. According to Table A9 in S1 Supporting Information, model (11) can be reduced to

$$Z_{hi} = \mu_i + s_{a1,a1} + s_{a2,a2} + s_{a1d1,a1d1} + s_{a2d2,a2d2} + s_{a1d1,a2d2} + s_{a2d2,a1d1} + e_{hi},$$

where the model mean $\mu_i = 3(\mu + \frac{1}{2}d_1 + \frac{3}{2}d_2)$. Genetic effects $a_1, a_2, l_{a1d1}, l_{a2d2}, l_{a1d2}, l_{a2d1}$ can be estimated directly.

In the same way, the phenotypic value of $Z_{6i}$ can be described as

$$Z_{6i} = \mu + d_1 + d_2 + t_{a1d1}, t_{a2d2}, t_{a1d2}, t_{a2d1} + e_{6i},$$

where $t_{a1d1}, t_{a2d2}, t_{a1d2}, t_{a2d1}$ are determined by the genotype of the $i$th RIL line (Table A10 in S1 Supporting Information), $e_{6i}$ is the residual error with an $N(0, \sigma_i^2)$ distribution. According to Table A10 in S1 Supporting Information, model (13) can be reduced to

$$Z_{6i} = \mu_i + t_{a1d1}, t_{a2d2}, t_{a1d2}, t_{a2d1} + e_{6i},$$

where the model mean $\mu_i = \mu + d_1 + d_2$. Genetic effects $i_{a1d1}, i_{a2d2}, i_{a1d2}, i_{a2d1}$ can be calculated directly.

Model parameter components for $Z_{1i}$, $Z_{2i}$, $Z_{3i}$, $Z_{4i}$, $Z_{5i}$, and $Z_{6i}$ in the RIL-based aTTC design under both the $F_{\infty}$ were listed in Table 2.

Genetic models for mapping heterotic QTL in $F_2$-based aTTC design

Genetic models for mapping heterotic QTL in the $F_2$-based aTTC design under both $F_{\infty}$ and $F_2$ metric can be found in S4 Supporting Information.
Parameter estimation

For a continuously distributed trait, the observed phenotypic value $y_i$ of individual $i$ can be described by the linear regression model

$$y_i = \mu + \sum_{k=1}^{q} x_{ik}a_k + \sum_{k=1}^{q} \sum_{l=k+1}^{q} x_{il}i_{kl} + e_i = \mu + \sum_{j=1}^{p} x_{ij}\beta_j + e_i, \quad i = 1, 2, \cdots, n, \quad (15)$$

where $q$ is the number of markers, $\mu$ is the overall mean, $x_{ik}$ denotes the genotype of marker $k$ for individual $i$ and is defined as $-1$ or $1$ for the two genotypes in the mapping population, and $x_{il}$ represents the epistatic genotype between the $k$th and $l$th QTL of individual $i$, and is obtained as the element-wise product of $x_{ik}$ and $x_{lj}$. In addition, $a_k$ and $i_{kl}$ are the corresponding augmented main and epistatic effects, respectively. $p = q + \frac{1}{2}q(q-1)$ is the total number of genetic effects and $x_{ij}$ and $\beta_j$ are the corresponding genotypes and coefficients, including the main and epistatic effects. $e_i$ is the residual error assumed to follow an $N(0,\sigma^2)$ distribution.

Model (15) can be written as

$$y = \mu + X_G\beta_G + X_{G\!G'}\beta_{G\!G'} + e, \quad (16)$$

where vectors $\beta_G$ and $\beta_{G\!G'}$ represent the augmented main and epistatic effects of all markers, respectively. $X_G$ and $X_{G\!G'}$ are corresponding design matrices of different effects and $e$ is the residual error that follows an $N(0,\sigma^2)$ distribution. Defining $\beta = [\beta^T_G, \beta_{G\!G'}^T]$ and $X = [X_G, X_{G\!G'}]$, model (16) can be written in a more compact form

$$y = \mu + X\beta + e. \quad (17)$$

Due to the physical linkage or epistatic interactions among multiple QTL, it is rational when taking a large number of loci into consideration simultaneously. However, the total number of genetic effects $p$ is very large because we set each marker as a QTL initially. Typically, we have $p >> n$. To handle such an oversaturated model, we employed a fast empirical Bayesian LASSO (EBLASSO) algorithm. Simulation studies demonstrate that the EBLASSO method can sharply reduce the computational burden by shrinking small effects into zero, and can detect more true QTL effects without increasing the false-positive rate. Details of the EBLASSO algorithm can be seen by reference to the work of Cai et al. [41]. At last, all remaining markers with $t_j = |\hat{\beta}_j|/\hat{\sigma}_j > 2.0$ are picked up, where $\hat{\sigma}_j$ is the standard deviation.
estimation for normal prior $\hat{\beta}_j \sim N(0, \sigma^2_j)$. The epistatic model is then established that only includes effects that pass the first round of selection. We also perform a usual likelihood ratio test on the model to obtain significant QTL and epistatic interactions. With transformation combinations ($Z_1$, $Z_2$, and $Z_4$), following the above steps, in the first round, the argument effects $d_k^*$, $i_{kl}$, $i_{kli}$, and $i_{kl}^*$ can be obtained; in the second round, main and epistatic effects of each QTL and interaction can be calculated according to equation transformations

$$a_k^* = a_k + \frac{1}{2} \sum_{i=1}^{q} (i_{a_i} - i_{a_i}), i_{kl} = i_{a_k} + i_{a_l}, d_k^* = d_k - \frac{1}{2} \sum_{i=1}^{q} (i_{a_i} - i_{a_i}), i_{kl} = i_{a_k} + i_{a_l}.$$ 

Take any two significant QTL in a model as an example to explain how we obtain genetic effects. After performing QTL mapping, genetic parameters $\overrightarrow{i}_{12}$ and $\overrightarrow{i}_{12}$ in $Z_{4h}$, $\overrightarrow{i}_{12}$ in $Z_{10}$, and $\overrightarrow{i}_{12}$ in $Z_{2l}$ can be obtained. For $\overrightarrow{i}_{12} = i_{a_{12}}$, $\overrightarrow{i}_{12} = i_{a_{12}} + i_{d_{12}}$, so $i_{a_{12}} = (\overrightarrow{i}_{12} + \overrightarrow{i}_{12})/2$, while $\overrightarrow{i}_{12} = i_{a_{12}} - i_{d_{12}}$ and $\overrightarrow{i}_{12} = i_{a_{12}} + i_{d_{12}}$; we then get $i_{a_{12}} = (\overrightarrow{i}_{12} + \overrightarrow{i}_{12})/2$, $i_{d_{12}} = (\overrightarrow{i}_{12} - \overrightarrow{i}_{12})/2$. Meanwhile, $a_1$, $a_2$, $d_1$, and $d_2$ are obtained after performing QTL mapping in $Z_{4h}$ and $Z_{2l}$ because $a_1^* = a_1 + \frac{1}{2} (i_{a_{12}} - i_{d_{12}}), a_2^* = a_2 + \frac{1}{2} (i_{a_{12}} - i_{d_{12}}), d_1^* = d_1 + \frac{1}{2} (i_{a_{12}} - i_{d_{12}})$, and $d_2^* = d_2 + \frac{1}{2} (i_{a_{12}} - i_{d_{12}})$, with the estimation value of $d_1$, $d_2$, and above $i_{a_{12}}, i_{a_{12}}, i_{d_{12}}, i_{d_{12}}$, main effects can be calculated by $d_1 = d_1^* - \frac{1}{2} (i_{a_{12}} - i_{d_{12}}), d_2 = d_2^* - \frac{1}{2} (i_{a_{12}} - i_{d_{12}}), a_1 = a_1^* - \frac{1}{2} (i_{a_{12}} - i_{d_{12}})$, and $a_2 = a_2^* - \frac{1}{2} (i_{a_{12}} - i_{d_{12}})$. All main and epistatic effects were dissected by the integration of augmented effects in $Z_{4h}, Z_{2l}$, and $Z_6$.

