Finding new QTL for yield traits based on a high-density genetic map in the super hybrid rice Nei2You No.6

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Abstract

Background Rice is one of the most important food crops in the world. To determine the genetic basis of yield components in super rice Nei2You No.6, 387 recombinant inbred sister lines (RISLs) were obtained for mapping quantitative trait loci (QTL) responsible for yield-associated traits, such as 1000-grain weight (TGW), grain number per plant (GNP), number of panicles per plant (NP), and grain yield per plant (GYP). Results Using whole genome re-sequencing, a high-density linkage map consisting of 3203 bin markers was constructed with total genetic coverage of 1951.1 cM and an average density of 0.61 cM. As a result of the multi-environment test, 43 yield-related QTL were mapped to all 12 chromosomes, among which 28 inherited from Nei2B showed a positive effect on yield traits. Nine QTL, qTGW-1a, qTGW-5, qTGW-7, qTGW-10b, qTGW-10c, qTGW-12, qNP-7, qGNP-6c, and qGYP-6b, showed stable effects across multiple environments. Five of the nine QTL were co-located with previously reported QTL, and four novel loci, qTGW-7, qTGW-12, qGNP-6c, and qNP-7, were identified in the present study. Subsequently, qNP-7, qTGW-12, and qTGW-7 were validated using corresponding paired lines which differed only in the target region. Conclusions the RISL population is an effective tool for mapping and validating QTL of complex traits, for instance, yield-associated traits, and newly detected QTL provide new genetic resources for research of yield components and molecular breeding in rice.

Background

Rice (*Oryza sativa* L.) is the staple food for more than half of the global population. With the reduction of available arable land and the increasing population, food security has become a worldwide problem. Increasing grain yield per plant (GYP) is an effective way to solve this problem. GYP consists of number of panicles per plant (NP), grain number per panicle (GNP), and 1000-grain weight (TGW), all of which are known to be controlled by quantitative trait loci (QTL) and influenced by the environment.

Up to date, hundreds of yield-related QTL have been mapped in rice. In total 239, 282, and 223 QTL were mapped on all 12 chromosomes, responsible for NP, GNP, and TGW, respectively (http://www.gramene.org/). Among these, the number of identified yield-related QTL has increased rapidly in the last two decades. For NP, the well-known *MOC1* and *IPA1/OsPIL14* are major factors regulating the formation of effective tillers in rice [1, 2]. Specially for *IPA1/OsSPL14*, a point mutation leads to an 'ideal' rice plant with a reduced tiller number, increased lodging resistance and enhanced grain yield, which has been wildly used in hybrid breeding. The *Gnla* gene is the first cloned gene regulating GNP [3]. In addition, *DEP1* is reported to improve rice GNP, and can be directly positively regulated by *IPA1* to influence plant height and panicle length [4]. *Ghd7*, *Ghd7.1* and *Ghd8* can affect not only heading date but also GNP [5-8]. For TGW, a total of 17 QTL showing large effect for grain size and shape were successfully isolated in rice [9, 10]. For example, *GS3*, a major QTL for grain weight and grain length, functions as a negative factor for grain size [11]. *GL7* encodes a protein homologous to LONGIFOLIA in *Arabidopsis thaliana* and increase grain length and improvement of grain appearance quality [12]. Higher expression of *GW8* can promote cell division and grain filling, which result in the increase in grain weight

and yield [13]. The recently reported *GL6* encodes a plant-specific PLATZ transcription factor and positively regulates grain length to increase TGW [10].

Genetic population is an essential and effective tool for mapping QTL in plants and can be divided into primary and advanced populations. F_2, recombinant inbred line (RIL), Backcross (BC), and double haploid (DH) are primary populations. Advanced populations include residual heterozygous line (RHL), chromosome segment substitution line (CSSL), and near-isogenic line (NIL). Generally, QTL are primarily localized using F_2, DH, RIL or BC populations, and then fine mapped by constructing advanced populations [3, 6, 8, 12, 14-17].

The efficiency of QTL mapping is determined by the accuracy and saturation of the genetic map. Recent advances in sequencing technology have enabled rapid, high-throughput genotyping of single nucleotide polymorphisms (SNPs), which provides saturated molecular markers for the anatomy of complex yield traits [18]. For example, there are 1,703,176 SNPs between Nipponbare and 9311, equivalent to one SNP per 268 bp [19]. Compared with low-throughput molecular markers like simple sequence repeats (SSRs), sequencing-based genotyping is a powerful tool with advantages of time-saving and high-density. Bin map refers to a certain number of continuous SNP as the unit for determining recombination events, and the parent source of each Bin is speculated to obtain the genome-wide physical map of the offspring. For example, a high-density genetic map consisting of 3016 bin was constructed and finally 26 QTL for six yield traits were located, among which two novel QTL, *qAGB6* and *qHI9*, were identified [20]. In another report, a total of 79 QTL for 15 yield traits were mapped using a high-density linkage map with 3569 bin markers [21].

Most studies focus on mapping yield-associated QTL differing between high and low yield varieties. Therefore, more attention should be paid in genetic factors varying in high-yielding lines. The three-line indica hybrid cultivar Nei2You No.6 is a super hybrid rice released by the China National Rice Research Institute (CNRRI) in 2006 that has a yield of up to 8.89 t/ha (http://www.chinariceinfo.com/variety/). To investigate the genetic basis of high yield, we constructed a RISL population derived from the two parents of Nei2You No.6. Using high-density bin-mapping by resequencing, QTL associated with NP, GNP, TGW, and GYP were scanned in two environments across two years. These QTL are helpful to elucidate the high yield mechanism of Nei2You No.6 and provide guidance for high-yielding rice breeding.

Methods

Mapping population

As shown in Fig. 1, the recombinant inbred sister lines (RISLs) were derived by a single-seed descent method (SSD) from a cross between the maintainer line Nei2B and restorer line Zhonghui8006 (R8006). At the F_7 generation, two individuals were selected randomly from each of 194 lines followed by selfing to F_{15}. The 387 RISLs were named Q plus line number.

Plant cultivation

387 RISLs and their parents were originally planted in the experimental field of the China National Rice Research Institute (CNRRI) in Fuyang (FY), Hangzhou, Zhejiang province (Longitude: 119.95, Latitude: 30.05) from May to October in 2015 and 2016, and in Lingshui (LS), Hainan province (Longitude: 110.03, Latitude: 18.05) from November to April of in 2015 and 2016. The four environments were abbreviated as LS15, FY15, LS16 and FY16. With a randomized complete block design of two replications, the parents and RISLs were transplanted about 25 days after germination with a plant density of 21 cm × 18 cm. And each line was represented by 16 plants in two rows. The field management followed the normal agricultural practice. At maturity, six middle individuals in each line were harvested for phenotyping. For QTL validation, the F_{17} generation of RISLs was planted in Fuyang, Zhejiang Province in 2018.

