

Population structure and association analysis of yield and grain quality traits in hybrid rice primal parental lines

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Abstract To make advances in rice molecular design breeding, it is important to understand the genetic background and phenotypes of the parental lines, to known superior allele of the breeding material. In this study, 34 pairs of insertion-deletion (InDel) markers were used to analyze the differentiation of *indica-japonica* properties in 277 primal parental lines of hybrid rice that are used in China and abroad. The genetic diversity and population structure

were assessed with simple sequence repeat (SSR) markers. We also evaluated the association between SSR markers and seven yield traits and four grain-quality traits of 256 *indica* rice lines. The main results are as follows. (1) Within the 277 lines there were two subgroups: *indica* and *japonica*. The 256 lines of the *indica*-subgroup were further divided into four sub-sub-groups: landrace, maintainer, restorer, and rice lines from the International Rice Research Institute. (2) We identified 29 loci associated with 11 agronomic traits, including one for plant height, two for tiller number, one for panicle length, three for grain length,

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eight for grain width, eight for grain length/width ratio, one for thousand grain weight, one for amylose content, two for gel consistency, one for gelatinization temperature and one for total protein content. (3) Among those loci, 16 markers were closely linked to previously identified genes. Superior allele carrier lines and allele effects were also analyzed. Our results provided the population structure and *indica-japonica* properties for parent matching, and the marker-trait association will also be useful for molecular design breeding.

Keywords Rice primal parental lines · *Indica-japonica* property · Rice yield · Grain quality · Population structure · Association analysis

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, and China is a large rice producer and consumer. Therefore, high and stable rice yields are important for the security of China's food supply. Moreover, the demand for high quality rice is continuously increasing as living standards improve. Hence, there is a need to breed high quality rice with large yields. Rice yield and grain quality are quantitative traits controlled by multiple minor-effect genes. Identifying these alleles in various lines and elucidating their functions will be important in hybrid rice breeding programs.

Yield-related traits are quantitative traits controlled by multiple minor-effect genes/loci (quantitative trait loci or QTLs) distributed in the 12 rice chromosomes (www.gramene.org). Many yield-related genes have been cloned, such as *GS3*, *GW2*, *GW5*, *GL7*, *Ghd7*, *OsSPL16*, *TGW6*, *GIF1*, *DTH8* and *Gnla* (Ashikari et al. 2005; Ishimaru et al. 2013; Mao et al. 2010; Song et al. 2007; Wang et al. 2008, 2012, 2015; Wei et al. 2010; Xue et al. 2008; Yan et al. 2011). Some of these genes have pleiotropic effects: for example, *GS3* controls both grain length and weight; *Ghd7* controls grain number per spike, plant height, and heading date; *GW2* controls grain width, grain weight, panicle number per plant, and heading date; and *GL7* controls grain length and width.

Grain quality traits, including rice milling quality, appearance quality, cooking and eating quality, and

nutritional quality, are also quantitative traits. Few QTLs for milling quality have been identified. Appearance quality is also associated with yield-related traits, and many QTLs for grain appearance have been identified (Mao et al. 2010; Song et al. 2007; Wang et al. 2008, 2015; Yan et al. 2011). Amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) are the three main properties related to rice cooking and eating quality. Many studies focused on the starch synthesis pathway and 11 genes have been cloned (<http://www.ricedata.cn/gene/index.htm>). AC is mainly controlled by *Wx*, *SSIIa*, and *SSIII* (Fujita et al. 2007; Sato et al. 2002; Zhang et al. 2011a). *Wx* is a pleiotropic gene, which also regulates GC. Map-based cloning has confirmed that *Wx* is the major QTL *qGC-6* for GC of *japonica/indica* cross (Su et al. 2011). More than 20 QTLs for GC have been identified, but only *Wx* has been cloned. GT is mainly controlled by *ALK/SSIIa* (Gao et al. 2003). The total protein content (TPC) is the second largest component by weight in the endosperm, but rice lines with protein contents >9 % in the endosperm have poor eating quality. None of the QTLs for TPC distributed throughout all 12 chromosomes have yet been cloned.

As rice breeding has become increasingly difficult, molecular design breeding has become more popular. Although many genes for rice yield and grain quality have been fine-mapped and cloned, more information is required for rice molecular design breeding. First, genes related to rice yield and grain quality are quantitative traits, and various alleles in the different lines cause different phenotypes, but little information is published. Second, the genetic background and phenotypes of parental lines, especially the primal parental lines, are not well known. However, understanding *indica-japonica* properties is important in parent matching for super hybrid breeding practices. Therefore, we need to understand the various alleles in the germplasm first, and then identify the effect of different alleles and determine the most important alleles controlling target traits.