Similarity, with transformation combination ($Z_1$, $Z_2$, and $Z_6$) and ($Z_1$, $Z_2$, and $Z_6$), we can also get the genetic effect of each QTL or interaction by QTL mapping under the aTTC design, respectively. More details are listed in S1 Supporting Information.

Simulation study

We took all the possible types of epistatic interaction patterns into consideration. The simulated genome, covered by 100 evenly spaced markers with a marker interval of 5 cM, was 495 cM in total length and comprised four chromosomes. For data sets $Z_{1}, Z_{2}$, or $Z_{6}$, six QTL positions were preset, of which three positions ($QTL_1$, $QTL_2$, and $QTL_4$) had main effects. Pairwise interactions were set between positions with main effects ($QTL_1$ and $QTL_2$), with and without main effects ($QTL_3$ and $QTL_4$), and without main effects ($QTL_5$ and $QTL_6$), respectively. The assumed QTL positions, parameters, and augmented effects (including main and epistatic effects) are listed in Table 3 and Tables A-B in S11 Supporting Information. The sample size ($n$) was set at three levels: 800, 400, and 200. The broad heritability ($h$) was also set at three levels: 0.8, 0.5, and 0.2, separately representing high, middle, and low heritabilities. The replication number of offspring ($m$) was set at two levels: 5 and 10. In three transformations, $Z_{1}, Z_{2}$, and $Z_{4}$, each treatment was replicated 100 times. Simulation study also be conducted for $Z_{5}$ and $Z_{6}$. The results of $Z_{6}$ and $Z_{6}$ were listed in Tables C and D in S11 Supporting Information.

QTL mapping in simulation study. All independent variable $p = 100 + \frac{1}{2} \times 100 \times (100 - 1) = 5050$ for $Z_{1}$, $Z_{2}$, $Z_{4}$ in simulation study were simultaneously included in one genetic model, which was much larger than the sample size. Data sets were implemented in R (version 3.0) with the EBLASSO package obtained from Cai et al. [41]. Hyper-parameters $a$ and $b$ were obtained by three-fold cross-validation (by default) in each individual model; after 100 replications, hyper-parameters with minimum predicted errors were fixed to estimate parameters. The time was approximately 5 minutes in each transformation in a stand-alone
Table 3. QTL mapping results for Z₄ in RIL-based aTTC design under the F₁ metric model in simulation study.

| n   | h   | m   | QTL₁ × QTL₂ | QTL₁ × QTL₃ | QTL₂ × QTL₃ | QTL₃ × QTL₄ |
|-----|-----|-----|-------------|-------------|-------------|-------------|
|     |     |     | l₁⁺⁺⁺⁺⁺⁺⁺⁺ | l₂⁺⁺⁺⁺⁺⁺⁺⁺ | l₃⁺⁺⁺⁺⁺⁺⁺⁺ | l₄⁺⁺⁺⁺⁺⁺⁺⁺ |
| 800 | 0.8 | 10  | 3.204       | 2.399       | 1.994       | 2.204       |
|     |     |     | (0.062)     | (0.062)     | (0.035)     | (0.062)     |
| 5   | 20  | 36  | 3.191       | 2.192       | 2.989       | 3.191       |
|     |     |     | (0.075)     | (0.085)     | (0.048)     | (0.093)     |
| 0.5 | 10  | 3.175 | 2.37    | 2.178       | 2.985       | 3.175       |
|     |     |     | (0.118)     | (0.109)     | (0.065)     | (0.109)     |
| 0.2 | 10  | 3.146 | 2.333   | 2.141       | 3.336       | 3.146       |
|     |     |     | (0.233)     | (0.141)     | (0.232)     | (0.232)     |
| 400 | 0.8 | 10  | 3.183       | 2.385       | 2.989       | 3.183       |
|     |     |     | (0.081)     | (0.089)     | (0.066)     | (0.088)     |
| 5   | 20  | 36  | 3.19        | 2.374       | 2.999       | 3.19        |
|     |     |     | (0.141)     | (0.138)     | (0.069)     | (0.130)     |
| 0.5 | 10  | 3.160 | 2.381   | 2.15        | 3.345       | 3.160       |
|     |     |     | (0.156)     | (0.158)     | (0.099)     | (0.173)     |
| 0.2 | 10  | 3.118 | 2.283   | 2.066       | 3.316       | 3.118       |
|     |     |     | (0.354)     | (0.345)     | (0.197)     | (0.337)     |
| 200 | 0.8 | 10  | 3.180       | 2.386       | 2.993       | 3.180       |
|     |     |     | (0.118)     | (0.126)     | (0.065)     | (0.139)     |
| 5   | 20  | 36  | 3.192       | 2.342       | 2.947       | 3.192       |
|     |     |     | (0.166)     | (0.181)     | (0.098)     | (0.174)     |
| 0.5 | 10  | 3.087 | 2.317   | 2.12        | 3.321       | 3.087       |
|     |     |     | (0.253)     | (0.224)     | (0.147)     | (0.268)     |
| 0.2 | 10  | 3.068 | 2.329   | 2.05        | 3.308       | 3.068       |
|     |     |     | (0.47)      | (0.859)     | (0.263)     | (0.829)     |
| 5   | 20  | 36  | 3.591       | 2.834       | 3.044       | 3.591       |
|     |     |     | (0.612)     | (0.624)     | (0.359)     | (0.527)     |

Epistatic effects estimated in Z₄ were multiplied by 2; n: sample size; h: broad heritability; m: the replication number of offspring.

Epistatic effects estimated in Z₄ were multiplied by 2; n: sample size; h: broad heritability; m: the replication number of offspring.

₁⁺⁺⁺⁺⁺⁺⁺⁺ ₂⁺⁺⁺⁺⁺⁺⁺⁺ ₃⁺⁺⁺⁺⁺⁺⁺⁺ ₄⁺⁺⁺⁺⁺⁺⁺⁺
personal computer (Intel Pentium CPU 2.9 GHz; memory 4 GB); therefore, the EBLASSO algorithm presented high efficiency and saved time.

Real data analysis
Considering the unbiased estimate of coefficients and the excellent detection power in the simulation study, we further applied the proposed approach to a real mapping population and presented a comparison to previous mapping results.

Populations. Two elite rice hybrids, one inter-subspecific between 9024 \((\textit{indica})\) and LH422 \((\textit{japonica})\) and one intra-subspecific between Zhenshan97 \((\textit{indica})\) and Minghui63 \((\textit{indica})\), were analyzed, and details were documented in our previous study [24]. For convenience, we designated the two hybrids as IJ and II hybrids, respectively. The RIL were derived from the cross of a random sample of F\(_2\) individuals to their parental lines (194 F\(_7\) lines for the IJ hybrid and 222 F\(_12\) lines for the II hybrid, respectively).

Genetic linkage maps. For the II hybrid, the linkage map comprised 221 marker loci and covered 1796 cM in total [42]. For the IJ hybrid, Xiao et al. [8] constructed a linkage map of the recombinant population in which a subset of 141 polymorphic restriction fragment length polymorphism markers was used.

Phenotypic traits. Nine quantitative traits, including heading date (HD, in days), plant height (PH, in centimeters), tillers per plant, panicle length (PL, in centimeters), filled grains per panicle (FGPP), percentage of seed set, grain density (GD, in grain numbers per centimeter of panicle length), 1000-grain weight (KGW, in grams), and grain yield (YD, in tons/hectare) were investigated in RIL, Z\(_1\), Z\(_2\), and Z\(_4\) respectively. All the materials described above were laid out in a field in a randomized complete block design with two replications (plots) for phenotypic evaluation.

QTL mapping in real data analysis. Data sets Z\(_1\), Z\(_2\) and Z\(_4\) were implemented in R (version 3.0) with the EBLASSO package obtained from Cai et al. [41] for QTL mapping.