Evaluation of yield-related traits

After drying at 37°C for 2 weeks, all harvested individuals were measured for four yield-associated traits, such as TGW, GNP, NP, and GYP. The filled grains of each plant were weighed to measure GYP and effective panicles were counted manually for NP. GNP and TGW were analyzed using an automatic seed examination analysis meter (Wanshen SC-G).

DNA extraction and population resequencing

At the F_{15} generation, DNA from leaves of Nei2B, R8006, and 387 RISLs were sampled for re-sequencing at the tillering stage. To obtain high-quality DNA, a cetyltrimethylammonium bromide (CTAB) method was used to extract the genomic DNA [22]. DNA quality and concentration were assessed using the NanoDrop 2000C Spectrophotometer.

High-throughput sequencing was conducted by Berry Genomics company using Illumina HiSeq X Ten sequencing platforms (Illumina). The sequencing depth of Nei2B and R8006 were 60.48-fold and 79.38-fold, respectively. In addition, the average sequencing depth of the RISL population was 8.01-fold. For SNP calling, the Burrows-Wheeler Aligner (BWA) with default parameters was applied for sequencing alignment between clean data and the R498 reference genome sequence (http://www.mbkbase.org/R498/) [23]. The Genome Analysis Toolkit (GATK) was used to detect SNP and InDel loci [24]. Annovar software was used to annotate SNPs [25]. In addition, Clipping REveals STructure (CREST) and CNVnatoor were used to detect and annotate structural and copy number variations, respectively [26, 27].

Linkage map construction

We used a sliding window method to incorporate continuous non-recombinant SNPs on the genome into a bin [28]. The window size for genotyping was 15 continuous SNPs without missing data. According to the ratio of SNPs in the sliding window coming from the parents, the genotype of this window was determined. Recombination breakpoints were determined by the join point of two different genotypic regions. A bin map was constructed according to the recombination breakpoints of all individuals in this study. Bin markers were screened to filter out distorted and missing markers. To filter partial separation

markers, 3273 candidate markers were filtered at a ratio of 1:3, and 25 biased separation markers were filtered out. Markers with genotypes covering at least 75% of 387 RISLs were retained. Heterozygous genotypes were deleted. The Kosambi algorithm was used to calculate the genetic distance between the markers.

Phylogenetic analysis of RISLs

To evaluate constructed RISLs, we conducted phylogenetic analysis using Tassel 3 software (https://tassel.bitbucket.io/) on Linux system. Cluster analysis were performed on bin marker data of the RISL population. Parameters were set as follows: perl run_pipeline.pl -Xms512m -Xmx10g -fork1 -p Bin_data -tree Neighbor -treeSaveDistance true -export Bin_data_NJ_tree_distance -runfork1. MEGA7 software (https://www.megasoftware.net/) was used to display the clustering results.

QTL analysis

Additive QTL were analyzed using the composite interval mapping (CIM) model in Windows QTL Cartographer v.2.5 software (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The threshold for declaring a putative QTL ($P < 0.05$) was estimated using 1000 permutations with 1 cM as a search step. The percentage of phenotypic variation explained (R^2) and additive effects (AE) were also obtained using this software. The location of the highest LOD value was taken as the interval of QTL. The QTL nomenclature followed the recommendations reported by Mccouch [29], specifically, q plus trait abbreviation and chromosome number; if more than one QTL are detected on the same chromosome for one trait, the end of the QTL will be plus a, b, etc.

Statistical analyses

Statistical analyses were carried out by comparing the raw data of all individuals using Microsoft Excel (2016) and Prism 5 software (GraphPad). Frequency distribution for each trait was drawn in Microsoft Excel 2016 to identify the pattern of variation of each trait within the population. Furthermore, correlations among the four traits in two year were estimated using the Pearson correlation coefficient. For QTL validation, data of RISLs were compared using Student's t-tests and significance levels were determined according to the Student's t-test: * $P < 0.05$, ** $P < 0.01$.

Results

Phenotypic variation and correlation analysis of RISLs

Phenotypic variation of the RISLs and two parents are illustrated in Fig. 2. All four traits of 387 RISLs displayed continuous distributions and transgressive segregation across four environments, indicating their quantitative inheritance. Coefficient of variation (CV) for GYP, GNP, and NP ranged from 23.8-27.9%, 17.8-25.7%, and 21.9-27.6% in four conditions, respectively, which meant a wide range of variation among the RISL. According to the results of CV, GYP, GNP, and NP were vulnerable to environmental influences. In

particular, the CV of 1000-grain weight (TGW) ranged from 6.2-9.5%, which indicated that TGW was a stable quantitative trait.

The correlation among the four yield-related traits is shown in Fig. 3. GYP was positively correlated with NP and GNP while NP and GNP were negatively correlated. TGW was negatively correlated with GNP and NP. However, there was no significant correlation between TGW and GYP. The high yield performance of Nei2You No.6 may be mainly caused by the increase in GNP and NP.

Population sequencing and linkage map construction

In total, 221,340,292 high-quality reads were generated for Nei2B and 303,049,072 high-quality reads for R8006, with average sequencing depths of 60.48-fold and 79.38-fold, respectively. A total of 1,589,836 single nucleotide polymorphisms (SNPs) were identified between Nei2B and R8006.All of 387 RISLs were used for whole-genome sequencing and resulted in a total of 1.05T of clean data, with approximately 8.10-fold depth for each RISL. Using a sliding-window method, a total of 59,890 recombination breakpoints were generated, with an average of 155.16 breakpoints per line. We preliminary obtained 3,273 bin markers with 930,361 high-quality SNPs. After filtering out segregation distortion and low coverage markers, 3,203 effective markers were selected and then used for linkage analysis to construct a genetic linkage map (Fig. 4). The length of bin markers ranged from 50 kb to 1.4 Mb, with a mean of 119 kb. The number of SNPs and bins of each chromosome are shown in Table 1. The total genetic distance of the map was 1951.1 cM, with an average linkage distance of 0.61 cM between adjacent markers. The largest linkage group was Chr.1 with 419 bin markers and a length of 263.6 cM and the smallest Chr. 9 with 163 bin markers and a length of 69.3 cM. The ratio of linkage distance to physical distance ranged from 2.8 to 6.1 cM/Mb, with a mean ratio of 5.0 cM/Mb.