Genome wide association analysis had been widely conducted on rice to detect complex trait variation (Chen et al. 2014; Huang et al. 2011; Zhao et al. 2011). In this study, we used 277 primal parental lines of hybrid rice and intermediate materials from more than ten provinces in China and Southeast Asian countries that are major rice producing areas. Thirty-four insertion-deletion (InDel) markers were used to

analyze the differentiation of *indica-japonica* properties, and 193 simple sequence repeats (SSR) markers were used to assess the genetic diversity and a SSR subset was applied to population structure. Moreover, association analysis was undertaken between SSR markers, seven yield traits and four grain quality traits of 256 *indica* rice lines. Our goals were to (1) clarify the *indica-japonica* properties and distinguish the population structure, (2) identify SSR markers associated with rice yield and grain quality-related traits, and (3) establish allele effects on phenotypes to lay the foundation for parent matching and molecular design breeding.

Materials and methods

Plant materials

A total of 277 hybrid rice primal parental lines and intermediate materials were collected from more than ten rice producing provinces in China, Pakistan, Indonesia, Vietnam and other countries (Table S1) as well as the International Rice Research Institute (IRRI, the Philippines).

Field experiments and phenotypes

During the 2013 and 2014 growing seasons, the 277 rice lines were planted in two field locations: Sanming City, Fujian Province and Sanya City, Hainan Province. The Sanming site was representative of a temperate climate and Sanya of a subtropical climate. The 22-d-old seedlings were planted at a spacing of 17 × 25 cm in all field experiments. Nitrogen (N) fertilizer was applied at a level of 150 kg ha⁻¹. Weeds and pests were controlled as locally recommended. Plant height (PH), tiller number (TN), panicle length (PL), thousand-grain weight (TGW), grain length (GL), grain width (GW), and the grain length-to-width ratio (L/W) were recorded at maturity. Three central plants in each plot were used to measure PH and TN. Then, the plants were harvested to measure grain traits. For TGW, GL, GW, and L/W, the data were collected using a SC-G rice grain appearance quality image analysis system (Wseen Detection Technology Co., Ltd, Hangzhou, China). Approximately 500 grains were spread on one 21 × 30 cm flat-bed surface, photographed using an Eloam high-speed photographic apparatus S500A3, and

the image analysis system calculated GL, GW and L/W (Yin et al. 2015). Next, the grains were collected and a high precision balance (Amput, China) was used to weigh the grains and calculate TGW.

In addition, 200 g of grain from each line was harvested at Sanming in 2014 for grain quality analysis. AC, GT, GC and TPC were measured with three independent replications at the State Key Laboratory of Rice Biology, China National Rice Research Institute. GT and GC were measured according to the method of Tan (Tan et al. 1999).

For AC analysis, 50 mg of rice flour was placed in a 100 mL volumetric flask and 1 mL of ethanol (95 %) was added. The volumetric flask was shaken to disperse the rice flour and 9 mL of sodium hydroxide (1 mol/L) was added. The volumetric flask was placed in a boiling water bath for 10 min, and distilled water was added to make up 100 mL after cooling. The sample or standard sample, acetic acid (1 mol/L) and iodine/potassium iodide solution were mixed using an AA3 Continuous Flow Analyzer (SEAL Analytical GmbH, Germany). Absorbance data were collected and AC content was calculated using a standard curve. For TPC analysis, 200 mg of rice flour was placed in a digestion tube, 5 mL of concentrated sulfuric acid was added and the tube was placed in a digestion furnace (SEAL Analytical) for 1.5 h at 220 °C. Then, 2 mL of hydrogen peroxide was added and the tube was reheated until the solution became colorless. The solution was transferred to a 100 mL volumetric flask and distilled water was added to make up 100 mL. The sample or standard, sodium salicylicum and sodium hypochlorite were mixed using an AA3 Continuous Flow Analyzer. Sodium nitroprusside was added to catalyze the reaction, generating a blue compound and 660 nm absorbance data were collected. The N content was calculated using a standard curve and TPC determined using the following formula:

$$TPC(\%) = \frac{c(mg/L) \times v(L) \times 6.25}{w(mg)}, \quad (1)$$

where c is N content, v is volume, and w is sample weight.