Results
Simulation study results

Augmented effects in simulation study. As shown in Table 3 and Tables A-B in S11 Supporting Information, the augmented additive \((a_1^* = a_1 + \frac{1}{2}(i_{a_1a_2} - i_{d_1d_2}))\) and epistatic effects \((i_{12}^* = (i_{a_1a_2} + i_{d_1d_2}))\) in Z\(_1\), augmented dominance \((d_1^* = d_1 - \frac{1}{2}(i_{a_1a_2} - i_{d_1d_2}))\) and epistatic effects \((i_{12} = (i_{a_1d_2} + i_{d_1a_2}))\) in Z\(_2\), augmented epistatic effects \((i_{12}^* = i_{a_1a_2} - i_{d_1d_2})\) in Z\(_4\) were rightly and unbiased estimated with a high statistical power in preset positions. The ratio of the number of samples, in which the LOD statistic was greater than 2.5, to the total number of replicates represented the empirical power of this simulated QTL or interaction.

In Z\(_1\) transformation (Table A in S11 Supporting Information, S1 Fig), when the sample size was 800 or 400, almost all augmented additive and epistatic effects were detected, except for the detection power of digenic interactions in 400 samples, 0.2 heritability, and 5 replications. This indicated that smaller heritability or less individual replication had little influence on the detection of QTL in a relatively large sample size. When the sample size was reduced to 200, all the preset QTL were detected successfully with a heritability of 0.8 and 0.5; however, detection power decreased sharply to the level of 0.2, which is more true for the preset digenic interactions. When individual replication was 5, the detection power of the augment additive effect of QTL\(_3\) was 0.73, whereas it was 0.985 when the individual replication was 10, and the
detection power of augmented epistatic effects dropped to 0.59 for interaction QTL$_5$ × QTL$_6$.

Similar results could be found in $Z_2$ (Table B in S11 Supporting Information, S2 Fig): all the preset QTL were precisely detected and the QTL effects were estimated in an unbiased manner, even on the level of the smallest sample size (200) or the lowest heritability (0.2). In addition, all the augmented epistatic effects estimated in an unbiased manner in $Z_4$ (Table 3, Fig 1). The poor detection power occurred only on a low heritability level (0.2) with sample sizes 400 or 200.

**Main and epistatic effects in simulation study.** Table 4 shown the main and epistatic effects of QTL$_1$ and QTL$_2$ in the in RIL-based aTTC design using the two-step approach under the $F_\infty$ metric model. Other pairs of interactions are listed in Tables 5 and 6 for the interaction between QTL$_3$ and QTL$_4$ and QTL$_5$ and QTL$_6$, respectively. We can see that the main-effect and epistatic effects of QTL were very close to set value when sample size is big (800) and heritability is high (0.8). all the preset QTL were precisely detected and the QTL effects were estimated in an unbiased manner, except on the level of the smallest sample size (200) and the lowest heritability (0.2) with 5 replications in $Z_1$, $Z_2$ and $Z_4$.

**Real data analysis results**

**QTL mapping in II and II hybrid.** In the II hybrid, all $p = 221 + \frac{1}{2} \times 221 \times (221 - 1) = 22321$ were simultaneously included in the genetic model, about 115 times as large as the sample size, while in II hybrids, $p = 141 + \frac{1}{2} \times 141 \times (141 - 1) = 10011$, which was about 45 times bigger than the sample size. QTL effect-explained 1% phenotypic variation was set as a threshold for declaring the presence of QTL. QTL mapping results for the II and II hybrids are listed in Tables A and B in S12 Supporting Information, respectively.

**Augmented effects in II and II hybrid.** As shown in Table A in S12 Supporting Information, 14 QTLs and 36 digenic interactions were detected in the II hybrid, and the explained variation of a single QTL or interaction varied from 1.13% to 7.67%. In the RIL mapping population, 8 QTL (16%) were revealed, of which 2 QTLs (25%) were detected in trait GD with relative small phenotypic variation. In trait YD, a digenic interaction of marker C1016 and C483 explained the maximum (7.49%) phenotypic variation. In $Z_1$, 11 QTL (22%) were detected,
and one or more QTL were revealed in each trait. The explained variation in \( Z_1 \) varied from 1.21% to 7.67%. The interaction between marker R3166 and RZ667 was also detected in traits FGPP and GD, and explained 7.67% and 7.33% variation, respectively. In \( Z_2 \), 10 QTLs were identified. There was no QTL detected in trait PL. The explained variation in \( Z_2 \) varied from 1.13% to 5.91%. In \( Z_4 \), 11 interactions were found, and at least one QTL or interaction was revealed in each trait. The maximum explained variation was in trait HD (6.05%). Ten interactions were also dissected, and the explained variation of a single interaction varied from 1.69% to 7.09%.

Table 4. Dissected main and epistatic effects of QTL1 and QTL2 in the in RIL-based aTTC design using the two-step approach under the F1 metric model in simulation study.

| Parameter values | N | h | m | statistics | QTL1 | QTL2 | QTL1×QTL2 |
|------------------|---|---|---|------------|------|------|-----------|
|                  |   |   |   |            | \( a_1 \) | \( d_1 \) | \( a_2 \) | \( d_2 \) | \( a_1 d_2 \) | \( a_2 d_1 \) | \( d_1 d_2 \) |
| 800              | 0.8 | 10 | mean | 1.802 | 3.204 | 2.795 | -1.501 | 2.699 | 3.399 | 0.999 | -0.506 |
|                  |   |   | sd  | (0.022) | (0.038) | (0.021) | (0.036) | (0.021) | (0.030) | (0.033) | (0.049) |
| 5                | mean | 1.804 | 3.192 | 2.794 | -1.506 | 2.698 | 3.403 | 1.004 | -0.492 |
|                  | sd  | (0.032) | (0.047) | (0.030) | (0.051) | (0.026) | (0.047) | (0.045) | (0.061) |
| 0.5              | 10 | mean | 1.807 | 3.183 | 2.789 | -1.511 | 2.686 | 3.370 | 1.000 | -0.489 |
|                  | sd  | (0.049) | (0.071) | (0.043) | (0.069) | (0.045) | (0.066) | (0.061) | (0.093) |
| 5                | mean | 1.825 | 3.181 | 2.758 | -1.523 | 2.672 | 3.356 | 1.015 | -0.498 |
|                  | sd  | (0.058) | (0.124) | (0.064) | (0.120) | (0.068) | (0.095) | (0.097) | (0.155) |
| 0.2              | 10 | mean | 1.830 | 3.158 | 2.748 | -1.526 | 2.653 | 3.350 | 1.017 | -0.493 |
|                  | sd  | (0.084) | (0.154) | (0.089) | (0.154) | (0.092) | (0.131) | (0.141) | (0.177) |
| 5                | mean | 1.827 | 3.142 | 2.688 | -1.492 | 2.614 | 3.333 | 1.067 | -0.557 |
|                  | sd  | (0.151) | (0.222) | (0.122) | (0.228) | (0.123) | (0.178) | (0.175) | (0.284) |
| 400              | 0.8 | 10 | mean | 1.808 | 3.184 | 2.795 | -1.515 | 2.689 | 3.388 | 1.002 | -0.494 |
|                  | sd  | (0.035) | (0.052) | (0.030) | (0.051) | (0.031) | (0.042) | (0.044) | (0.064) |
| 5                | mean | 1.810 | 3.187 | 2.789 | -1.506 | 2.688 | 3.385 | 1.011 | -0.501 |
|                  | sd  | (0.044) | (0.084) | (0.044) | (0.087) | (0.050) | (0.061) | (0.065) | (0.103) |
| 0.5              | 10 | mean | 1.807 | 3.156 | 2.786 | -1.525 | 2.673 | 3.378 | 0.998 | -0.486 |
|                  | sd  | (0.066) | (0.096) | (0.062) | (0.099) | (0.063) | (0.084) | (0.084) | (0.126) |
| 5                | mean | 1.831 | 3.168 | 2.744 | -1.518 | 2.660 | 3.354 | 1.041 | -0.494 |
|                  | sd  | (0.099) | (0.166) | (0.090) | (0.164) | (0.098) | (0.132) | (0.143) | (0.201) |
| 0.2              | 10 | mean | 1.842 | 3.118 | 2.698 | -1.559 | 2.625 | 3.305 | 1.022 | -0.494 |
|                  | sd  | (0.176) | (0.216) | (0.179) | (0.219) | (0.153) | (0.244) | (0.226) | (0.313) |
| 5                | mean | 1.946 | 2.993 | 2.568 | -1.629 | 2.399 | 3.206 | 1.138 | -0.471 |
|                  | sd  | (0.251) | (0.513) | (0.257) | (0.491) | (0.461) | (0.311) | (0.375) | (0.687) |
| 200              | 0.8 | 10 | mean | 1.805 | 3.184 | 2.785 | -1.500 | 2.688 | 3.391 | 1.006 | -0.492 |
|                  | sd  | (0.050) | (0.072) | (0.044) | (0.073) | (0.042) | (0.071) | (0.058) | (0.096) |
| 5                | mean | 1.816 | 3.190 | 2.771 | -1.484 | 2.668 | 3.360 | 1.018 | -0.525 |
|                  | sd  | (0.059) | (0.113) | (0.069) | (0.099) | (0.059) | 0.086 | 0.083 | 0.133 |
| 0.5              | 10 | mean | 1.834 | 3.137 | 2.749 | -1.557 | 2.635 | 3.355 | 1.038 | -0.451 |
|                  | sd  | (0.098) | (0.153) | (0.092) | (0.151) | (0.088) | 0.141 | 0.135 | 0.201 |
| 5                | mean | 1.862 | 3.129 | 2.684 | -1.510 | 2.611 | 3.288 | 1.054 | -0.531 |
|                  | sd  | (0.194) | (0.210) | (0.208) | (0.219) | (0.146) | 0.244 | 0.231 | 0.329 |
| 0.2              | 10 | mean | 1.991 | 3.057 | 2.498 | -1.588 | 2.474 | 3.156 | 1.239 | -0.502 |
|                  | sd  | (0.312) | (0.393) | (0.290) | (0.397) | (0.398) | 0.359 | 0.376 | 0.602 |
| 5                | mean | 2.169 | 2.626 | 2.099 | -1.983 | 1.830 | 2.770 | 1.423 | -0.908 |
|                  | sd  | (0.474) | (0.958) | (0.489) | (0.961) | (0.515) | 0.569 | 0.505 | 1.330 |