Coverage of genotypic difference in paired RISLs

To verify that if the construction of RISL population was successful, phylogenetic analysis was performed. The phylogeny showed that 387 RISLs were clustered in pairs (Additional file 1: Figure S1). Then, we compared the genome of each paired sister line. To determine the coverage of genotypic difference, we counted the different bins between each of paired RISLs in F_{15} generation (Fig. 5 and Additional file 2: Table S1). Each bin was covered by 77 paired RISLs on average, with the maximum of 119 paired and minimum of 54 paired. The maximum different bins between paired lines was 52.1%, and minimum of that was 8.6%, and the average different bins accounted as 39.1%. In F_{15} generation, the heterozygous region ranged from 0 to 27.5% with an average of 2.6%. This meant that the genome is not nearly homozygous until F_{15} generation. Some lines with high heterozygous rate may be caused by cross-pollination in high generation, and the homozygous degree of the whole population conforms to the stable genetic population. Therefore, we can conclude that the heterozygosity exists longer than in theory.

QTL analysis using the RISL population

As a result of QTL analysis, a total of 43 QTL were identified on all of twelve chromosomes, nine of which were repeatedly detected in multiple environments. Detailed information about all 43 QTL are summarized in Additional file 3: Table S2 and Fig. 6.

Eight QTL for GYP were detected on chromosomes 1, 6, 9, 10, and 12, and the total PVE ranged from 0.0 to 10.9% of the total phenotypic variation across four environments. *qGYP-6b* was detected in LS15 and FY15 with contributions to phenotypic variance of 4.3% and 4.2%, respectively. The other 7 QTL were detected in one environment. The positive alleles for five out of eight QTL for GYP were derived from Nei2B.

With PVE varying from 3.2 to 5.4%, four QTL related to NP were identified on chromosomes 3, 5, 7, and 9, respectively. In detail, *qNP-7* was detected in two environments, which explained 7.1% and 3.7% of the phenotypic variance in LS15 and FY15, respectively. *qNP-3*, *qNP-5*, and *qNP-9* were detected in only one environment. The positive alleles of *qNP-3* and *qNP-9* were from R8006 while *qNP-5* and *qNP-7* from Nei2B.

For GNP, 13 QTL were detected on chromosomes 1, 3, 4, 5, 6, 7, 8, and 12 with the phenotypic variance explained by QTL ranged from 3.2 to 10.2%. The total of PVE (%) of These 13 QTL for GNP ranged from 8.6 to 24.0%. *qGNP-6c* was repletely detected in FY16 and LS16 environments and explained 4.8% and 2.7% of phenotypic variance, respectively.

In addition, a total of 18 QTL associated with TGW were distributed on chromosomes 1, 2, 3, 5, 6, 7, 8, 10, and 12, totally explaining 13.9 to 37.4% of the phenotypic variation. The phenotypic variance explained by each QTL ranged from 2.4 to 6.1%, indicating multiple minor-effect genes for TGW in Nei2You No.6. All of these 13 QTL associated with TGW inherited from the big grain parent Nei2B. Among these, six loci were detected in multiple environments, such as *qTGW-1a*, *qTGW-5*, *qTGW-7*, *qTGW-10b*, *qTGW-10c*, and *qTGW-12*.

QTL verification

For QTL validation, sister lines were selected for phenotyping in Fuyang, 2018 (Fig. 7). For NP, lines Q277 and Q278 shared the same genetic background harboring on QTL other than *qNP-7* detected were selected. The phenotypic data showed a significant difference between Q277 and Q278 in NP, which led to a significant improvement in GYP (Fig. 7a, b). Similarly, lines Q239 and Q240 differed in *qNP-7* also showed a significant difference in NP.

For *qTGW-1a*, lines Q124 and Q125 shared the same genetic background besides the target region. The phenotypic data showed that there was a significant difference between Q124 and Q125 in TGW (Fig. 7c). For *qTGW-7*, it showed a similar result as *qTGW-1a* (Q378 vs Q379, Fig. 7d). For *qTGW-10b* and *qTGW-10c*, lines Q317 and Q318 showed significant difference in TGW (Fig. 7e). The *qTGW-12* was validated using lines Q70 and Q71 for TGW (Fig. 7f). The phenotypic date showed that there was a

significant difference between Q70 and Q71 in TGW. Similarly, lines Q146-Q147 and Q380-Q381 also contained *qTGW-12* and had a significant difference in TGW.

Discussion

High-density genetic linkage map is an effective tool for QTL mapping. Genetic maps constructed with traditional molecular markers have low density and too large a localization range, which leads to overestimation of phenotypic variance explained by QTL and difficulty of gene cloning and developing markers for pyramiding breeding [30, 31]. With the development of sequencing technology, whole-genome sequencing has been widely used in rice studies. Through bioinformatics analysis of the rice genome, many SNPs, InDels and structural variation (SV) can be exploited, which can be used to develop numerous molecular markers to build a high-density genetic map. In this study, 387 RISLs were selected for whole genome resequencing. A high-density genetic linkage map was constructed using 3203 bin markers for QTL analysis for yield associated traits.

A set of primary and secondary population were used for genome-wide QTL analysis, QTL validating and fine mapping. NILs are widely used in QTL validating and fine mapping, then came the residual heterozygous lines. The NILs are usually constructed by continuously back cross while residual heterozygous lines by selfing in advanced generation, both of which depending on the marker assisted selection strategy. We constructed recombinant inbred sister lines, and “sister” means that the two individuals used to construct the population originate from the same family in the F_7 generation. Comparing with NILs and RHL, RISLs showed advantage of more efficient and labor-saving with omission of MAS. As well as QTL mapping, the RISLs can be used in QTL validation efficiently. As a supplement, a library consisting of 1700 F_{11}, 2780 F_{12}, and 2464 F_{13} lines were obtained during constructing the RISLs, in which the target residual heterozygous line can be selected by library-screening and used for fine mapping.

A total of 43 QTL were identified in four environments for four yield-related traits and distributed in different regions of 12 chromosomes, and the phenotypic variance explained by a single QTL ranged from 2.4 to 10.2%. Among the 43 QTL, 65.1% of the positive alleles inherited from the parent Nei2B. Consistent with the probably existence of major QTL in both of two parents, the above results illustrate that yield-associated traits of Nei2You No.6 are mainly controlled by multiple minor- or middle-effect QTL. On another hand, the QTL effect or PVE are always influenced by the genetic background. For example, *Gn1a* and *DEP1* in japonica and indica genetic backgrounds showed different degrees of phenotypic variance [21]. Additionally, it has been realized that both major- and minor-effect QTL play important roles in the controlling of complex traits [32]. More and more attentions have been paid to QTL with minor- or middle- effects in recent years [33-35].