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In the I/J hybrid, as shown in Table B in S12 Supporting Information, a total of 46 QTL and 75 interactions was detected. Of the detected QTL, the majority was detected in RIL (39.37%) and Z4 (41.73%). The explained variation of a single QTL in the RIL ranged from 1.09% to 27.48%. In Z4, the detected QTL-associated marker RG333 affected HD in chromosome 8 explained 27.48% of phenotypic variation. It was also found simultaneously in Z1 and Z2 data sets. Eighteen QTLs in Z1 and 9 QTLs in Z2 were identified. In Z1, QTL-associated marker RG333 influenced HD, accounting for 36.58% of variation, which also explained 8.47% of

### Table 5. Dissected main and epistatic effects of QTL3 and QTL4 in the in RIL-based aTTC design using the two-step approach under the F1 metric model in simulation study.

| Parameter values | N | h | m | statistics | a3 | d3 | a4 | d4 | a3,a4 | d3,d4 | d3a4 | d4a3 |
|------------------|---|---|---|-----------|----|----|----|----|-------|-------|-------|-------|
| 800 0.8 10 mean | 0.001 | 0.003 | -2.003 | 2.097 | -0.698 | -1.605 | -3.798 | 2.898 |
| sd (0.022) (0.038) (0.020) (0.042) (0.022) (0.035) (0.029) (0.048) |
| 5 mean | 0.001 | 0.002 | -2.006 | 2.100 | -0.702 | -1.602 | -3.794 | 2.892 |
| sd (0.033) (0.061) (0.034) (0.053) (0.028) (0.047) (0.044) (0.074) |
| 0.5 10 mean | 0.008 | 0.012 | -2.007 | 2.098 | -0.703 | -1.606 | -3.784 | 2.892 |
| sd (0.044) (0.073) (0.047) (0.075) (0.038) (0.062) (0.066) (0.086) |
| 5 mean | 0.010 | 0.021 | -2.007 | 2.126 | -0.683 | -1.614 | -3.792 | 2.855 |
| sd (0.063) (0.114) (0.054) (0.100) (0.067) (0.089) (0.088) (0.123) |
| 0.2 10 mean | 0.023 | 0.021 | -2.029 | 2.116 | -0.695 | -1.620 | -3.761 | 2.836 |
| sd (0.090) (0.158) (0.093) (0.150) (0.096) (0.136) (0.153) (0.206) |
| 5 mean | 0.029 | 0.002 | -2.047 | 2.111 | -0.745 | -1.664 | -3.708 | 2.821 |
| sd (0.193) (0.230) (0.166) (0.256) (0.187) (0.237) (0.226) (0.389) |
| 400 0.8 10 mean | 0.002 | 0.006 | -2.003 | 2.104 | -0.699 | -1.602 | -3.794 | 2.891 |
| sd (0.030) (0.053) (0.030) (0.059) (0.031) (0.040) (0.043) (0.072) |
| 5 mean | 0.008 | 0.001 | -2.008 | 2.116 | -0.698 | -1.611 | -3.788 | 2.872 |
| sd (0.040) (0.079) (0.047) (0.087) (0.049) (0.068) (0.063) (0.107) |
| 0.5 10 mean | 0.006 | 0.008 | -2.019 | 2.101 | -0.703 | -1.629 | -3.778 | 2.877 |
| sd (0.055) (0.101) (0.058) (0.096) (0.051) (0.087) (0.092) (0.134) |
| 5 mean | 0.025 | 0.014 | -2.047 | 2.121 | -0.687 | -1.658 | -3.755 | 2.833 |
| sd (0.099) (0.155) (0.094) (0.156) (0.092) (0.152) (0.132) (0.204) |
| 0.2 10 mean | 0.049 | 0.032 | -2.079 | 2.164 | -0.700 | -1.661 | -3.664 | 2.778 |
| sd (0.213) (0.226) (0.230) (0.225) (0.121) (0.255) (0.273) (0.274) |
| 5 mean | 0.244 | 0.010 | -2.333 | 2.142 | -0.727 | -1.920 | -3.419 | 2.749 |
| sd (0.402) (0.315) (0.444) (0.351) (0.224) (0.528) (0.495) (0.467) |
| 200 0.8 10 mean | 0.002 | 0.002 | -2.004 | 2.105 | -0.697 | -1.605 | -3.797 | 2.896 |
| sd (0.046) (0.079) (0.045) (0.079) (0.047) (0.065) (0.062) (0.098) |
| 5 mean | 0.011 | 0.016 | -2.011 | 2.110 | -0.693 | -1.615 | -3.778 | 2.867 |
| sd (0.065) (0.116) (0.064) (0.095) (0.066) (0.096) (0.089) (0.137) |
| 0.5 10 mean | 0.031 | 0.017 | -2.037 | 2.129 | -0.692 | -1.628 | -3.749 | 2.848 |
| sd (0.093) (0.157) (0.082) (0.149) (0.085) (0.121) (0.135) (0.182) |
| 5 mean | 0.127 | 0.005 | -2.164 | 2.111 | -0.700 | -1.751 | -3.588 | 2.852 |
| sd (0.307) (0.202) (0.313) (0.198) (0.131) (0.336) (0.352) (0.262) |
| 0.2 10 mean | 0.332 | 0.243 | -2.373 | 2.352 | -0.588 | -2.008 | -3.340 | 2.553 |
| sd (0.482) (0.599) (0.404) (0.594) (0.555) (0.493) (0.506) (0.674) |
| 5 mean | 0.255 | 0.485 | -2.637 | 2.808 | -0.737 | -2.363 | -3.001 | 2.351 |
| sd (0.825) (1.049) (0.614) (0.960) (0.909) (0.497) (0.559) (0.955) |

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variation for PH. In Z4, the majority of interactions were detected in trait FGPP in which 17 (58.6%) and 10 (41.7%) marker pairs were found in
\[ i_{12} = i_{a_1a_2} - i_{d_1d_2} \] and
\[ i_{12} = i_{a_1d_2} - i_{d_1a_2} \].