As shown in Additional file 4: Table S3, nine repeatedly detected loci are compared with those of previously identified QTL. For grain yield per plant, *qGYP-6b* is probably allelic with reported *GW6a* or *APO1* [16, 36]. For grain number per panicle, the interval of *qGNP-4b* flanking an unnamed QTL related to

GNP and a cloned gene, *GNP4* [37, 38]. For 1000-grain weight, the *qTGW-1a* overlaps with an unnamed QTL for TGW reported by Zhuang, and *qTGW-5* co-locates with *gw5b* reported by Hua [39, 40]. Similarly, *qTGW-10b* is probably allelic with *Qkw10*. And most evidently, *qTGW-10c* is at the same region as *qTGW-10*, *kw10-2*, *qTGW10-2*, and *gw10b* 32 [40-44]. Finally, these above consistent results indicate the high accuracy of mapping results in the present study.

According to the results of comparison between the QTL in our study with previous studies, four loci, namely *qNP-7*, *qGNP-6c*, *qTGW-7*, and *qTGW-12*, are probably novel QTL. And re-phenotyping of paired lines successfully proved that the stable existence of *qNP-7*, *qGNP-6c*, *qTGW-7*, and *qTGW-12*. In future study, these four QTL will be genetically dissected and fine mapped using secondary segregated population by screening RISLs or $F_{11}/F_{12}/F_{13}$ library. The fine mapping and map-based cloning of novel QTL detected in this study will provide genetic resources for rice high-yield breeding.

Conclusions

A RISL population was obtained from the cross between Nei2B and R8006. After sequencing the genome of RISLs, a total of 3202 effective SNPs was used to construct a high-density genetic linkage map, totally spanning 1951.1 cM, with an average distance of 0.61 cM. In total 43 QTL were identified for GYP, NP, GNP, and TGW in the multi-environment test, tending to cluster into nine loci. After comparing with previous studies, there are four of nine novel QTL, such as qNP-7, qGNP-6c, qTGW-7, and qTGW-12. Subsequently, qNP-7, qTGW-7, and qTGW-12 were validated using corresponding paired lines. In brief, our results provide a new method for constructing QTL mapping population, and the RISLs used in present study is a useful tool for mapping and validating QTL of complex yield traits. Next, we aim to develop markers tightly linked with qNP-7, qTGW-7, and qTGW-12 and fine map these QTL using secondary segregated population. The QTL analysis results lay an important foundation for the candidate gene identification of yield-related traits for rice.

List Of Abbreviations

RISL: Recombinant inbred sister line

GYP: grain yield per plant

GNP: grain number per panicle

NP: the number of panicles

TGW: thousand grain weight

LS: Lingshui

FY: Fuyang

Declarations

Ethics approval and consent to participate

Field trials were conducted in accordance with local legislation.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files. Nei2You No.6 is an indica type hybrid rice bred by our research group. The seeds of recombinant inbred sister line population and the parents are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

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Author's contribution

Author Contributions: ZY[†, Ö], CS[†], CL[†] were responsible for the overall concepts and designing the experiments. ZY, ZZ, CYY, CYR, XP, ZX conducted population construction and phenotypic identification. ZM, ZY and DC conducted the genotyping of two parents and RISLs. ZM analyzed the data and drafted the manuscript; ZY, CL and GA revised the manuscript. All authors read and approved the final manuscript.

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Table

Table 1 Distribution and statistics of bin markers on the linkage groups of the RISL population.

Chr.	Bin Num[a]	Chr length(cM)[b]	Aver (cM)[c]	Max Gap (cM)[d]	Gap>5c[e]	cM/Mb[f]
Chr.1	419	263.594	0.629	6.60	1	5.94
Chr.2	235	132.559	0.564	12.615	1	3.51
Chr.3	308	200.907	0.652	17.556	2	5.08
Chr.4	314	189.697	0.604	8.067	2	5.29
Chr.5	251	146.324	0.583	6.971	1	4.68
Chr.6	302	171.419	0.568	4.205	0	5.28
Chr.7	249	164.317	0.660	6.185	2	5.43
Chr.8	244	173.622	0.712	3.973	0	5.80
Chr.9	163	69.280	0.425	2.525	0	2.80
Chr.10	230	120.633	0.524	11.559	2	4.72
Chr.11	243	193.636	0.797	8.44	3	6.09
Chr.12	245	125.140	0.511	4.137	0	4.70
Total	3203	1951.128	0.609	17.556	14	5.00

a The total number of bin markers on a chromosome; b Total genetic distance; c The average genetic distance of markers; d The largest gap in the linkage group; e The number of bin markers with gap greater than 5 cM; f The ratio of genetic distance to physical distance.

List Of Additional Files

Additional file 2: Figure S1. Phylogenetic analysis of RISLs. (png 1.0 Mb)

Additional file 2: Table S1. The number of paired RISLs in each bin. (xlsx 65.9 kb).

Additional file 3: Table S2. QTL identified from the analysis of the RISLs. a Chromosome region corresponding to the 95% confidence interval for the detected QTL. b Percentage of phenotypic variation explained by QTL. c Positive and negative values indicate additive effect contributed by the alleles of Nei2B and R8006, respectively. (xlsx 14.6 kb).

Additional file 4: Table S3. Comparison of QTL identified from this and previous studies. a The physical position of the bin corresponding to the 95% CI for the detected QTL. b Additive effects: negative values indicate that the resistant allele was inherited from R8006 and positive one from Nei2B. c Phenotypic variance explained by QTL. d The position of cloned gene is subject to Shuhui R498. (xlsx 11.8 kb).

Figures

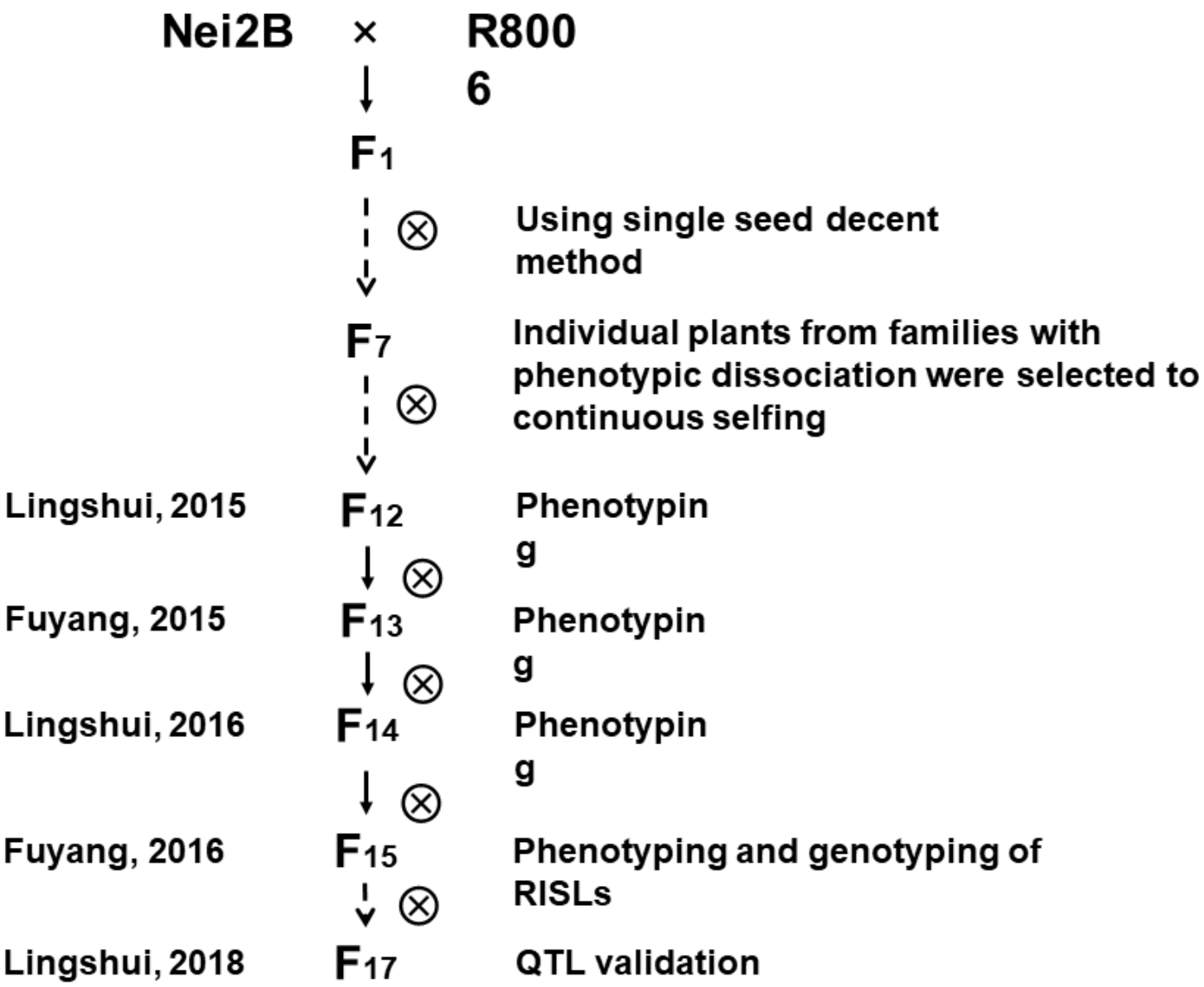

Figure 1

Work flow for RISL population development in the present study. LS, Lingshui Hainan Province; FY, Fuyang Hangzhou Province.

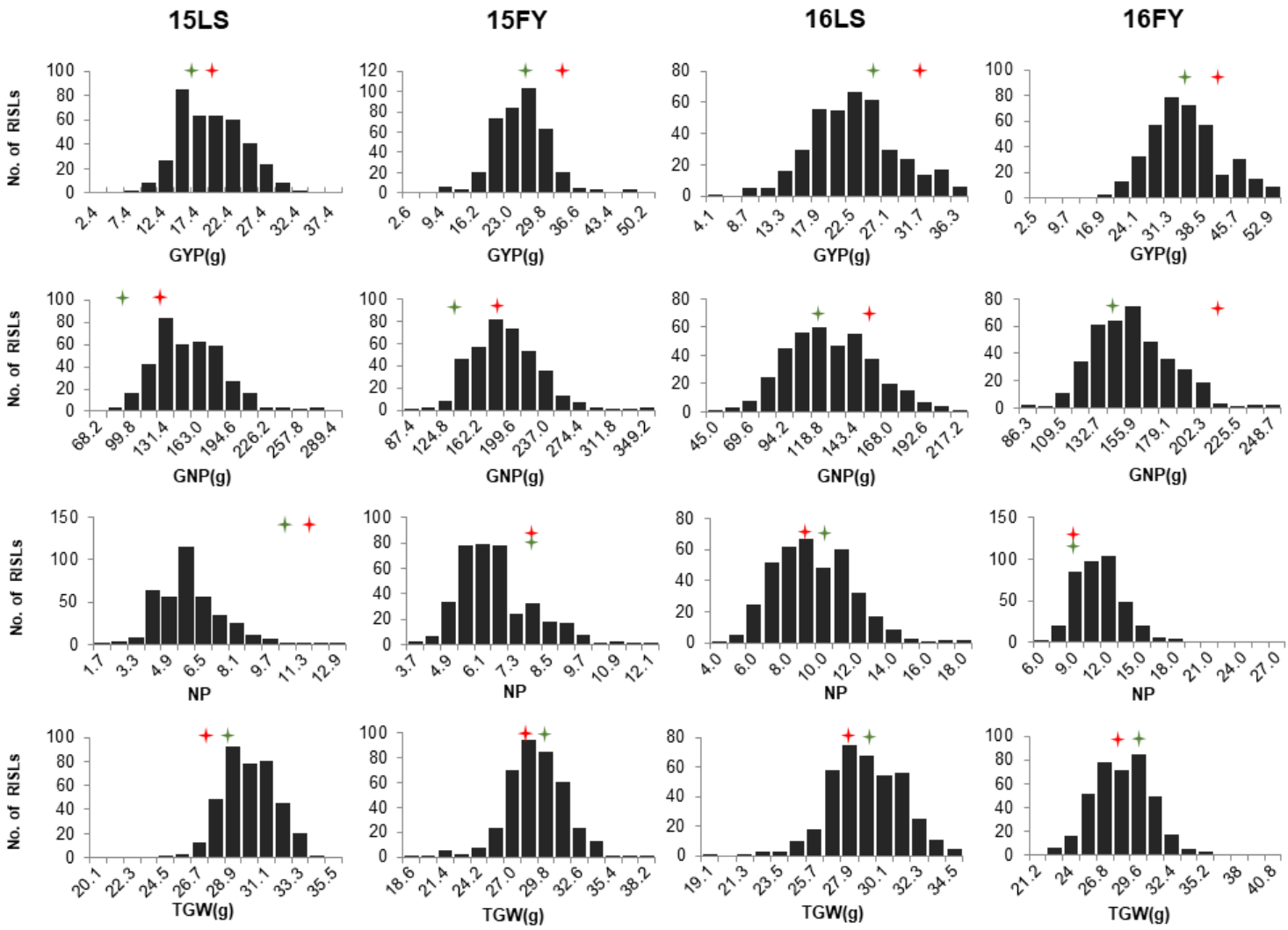

Figure 2

Phenotypic distributions of RISLs for four traits across four environments. Mean of the parents are at the top of each histogram, with red and green plus symbols representing R8006 and Nei2B, respectively. The numbers 15 and 16 represent the years 2015 and 2016, respectively.