Dissection of main and epistatic effects. Integrated in the QTL mapping result of Tables A and B in S12 Supporting Information, main and epistatic effects were dissected by the proposed approach distributed previous part 3.4. The results of the II and IJ hybrid are presented in Tables 7 and 8, respectively. For main effect QTL, we dissected the additive and dominance effects, whereas for interactions, additive × additive (aa), additive × dominance (ad),

| n   | h  | m | statistics | QTL5 | QTL6 | QTL5×QTL6 |
|-----|----|---|------------|------|------|-----------|
|     |    |   |            |      |      | a_s | d_s | a_s | d_s | a_s | d_s | a_s | d_s |
| 800 | 0.8| 10| mean       | 0.001| 0.008| 0.003| 0.006| 2.791| 3.897| 0.903| -0.594|
|     |    |   | sd         | (0.019)| (0.038)| (0.022)| (0.039)| (0.022)| (0.033)| (0.029)| (0.049)|
| 5   |    |   | mean       | 0.006| 0.010| 0.003| 0.008| 2.791| 3.894| 0.905| -0.588|
|     |    |   | sd         | (0.029)| (0.047)| (0.035)| (0.058)| (0.030)| (0.041)| (0.047)| (0.075)|
| 0.5 | 10|   | mean       | 0.001| 0.002| 0.006| 0.009| 2.788| 3.892| 0.903| -0.597|
|     |    |   | sd         | (0.046)| (0.075)| (0.045)| (0.067)| (0.038)| (0.059)| (0.063)| (0.088)|
| 0.2 | 10|   | mean       | 0.005| 0.006| 0.000| 0.003| 2.772| 3.888| 0.903| -0.600|
|     |    |   | sd         | (0.055)| (0.102)| (0.063)| (0.102)| (0.057)| (0.083)| (0.086)| (0.134)|
| 400 | 0.8| 10| mean       | 0.019| 0.031| 0.005| 0.021| 2.746| 3.847| 0.926| -0.590|
|     |    |   | sd         | (0.105)| (0.136)| (0.086)| (0.136)| (0.083)| (0.123)| (0.120)| (0.183)|
| 5   |    |   | mean       | 0.016| 0.022| 0.011| 0.011| 2.721| 3.841| 0.918| -0.618|
|     |    |   | sd         | (0.122)| (0.221)| (0.129)| (0.212)| (0.109)| (0.201)| (0.183)| (0.290)|
| 0.5 | 10|   | mean       | 0.002| 0.011| 0.005| 0.030| 2.764| 3.869| 0.889| -0.581|
|     |    |   | sd         | (0.067)| (0.115)| (0.059)| (0.102)| (0.059)| (0.097)| (0.095)| (0.141)|
| 5   |    |   | mean       | 0.020| 0.016| 0.005| 0.009| 2.736| 3.859| 0.916| -0.608|
|     |    |   | sd         | (0.095)| (0.182)| (0.084)| (0.180)| (0.097)| (0.142)| (0.138)| (0.225)|
| 0.2 | 10|   | mean       | 0.008| 0.018| 0.044| 0.010| 2.721| 3.833| 0.928| -0.595|
|     |    |   | sd         | (0.142)| (0.217)| (0.158)| (0.234)| (0.117)| (0.185)| (0.155)| (0.269)|
| 5   |    |   | mean       | 0.089| 0.316| 0.068| 0.282| 2.343| 3.791| 0.994| -0.329|
|     |    |   | sd         | (0.264)| (0.905)| (0.255)| (1.015)| (0.879)| (0.344)| (0.338)| (1.027)|
| 200 | 0.8| 10| mean       | 0.003| 0.005| 0.000| 0.003| 2.787| 3.896| 0.902| -0.603|
|     |    |   | sd         | (0.044)| (0.080)| (0.041)| (0.076)| (0.049)| (0.061)| (0.062)| (0.106)|
| 5   |    |   | mean       | 0.018| 0.018| 0.008| 0.029| 2.768| 3.871| 0.924| -0.578|
|     |    |   | sd         | (0.067)| (0.111)| (0.065)| (0.107)| (0.061)| (0.086)| (0.094)| (0.138)|
| 0.5 | 10|   | mean       | 0.023| 0.011| 0.001| 0.031| 2.740| 3.846| 0.903| -0.581|
|     |    |   | sd         | (0.099)| (0.151)| (0.097)| (0.169)| (0.085)| (0.136)| (0.140)| (0.209)|
| 5   |    |   | mean       | 0.010| 0.021| 0.004| 0.027| 2.723| 3.880| 0.939| -0.586|
|     |    |   | sd         | (0.129)| (0.232)| (0.141)| (0.225)| (0.123)| (0.163)| (0.194)| (0.281)|
| 0.2 | 10|   | mean       | 0.039| 0.132| 0.060| 0.114| 2.535| 3.806| 0.958| -0.520|
|     |    |   | sd         | (0.276)| (0.532)| (0.265)| (0.495)| (0.469)| (0.294)| (0.368)| (0.696)|
| 5   |    |   | mean       | 0.311| 0.573| 0.314| 0.517| 1.837| 3.201| 1.439| -0.776|
|     |    |   | sd         | (0.646)| (0.963)| (0.672)| (1.050)| (0.510)| (0.719)| (0.805)| (1.370)|

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Table 7. Dissected main and epistatic effects in II hybrid.

| Trait | Chr<sup>a</sup> | Marker | Chr<sup>a</sup> | Marker | QTL<sub>i</sub> | QTL<sub>j</sub> | QTL<sub>i</sub>×QTL<sub>j</sub> | Dominance degree<sup>b</sup> |
|-------|----------------|--------|----------------|--------|--------------|--------------|----------------|----------------|
| HD    | 12             | C996   |               |        | 2.09         | 0.00         | A              |                |
| HD    | 8              | C347   | 4             | C56    | 0.46         | -1.84        | 0.46           | 4.47           |
| HD    | 1              | R2632  | 10            | RM258  | -0.39        | 0.33         | 2.42           | -0.33          |
| HD    | 2              | RZ599  | 11            | R3203  | 0.04         | -1.89        | 0.04           | 1.89           |
| HD    | 3              | RM227  | 8             | C1121  | -0.27        | -1.56        | -0.27          | 1.56           |
| HD    | 6              | Waxy   | 6             | R2S49  | -0.83        | -0.15        | -0.83          | 0.15           |
| PH    | 7              | RM70   |               |        | 1.90         | 0.00         | A              |                |
| PH    | 3              | R1925  |               |        | 0.00         | 1.86         | OD             |                |
| PH    | 5              | RG360  | 8             | R1629  | 0.82         | -2.44        | -2.44          | 2.44           |
| PH    | 6              | RM204  | 10            | C962   | 1.67         | -0.26        | -0.26          | 1.67           |
| TP    | 2              | RM53   |               |        | 0.00         | 0.38         | OD             |                |
| TP    | 4              | R78    | 12            | C909B  | -0.07        | 0.33         | 0.33           | -0.07          |
| TP    | 4              | C2807  | 6             | P      | -0.01        | 0.60         | 0.60           | -0.01          |
| TP    | 2              | RG634  | 2             | R1738  | -0.81        | 0.09         | 0.09           | -0.09          |
| PL    | 6              | G342   |               |        | 0.30         | 0.00         | A              |                |
| PL    | 4              | G235   | 7             | RM70   | -0.07        | 0.46         | 0.46           | -0.19          |
| PL    | 3              | C316   | 8             | RM25   | 0.14         | 0.41         | -0.01          | 0.01           |
| PL    | 6              | RM204  | 12            | C909B  | -0.02        | 0.20         | 0.20           | -0.20          |
| FGP   | 5              | R3166  | 6             | RZ667  | -1.39        | 5.65         | 1.39           | -2.64          |
| FGP   | 8              | RM223  | 9             | RG570  | -0.78        | 0.17         | 0.17           | -2.92          |
| FGP   | 2              | RM48   | 9             | R1687  | 1.03         | 3.97         | -1.03          | 1.03           |
| FGP   | 8              | C1121  | 8             | RG978  | -3.82        | 0.00         | 0.00           | -3.82          |
| SS    | 12             | RM20b  |               |        | 2.62         | 0.00         | A              |                |
| SS    | 6              | RG424  | 13            | C933   | -0.76        | 0.97         | 0.97           | 3.13           |
| SS    | 2              | G1314a | 6             | R2549  | -1.00        | 2.94         | 2.94           | -1.00          |
| SS    | 9              | C153B  | 14            | C477   | -7.01        | 1.55         | 1.55           | 1.55           |
| GD    | 5              | R3166  | 6             | RZ667  | -0.05        | 0.19         | 0.19           | -0.05          |
| GD    | 1              | C161   | 1             | RM243  | 0.05         | 0.22         | 0.22           | -0.05          |
| GD    | 2              | RM48   | 9             | R1687  | 0.03         | 0.17         | 0.17           | -0.03          |
| GD    | 2              | RZ324  | 7             | RM234  | -0.12        | 0.08         | 0.08           | -0.12          |
| KGW   | 3              | C1176  |               |        | 0.00         | 0.33         | OD             |                |
| KGW   | 8              | L363A  |               |        | 0.00         | 0.22         | OD             |                |
| KGW   | 5              | RG360  | 8             | C347   | 0.02         | -0.01        | -0.01          | -0.01          |
| KGW   | 8              | RM25   | 9             | RZ404  | 0.18         | 0.40         | 0.40           | -0.18          |
| KGW   | 1              | RG101  | 4             | RM241  | -0.13        | 0.33         | 0.33           | -0.13          |
| KGW   | 1              | G393   | 1             | R2201  | 0.75         | -0.87        | -0.87          | 0.75           |
| YD    | 10             | C153A  |               |        | 1.50         | 0.00         | A              |                |
| YD    | 7              | R1789  |               |        | 0.00         | 1.67         | OD             |                |
| YD    | 4              | C2807  | 14            | C477   | -0.08        | 0.32         | 0.32           | -1.86          |
| YD    | 8              | R1399  | 9             | RM215  | -0.77        | 1.99         | 1.99           | 0.77           |
| YD    | 9              | R1952b | 10            | C153A  | 1.37         | 0.01         | 0.01           | 1.37           |
| YD    | 11             | L1044  | 11            | Y6854L | 4.53         | 1.02         | 1.02           | 4.53           |