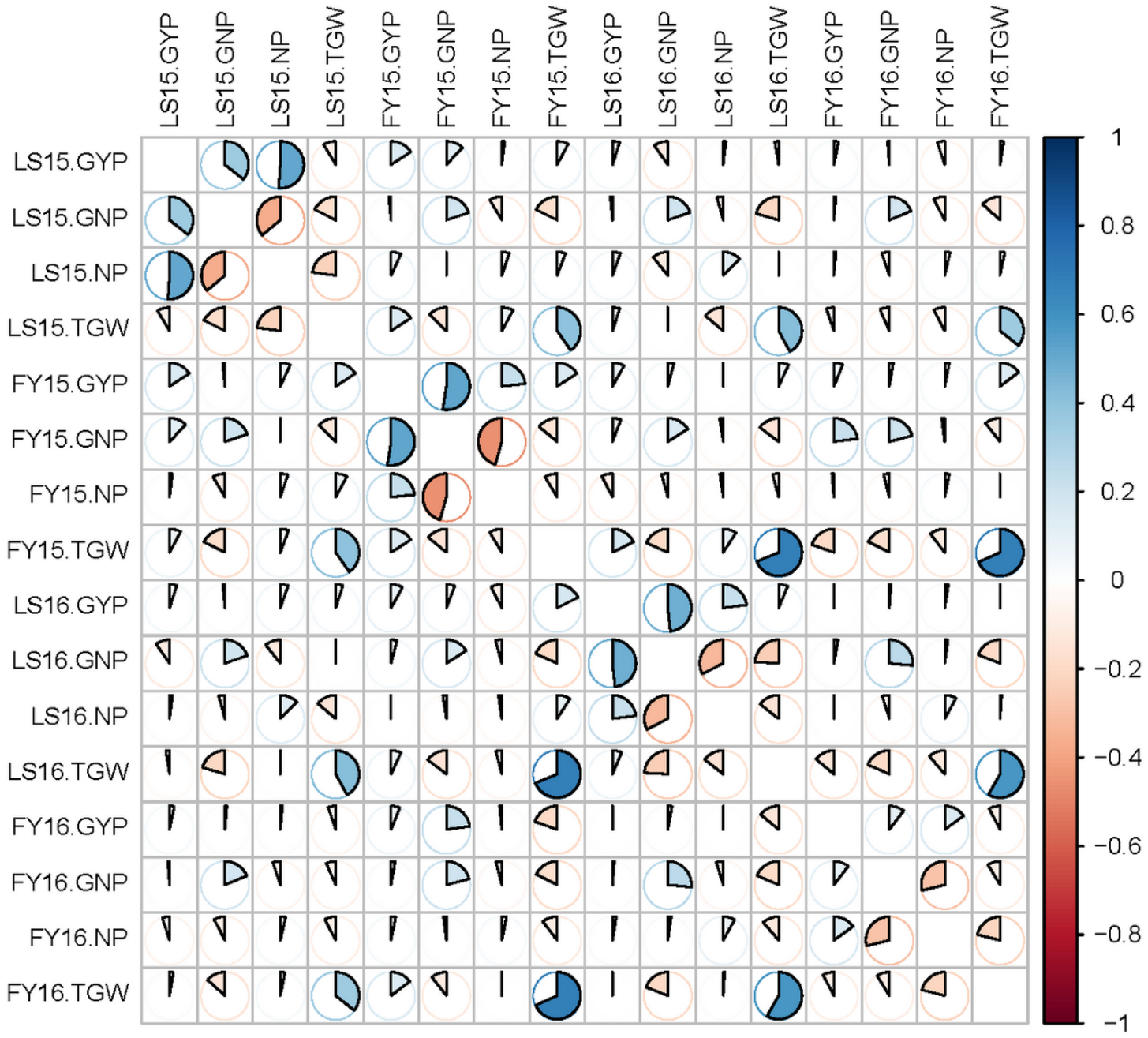

Figure 3

Correlation analysis of four yield associated traits in RISLs. Trait names were listed on the top and left of the picture. Blue and red pies indicate positive and negative correlations, respectively. The depth of pie color reflects the intensity of the correlation.