<sup>a</sup> Chromosome where the detected QTL located in.

<sup>b</sup>Main effect QTL can be classified as additive (A) (|d<sub>i</sub>/a<sub>i</sub>| < 0.2), partial dominance (PD) (0.2 < |d<sub>i</sub>/a<sub>i</sub>| < 0.8), dominance (D) (0.8 < |d<sub>i</sub>/a<sub>i</sub>| < 1.2), and overdominance (OD) (|d<sub>i</sub>/a<sub>i</sub>| ≥ 1.2). Epistatic QTL can be classified as additive (A) (|d<sub>i</sub>d<sub>j</sub>/a<sub>i</sub>a<sub>j</sub>| < 0.2), partial dominance (PD) (0.2 < |d<sub>i</sub>d<sub>j</sub>/a<sub>i</sub>a<sub>j</sub>| < 0.8), dominance (D) (0.8 < |d<sub>i</sub>d<sub>j</sub>/a<sub>i</sub>a<sub>j</sub>| < 1.2), and overdominance (OD) (|d<sub>i</sub>d<sub>j</sub>/a<sub>i</sub>a<sub>j</sub>| ≥ 1.2).

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Table 8. Dissected main and epistatic effects in IJ hybrid.

| Trait | Chr<sup>a</sup> | Marker | Chr<sup>a</sup> | Marker | QTL<sub>i</sub> | QTL<sub>j</sub> | QTL<sub>i</sub>×QTL<sub>j</sub> | Dominance degree<sup>b</sup> |
|-------|----------------|--------|----------------|--------|----------------|----------------|----------------|-------------------|
| HD 1  | RG811         |        |                |        | 0.73           | 0.00           | 0.00           | A                 |
| HD 3  | XNPB249       |        |                |        | 0.83           | 0.00           | 0.00           | A                 |
| HD 7  | RG711         |        |                |        | 0.60           | 0.00           | 0.00           | A                 |
| HD 8  | RG333         |        |                |        | 2.73           | 0.96           | 0.00           | D                 |
| HD 5  | RZ556         | 8      | RZ562          |        | 0.80           | 0.55           | 0.80           | 1.10             | OD                |
| HD 8  | RZ562         | 9      | RG667          |        | 0.85           | 1.01           | 0.85           | 1.01             | D                 |
| HD 2  | TW500         | 5      | RG480          |        | 0.43           | 0.24           | 0.43           | 0.24             | D                 |
| HD 6  | RZ450         | 6      | CDO204         |        | 6.66           | 0.00           | 6.66           | 0.00             | A                 |
| HD 8  | RG333         | 11     | CDO354         |        | 3.80           | 0.28           | 1.07           | 0.45             | D                 |
| PH 8  | RG333         |        |                |        | 3.47           | 0.00           | 3.47           | 0.00             | A                 |
| PH 2  | RZ987         | 10     | RZ982          |        | 0.26           | 1.34           | 0.26           | 3.95             | OD                |
| PH 2  | RG152         | 4      | XNPB271        |        | 0.12           | 0.79           | 0.12           | 3.79             | OD                |
| PH 2  | TW500         | 3      | RG510          |        | 0.03           | 1.35           | 0.03           | 1.35             | D                 |
| PH 1  | RG173         | 2      | TW500          |        | 1.15           | 1.02           | 1.15           | 1.02             | D                 |
| PH 4  | RG449         | 7      | RZ711          |        | 1.63           | 0.03           | 1.63           | 0.03             | D                 |
| PH 5  | RZ390         | 9      | RZ12           |        | 0.90           | 0.31           | 0.90           | 0.31             | D                 |
| PH 5  | RZ70          | 8      | RZ562          |        | 1.68           | 0.18           | 1.68           | 0.18             | D                 |
| TP 1  | RG541         | 7      | RZ262          |        | 0.01           | 0.24           | 0.01           | 0.41             | OD                |
| TP 9  | RZ422         | 11     | XNPB179        |        | 0.04           | 0.25           | 0.04           | 0.45             | OD                |
| TP 2  | RG555         | 7      | CDO405         |        | 0.15           | 0.00           | 0.15           | 0.00             | D                 |
| TP 6  | RZ450         | 9      | RZ12           |        | 0.03           | 0.05           | 0.03           | 0.06             | D                 |
| TP 2  | TW500         | 6      | RZ282          |        | 0.04           | 0.29           | 0.04           | 0.29             | D                 |
| TP 3  | RG1356        | 6      | RZ213          |        | 0.12           | 0.00           | 0.12           | 0.00             | D                 |
| TP 4  | RG908         | 9      | XNPB295        |        | 0.10           | 0.08           | 0.10           | 0.08             | D                 |
| PL 5  | RZ296         | 11     | CDO354         |        | 0.05           | 0.32           | 0.05           | 0.32             | OD                |
| PL 4  | RZ262         | 12     | XNPB189        |        | 0.12           | 0.45           | 0.12           | 0.45             | D                 |
| PL 9  | RZ404         | 12     | RG98           |        | 0.30           | 0.12           | 0.30           | 0.12             | D                 |
| FGPP 3| CDO1081       |        |                |        | 6.12           | 0.00           | 6.12           | 0.00             | A                 |
| FGPP 1| RG541         | 4      | XNPB271        |        | 1.76           | 0.66           | 1.76           | 0.66             | D                 |
| FGPP 1| RG469         | 5      | RZ390          |        | 0.60           | 3.18           | 0.60           | 3.18             | D                 |
| FGPP 1| RG737         | 8      | RZ333          |        | 0.28           | 4.18           | 0.28           | 4.18             | D                 |
| FGPP 1| RG375         | 10     | RZ811          |        | 2.59           | 5.47           | 2.59           | 5.47             | D                 |
| FGPP 2| RZ913         | 2      | RG544          |        | 0.01           | 2.71           | 0.01           | 2.71             | D                 |
| FGPP 2| CDO395        | 7      | CDO405         |        | 0.22           | 4.98           | 0.22           | 4.98             | D                 |
| FGPP 2| CDO395        | 8      | RG333          |        | 0.22           | 2.14           | 0.22           | 2.14             | D                 |
| FGPP 2| RZ986         | 6      | WAXY           |        | 0.14           | 2.28           | 0.14           | 2.28             | D                 |
| FGPP 2| RZ987         | 10     | RZ892          |        | 2.27           | 4.02           | 2.27           | 4.02             | D                 |
| FGPP 2| XNPB132       | 10     | RZ811          |        | 1.97           | 3.42           | 1.97           | 3.42             | D                 |
| FGPP 2| RG454         | 5      | RG480          |        | 2.07           | 4.65           | 2.07           | 4.65             | D                 |
| FGPP 2| TW500         | 8      | RZ562          |        | 0.36           | 5.19           | 0.36           | 5.19             | D                 |
| FGPP 3| XNPB249       | 8      | RG136          |        | 0.19           | 2.33           | 0.19           | 2.33             | D                 |
| FGPP 3| RZ16          | 10     | RZ561          |        | 0.59           | 3.01           | 0.59           | 3.01             | D                 |
| FGPP 4| CDO456        | 11     | XNPB320        |        | 1.55           | 3.61           | 1.55           | 3.61             | D                 |