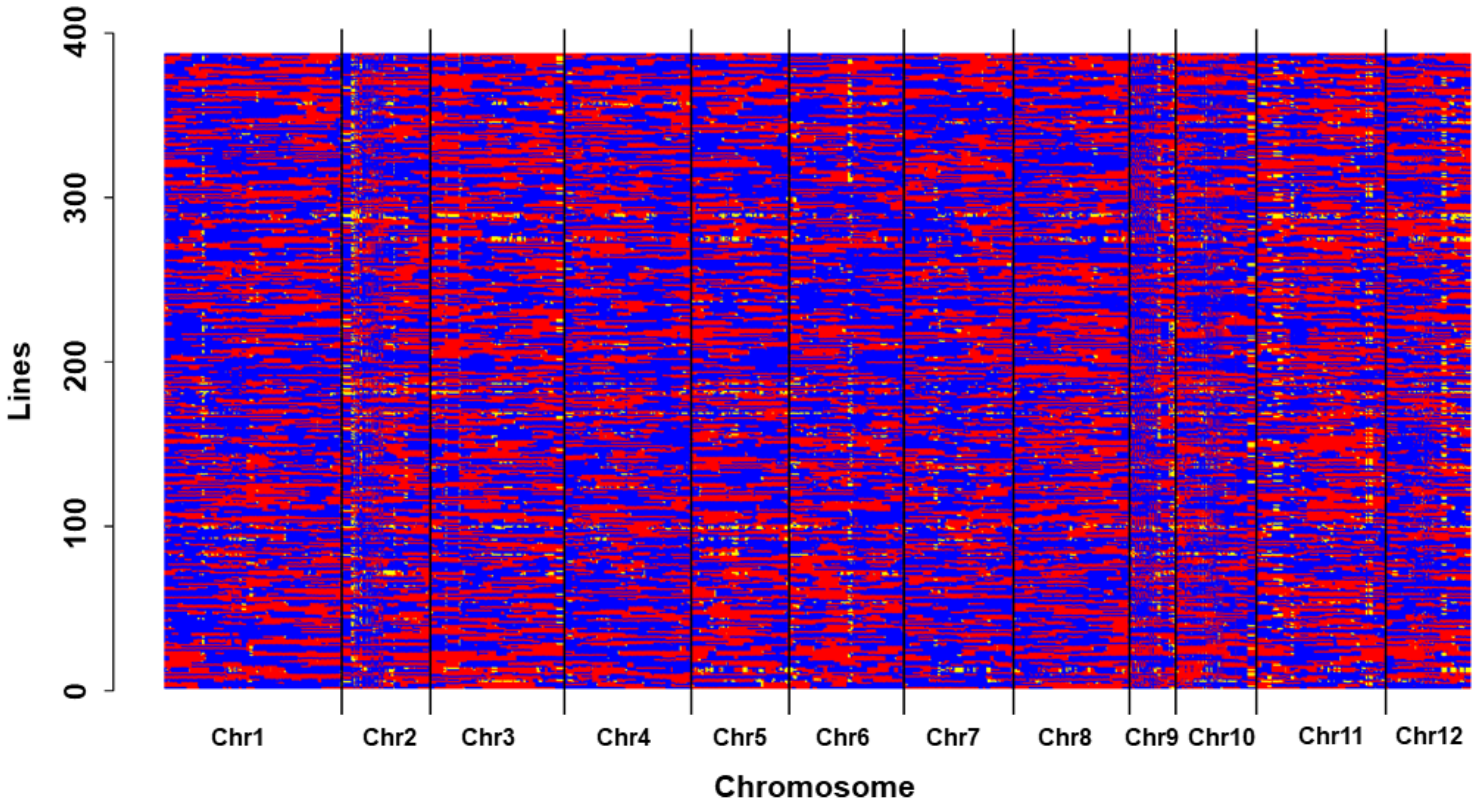

Figure 4

The bin map of 387 RISLs. The horizontal axis shows the twelve chromosomes and the vertical represents line list. Red, blue, and yellow means the genotype is consistent with Nei2B, R8006, and heterozygosity, respectively.

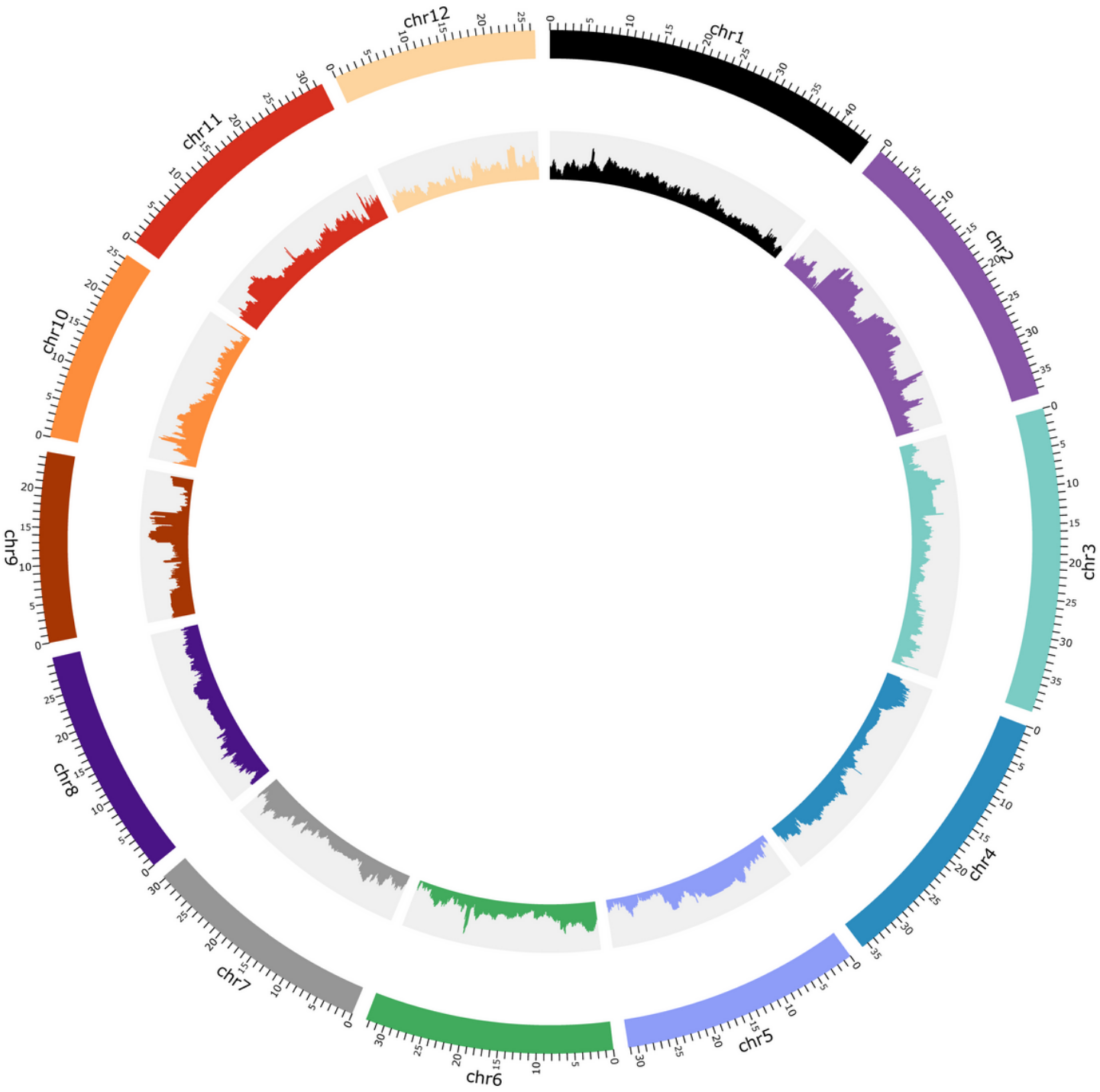

Figure 5

Overview of genotypic difference in RISLs. The inner track indicates the number of paired sister lines whose difference interval can cover the bin in whole genome.