(Continued)
dominance × additive (da), dominance × dominance (dd), effects were dissected. The dominance degree of each QTL or interaction was separately calculated by |d1/a1| and |d1d2/a1a2|, respectively. Where d1, a1, d2, a2 denote the dissected dominance effect, additive effect, dominance × dominance epistatic effect, additive × additive epistatic effect, respectively. According to Stuber et al. [12], main effect QTL can be classified as additive (|d1/a1|<0.2), partial dominance (0.2≤|d1/a1|<0.8), dominance (0.8≤|d1/a1|<1.2), and overdominance (|d1/a1|≥1.2). Epistatic QTL can be classified as additive (|d1d2/a1a2|<0.2), partial dominance (0.2≤|d1d2/a1a2|<0.8), dominance (0.8≤|d1d2/a1a2|<1.2), and overdominance (|d1d2/a1a2|≥1.2).

HD: In the II hybrid, the only main effect QTL was classified as additive; in the other five epistatic QTL, most were classified as dominance, except one that showed overdominance. In the IJ hybrid, four main effect QTL were classified as additive, and only one main effect QTL was classified as partial dominance; four epistatic QTL were classified as dominance, and one epistatic QTL was classified as overdominance.
PH: In the II hybrid, two main effect QTL were classified as additive and overdominance, respectively; two epistatic QTL were classified as dominance. In the IJ hybrid, two main effect QTL were classified as additive and overdominance, respectively; in seven epistatic QTL, two were classified as overdominance and the other five were dominance.

Tillers per plant: In the II hybrid, the only main effect QTL was classified as overdominance; two of three epistatic QTL were classified as dominance and the remaining one showed overdominance. In the IJ hybrid, no main effect QTL was found; in four epistatic QTL, one was identified as overdominance and the rest were dominance.

PL: In the II hybrid, the only main effect QTL was classified as additive; two of three epistatic QTL were classified as dominance and the remaining one showed overdominance. In the IJ hybrid, no main effect QTL was found; in four epistatic QTL, one was identified as overdominance and the rest were dominance.

FGPP: In the II hybrid, no main effect QTL was found; in four epistatic QTL, one was classified as overdominance and the rest were dominance. In the IJ hybrid, the only main effect QTL was classified as additive; a total of 28 epistatic QTL was dissected, all of which showed dominance.

Percentage of seed set: In the II hybrid, the only main effect QTL was classified as additive and the three epistatic QTL were classified as dominance. In the IJ hybrid, only three epistatic QTL were dissected and all of them were classified as dominance.

GD: In the II hybrid, no main effect QTL was found; among four epistatic QTL, one was classified as overdominance and the remaining three were dominance. In the IJ hybrid, the only main effect QTL was classified as overdominance; among four epistatic QTL, three were classified as dominance and the remaining one was classified as overdominance.

KGW: In the II hybrid, two main effect QTL were classified as overdominance; in four epistatic QTL, one showed overdominance and the remaining three showed dominance. In the IJ hybrid, two main effect QTL were classified as additive and overdominance, respectively; two epistatic QTL showed dominance.

YD: In the II hybrid, two main effect QTL were classified as additive and overdominance, respectively; all four epistatic QTL were classified as dominance. In the IJ hybrid, the only main effect QTL showed overdominance, and the three epistatic QTL were classified as dominance.

From Tables 7 and 8, we can see that little common loci were found. This phenomenon partially results from the mapping markers in IJ and II hybrid are different. But on the same chromosome, we found some nearby loci affected same trait in both II and IJ hybrids.

Table 9 summarizes the main and epistatic QTL revealed in the II and IJ hybrids. For main effect QTL, 10 QTL were identified in the II hybrid; five were classified as additive, and the rest were classified as overdominance. In the IJ hybrid, 12 QTL were found, more additive (58.33%) loci were identified than overdominance in number (33.34%). For epistatic QTL, dominance or overdominance are found in two hybrid combinations, and dominance played a leading role in

Table 9. Summary of main and epistatic effects in II and IJ hybrids.

|       | II Main-effect QTL | IJ Main-effect QTL | II Epistatic-effect QTL | IJ Epistatic-effect QTL |
|-------|--------------------|--------------------|-------------------------|-------------------------|
| No.   | Rate(%)           | No.                | Rate(%)                 | No.                     |
| A     | 5                  | 8                  | 57.14                   |                         |
| PD    | 1                  | 7.14               |                         |                         |
| D     |                    | 26                 | 81.25                   | 56                      |
| OD    | 5                  | 5                  | 35.72                   | 6                       |
| SUM   | 10                 | 14                 | 32                      | 65                      |

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epistatic QTL. Dominance accumulation and overdominance were the major genetic basis of heterosis.

Discussion
Models comparison
Based on the aTTC design, this paper developed a QTL mapping method that fit for many base populations (RIL, F2, and DH); by employing multiple data set transformations (Z1i, Z2i, Z3i, Z4i, Z5i, and Z6i), many types of main and epistatic effects can be dissected. This paper took one combination (Z1, Z2, and Z4) of the aTTC design as an instance and proposed a two-step approach to dissect additive, dominance, and epistatic effects of QTL in the RIL-based aTTC design. A series of Monte Carlo simulation studies were carried out to confirm the proposed approach. Compared to previous studies on our methodologies, the proposed approach offered great advantages over previous methods.

aTTC design has many more transformations than do the TTC or NCIII designs, and with a series of transformation combinations (Z1, Z2, and Z4), (Z1, Z2, and Z5), or (Z1, Z2, and Z6), we can dissect main and epistatic effects of individual QTL or interactions by QTL mapping. It provides a new method for quantitative genetics research and especially for allowing scientists and breeders to understand the genetic basis for plant heterosis. In our study, we took the transformation combination Z1, Z2, and Z4 of RIL-based aTTC design as an instance to dissect genetic effects. There were some advantages when taking RIL as the base population. The genetic expectation mean of RIL was equivalent to L4i; therefore, there was no need to self-mate the base population. We simply used the RIL population data set substitute L4i, which saved labor and time. When using RIL-based TTC design for QTL mapping, we need generate four populations RIL, L1, L2 and L3. However, when using RIL-based aTTC design, breeders only need generate three populations RIL, L1 and L2. With combination (Z1 = L1+L2, Z2 = L1−L2 and Z4 = L1+L2−L4), we can dissect additive, dominance, and epistatic effects of QTL with high statistical powers and accuracies. In addition, many real mapping populations that derived from RIL-based NCIII design can be re-analyzed by the proposed method to develop main and epistatic effects to clearly decipher a genetic basis for heterosis.

In the present study, we used three different interaction patterns in one genetic model, which was much more complicated than that proposed by He et al. [37,39]. As shown in Tables A and B in S11 Supporting Information, with high detection power, all the augmented main effects in QTL (QTL1-6) and epistatic effects in digenic interactions (QTL1 and QTL2, QTL3 and QTL4, and QTL5 and QTL6) were estimated in an unbiased manner in Z1 and Z2. In Z4, two augmented epistatic effects $\overline{t}_{12} = i_{1a2} - i_{1d2}$ and $\overline{t}_{12} = i_{1a2} - i_{1d2}$ were further estimated precisely (shown in Table 3).

Actually, for the detection of small and linked QTL, low powers were observed. EBLASSO can handle the model that includes many effects [37, 39, 43]. In this study, we use a large number of effects, including main and epistatic QTL effects, simultaneously. EBLASSO shrinks weak effect into zero, which has little influence on large effect QTL. Simulation studies demonstrated that the fast EBLASSO greatly improved calculated speed and detected more true QTL effects without increasing the false-positive rate.

Comparison of QTL mapping results in II and IJ hybrid with previous mapping results
The QTL mapping results of this paper were compared with those of our previous study [24] in which the CIM was employed to mapping main effect QTL [44], and the mixed linear
approach [45] was used to estimate epistatic QTL. QTL detected by both studies are listed in Tables 10 and 11 for II and IJ hybrids, respectively. As shown in Table 10, a total of nine main effects QTL and four epistatic QTL was found simultaneously in two studies; only one QTL revealed in trait PH showed opposite dominance degree. In an RIL mapping population, three main effects QTL were detected; two main effects QTL were detected in Z1, and both of them were identified as additive. Eight QTL were found in Z2, and half of them were main effects; dominance degree revealed by two methods was similar, except for marker R1925 in trait PH. For the IJ hybrid, shown in Table 11, no epistatic QTL was simultaneously detected. The number of main effects QTL detected by both studies was 17, 3, and 3 for RIL, Z1, and Z2, respectively. Except for marker CDO533 in trait PH, the detected main effects QTL showed the same dominance degree. If not taking threshold into consideration, the ratio of same main effect QTL detected by the fast EBLASSO algorithm to previous mapping results were 23.08%, 22.22%, and 50% for RIL, Z1, and Z2, respectively in the II hybrid, whereas in the IJ hybrid, they were 54.84%, 17.65%, and 21.430%.

Among the identified QTL, some of them were pleiotropic. In the IJ hybrid, marker RG333 on chromosome 8 was simultaneously revealed in traits HD, PH, KGW, and YD; marker CDO1081 on chromosome 3 was simultaneously identified for traits HD, FGPP, and YD. These markers, especially for marker RG333 and marker CDO1081, were also found pleiotropic in the work of Xiao et al. [8] and Li et al. [24]. These regions deserve further attention, especially in marker-assisted breeding.

| Trait | Chr | Marker | Chr | Marker | Method A | Method B | Method A | Method B | Method A | Method B | Method A | Method B | Dominance degree |
|-------|-----|--------|-----|--------|----------|----------|----------|----------|----------|----------|----------|----------|------------------|
|       |     |        |     |        | beta     | h\(^2\)(%) | beta     | h\(^2\)(%) | beta     | h\(^2\)(%) | beta     | h\(^2\)(%) |                  |
| HD 12 | C996| 6.10   | -2.09| 2.86   |          |          |          |          |          |          |          |          |                  |
| HD 10 | R2632| 2.11 | 6.27 | 2.03   | 5.91     |          |          |          |          |          |          |          |                  |
| PH 20 | RG561| 2.27 | 3.6  | 1.27   | 2.16     |          |          |          |          |          |          |          |                  |
| PH 3  | R1925| -2.86| 4.60 | -1.86  | 1.99     |          |          |          |          |          |          |          |                  |
| PL 5  | R3166| 0.43 | 4.2  | -0.30  | 1.21     |          |          |          |          |          |          |          |                  |
| PL 6  | G342 | 0.62 | 5.20 | 0.30   | 1.21     |          |          |          |          |          |          |          |                  |
| FGPP 8 | RM223| -3.75| 3.08 | -3.70  | 3.30     |          |          |          |          |          |          |          |                  |
| SS 6  | RG424| 2.18 | 3.56 | 2.37   | 4.45     |          |          |          |          |          |          |          |                  |
| GD 6  | R1014| 0.2  | 5.5  | 0.10   | 1.64     |          |          |          |          |          |          |          |                  |
| KGW 3 | C1176| 0.42 | 3.80 | -0.33  | 2.48     |          |          |          |          |          |          |          |                  |
| KGW 8 | L363A| 0.50 | 5.50 | -0.22  | 1.13     |          |          |          |          |          |          |          |                  |
| YD 7  | R1789| -1.99| 5.20 | 1.67   | 3.67     |          |          |          |          |          |          |          |                  |
| YD 4  | C2807| -2.77| 8.24 | -1.94  | 5.33     |          |          |          |          |          |          |          |                  |

a See footnote of Table 7.
b See footnote of Table 7.
c Method A: Composite-interval mapping, with WinQTLcart (Zeng 1994) for main effect QTL mapping; Mixed linear approach, with QTLMAPPER ver.1.0 (Wang et al. 1999) for epistatic effect QTL mapping.
d Method B: Proposed method in this paper.
e Variation contributed by QTL or digenic interaction.
Genetic basis of heterosis with real data analysis

With our proposed approach, we dissected genetic effects of QTL and interactions for the $II$ and $IJ$ hybrids, respectively, and calculated the dominance degree of each QTL or digenic interaction (Tables 7 and 8). We summarized the classified dominance degree of real mapping populations (Table 9) and found that dominance degree in the $Z_2$ data set that mainly characterized the heterosis showed overdominance and dominance for QTL and digenic interactions, and the ratio of dominance is greater than overdominance. Therefore, we conclude that dominance accumulation and overdominance are the major genetic basis of heterosis. This finding is consistent with Huang et al. [4], who pointed out that the accumulation of numerous rare superior alleles with positive dominance was an important contributor to heterotic phenomena after genomic analysis of hybrid rice varieties.

To explicitly elucidate the influence of single-locus (additive and dominance) and two-loci ($aa$, $ad$, $da$, and $dd$ epistatic effect) genetic effects conditioning the heterosis of agronomic traits, models or genetic mating design (e.g., RIL-based TTC design) [30, 38], which can be used to study how interactions among multiple genes can lead to the phenotypic manifestations of heterosis, are probably the most relevant. Recent findings from genomic, proteomic,
metabolic, epigenetic, and network studies in hybrids and polyploids also highlight some testable models for heterosis [46].

Supporting information

S1 Supporting Information. Expected genetic values of $Z_{1i}$, $Z_{2i}$, $Z_{3i}$, $Z_{4i}$, $Z_{5i}$ and $Z_{6i}$ under both the $F_1$ and $F_2$ metric models in RIL-based aTTC design. (DOCX)

S2 Supporting Information. Expected genetic values of $Z_{1i}$, $Z_{2i}$, $Z_{3i}$, $Z_{4i}$, $Z_{5i}$ and $Z_{6i}$ under both the $F_\infty$ and $F_2$ metric models in $F_2$-based aTTC design. (DOCX)

S3 Supporting Information. Statistical genetic models for mapping heterotic QTL in the RIL-based aTTC design under the $F_2$ metric model. (DOC)

S4 Supporting Information. Statistical genetic models for mapping heterotic QTL in the $F_2$-based aTTC design under the $F_\infty$ metric model. (DOC)

S5 Supporting Information. Simulation data generate script. (ZIP)

S6 Supporting Information. Simulation data of $Z_1$. (ZIP)

S7 Supporting Information. Simulation data of $Z_2$. (ZIP)

S8 Supporting Information. Simulation data of $Z_4$. (ZIP)

S9 Supporting Information. Simulation data of $Z_5$. (ZIP)

S10 Supporting Information. Simulation data of $Z_6$. (ZIP)

S11 Supporting Information. Simulation results of $Z_1, Z_2, Z_3, Z_6$. (DOC)

S12 Supporting Information. Real data augment effect results. (DOC)

S1 Fig. The mean statistic power of augmented main and epistatic effect interactions in $Z_1$. (TIF)

S2 Fig. The mean statistic power of augmented main and epistatic effect interactions in $Z_2$. (TIF)

S3 Fig. The mean statistic power of augmented main and epistatic effect interactions in $Z_5$. (TIF)

S4 Fig. The mean statistic power of augmented main and epistatic effect interactions in $Z_6$. (TIF)
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