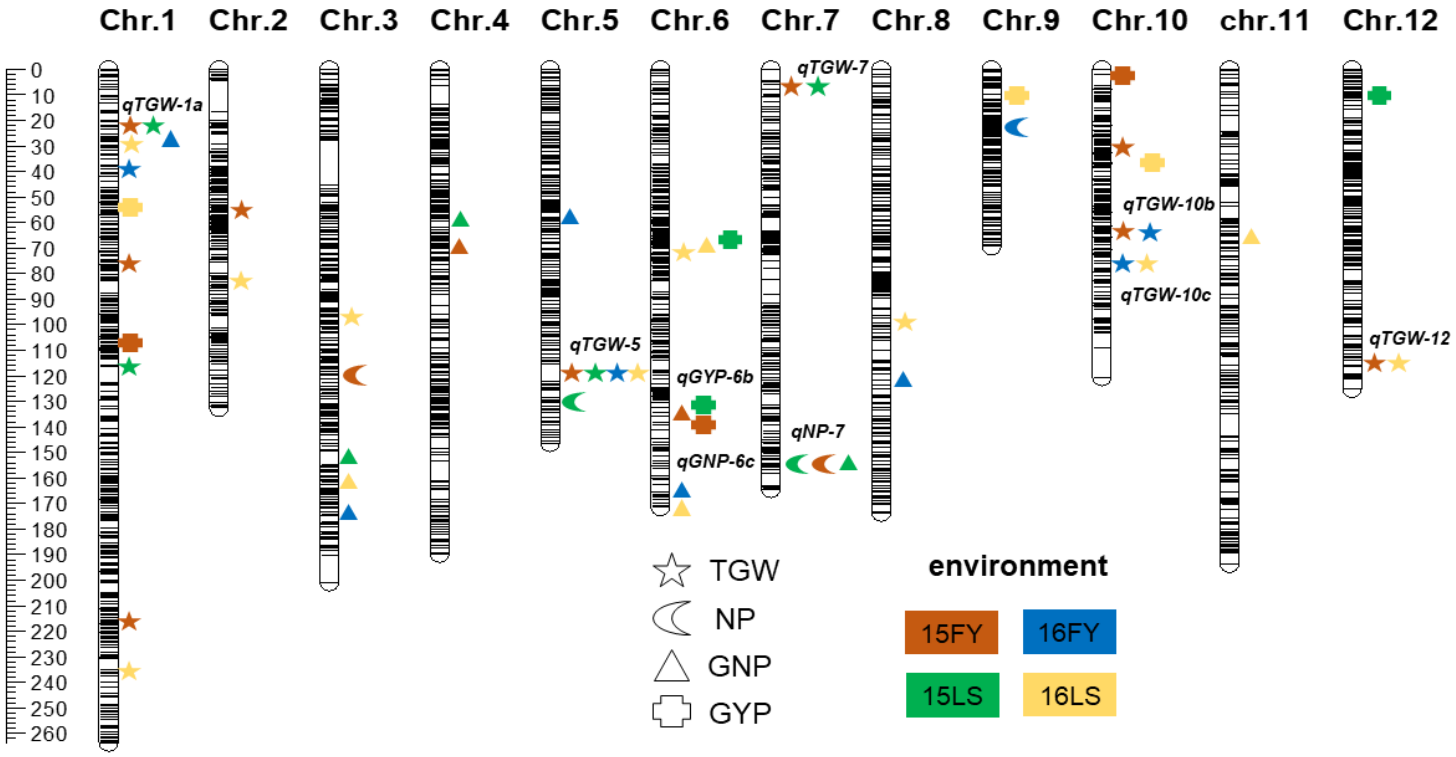

Figure 6

Graphical representation of QTL detected using RILSs. The scale on the left indicates map distance in centimorgans (cM). The darker bands indicate the markers. Graphics with different colors and shapes indicate the different traits and environments of detected QTL. Names of stable detected QTL are marked in the figure.

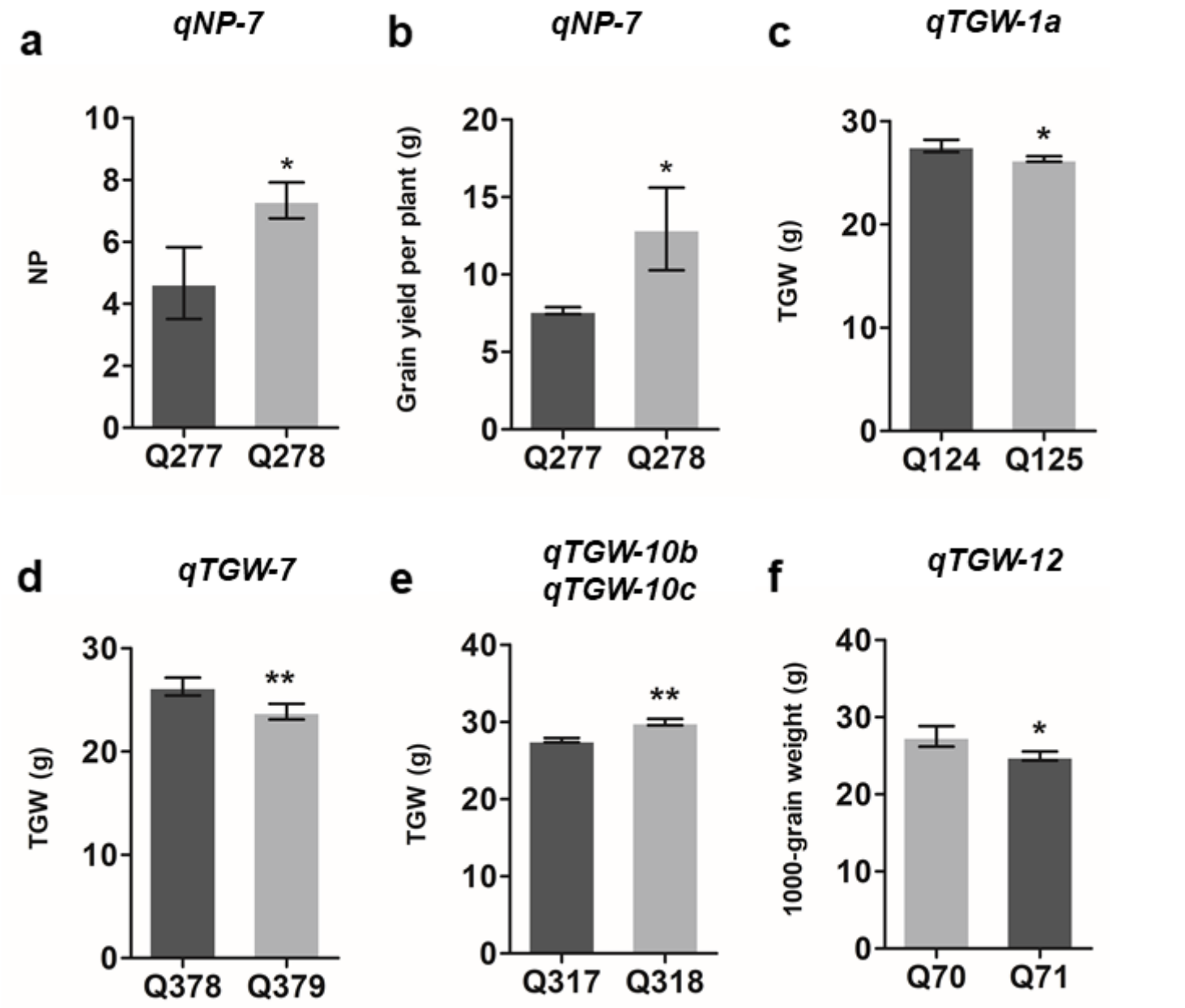

Figure 7

Validation of identified QTL using paired lines of RISLs. (A-D) The TGW of Q124-Q125, Q317-Q318, Q378-Q379, and Q70-Q71. (E-F) The GNP, TGW, NP, and GYP of Q277-Q278. The QTL for each pair of lines is on the top of the corresponding histogram. The data are presented as mean ± s.d. * $P < 0.05$ ** $P < 0.01$ (n = 6 plants; Student's t-test).

Supplementary Files

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- Additionalfile1.png
- Additionalfile2.xlsx

- Additionalfile3.xlsx
- Additionalfile4.xlsx