Genotyping

A total of 193 SSRs distributed on the 12 chromosomes and another 34 InDel *indica-japonica* identification

markers (Lu et al. 2009; Shen et al. 2004) were used in this study. Exactly 22, 27, 22, 11, 16, 15, 18, 22, 14, 10, 8, and 8 SSRs were mapped to chromosomes 1–2, respectively; and the corresponding average distances between SSR markers were 7.8, 7.6, 10.3, 14.8, 9.3, 9.2, 6.8, 6.1, 8.5, 11.3, 16.1, and 13.9 cM.

DNA was extracted using the CTAB method (Murray and Thompson 1980). The SSR and InDel markers were all amplified and separated by the following method. The PCR volume was 10 µL and the reaction was conducted on a 9700 PCR system (ABI) programmed as follows: 94 °C for 5 min; 30 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 40 s; and 7 min of final extension at 72 °C. The amplified products were size-separated using 8 % polyacrylamide gel electrophoresis and detected by SYBR Green Gel Stain (Zeng et al. 2013). The alleles were recorded by the Imagine System (GeneSys, USA).

Data analysis

Phenotype profiling

The phenotype data were managed using Excel 2007 (MS Excel), and the descriptive statistics and variance analysis were produced using SPSS 17.0 (IBM SPSS). Broad-sense heritability was calculated using the formula: $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the genotypic variance and σ_e^2 is the environmental variance (Wang et al. 2007).

Differentiation of indica-japonica rice

The *indica* gene frequency (F_i) and *japonica* gene frequency (F_j) were calculated using the method described by Lu et al. (Lu et al. 2009).

Genetic diversity

PowerMarker 3.25 (Liu and Muse 2005) was used to calculate the average number of alleles per locus, gene diversity (H_e), and the polymorphism information content (PIC) values.

Population structure

A subset of 90 SSR markers was chosen for population structure analysis, with a genetic distance far more than 10 cM between any two markers. The

model-based program STRUCTURE 2.2 (Earl and vonHoldt 2012) was used to infer the population structure of the 277 rice accessions using a burn-in period of 10,000 and Monte Carlo Markov Chain replicates of 100,000. The number of groups (K) was set from 1 to 15, with seven independent runs each. Evanno's method (Evanno et al. 2005) was used to calculate the most probable structure number for K. The rice lines were divided into different subgroups with a membership probability threshold (Q) of 0.80 (Zhang et al. 2011b). The admixed group (AD) consisted of those lines with $Q < 0.80$. PowerMarker was also used to calculate Nei's genetic distance and to construct the Neighbor-Joining tree (Liu and Muse 2005). MEGA 4 (Tamura et al. 2007) was used to visualize the phylogenetic distribution tree for lines and groups. The main variation patterns for the alleles were generalized by principal coordinate analysis (PCoA) performed by NTSYSpc 2.10 (Rohlf 2002).

Linkage disequilibrium (LD) analysis

The software TASSEL 2.1 (<http://www.maizegenetics.net/>) was used to calculate the level of LD between pairs of loci both in the entire population and the *indica* population. For each SSR, the rare alleles with minor allele frequency less than 0.05 were pooled into one allelic class (Jin et al. 2010; Maccaferri et al. 2005). The relationship between LD decay and physical distance was estimated by a plot drawn using squared correlations of allele frequencies (r^2) against physical distance between linked loci.

Association analysis

The association analysis was undertaken using the mixed linear model (MLM) procedure in TASSEL 2.1 (<http://www.maizegenetics.net/>) (Bradbury et al. 2007). Since the phenotypic data were collected at two geographical locations in 2 years, we used best linear unbiased predictors (BLUPs) to integrate the data. The kinship was calculated using the software SPAGeDi (Hardy and Vekemans 2002). The model MLM_Q + K, using kinship and Q as the concomitant variable, was used to test association between genotypes and phenotypes. False discovery rate (FDR) was applied to identify statistically significant loci.

Identification of superior loci and alleles

Allele effects were estimated using the null allele method described by Bresegheello (Bresegheello and Sorrells 2006).

Results

Morphological analysis

Seven yield traits were investigated at two geographical locations in 2013 and 2014 (Fig. 1). Statistical analysis demonstrated a high level of variation for all seven yield traits, with variable coefficients in the range of 9.81–30.02 %. The broad-sense heritability

of the traits was >90 % except for TN, which was only 57.39 %, suggesting that TN was strongly affected by environment. Genotype had a highly significant effect ($P < 0.01$) for all seven traits (Table S2). Location had a significant effect for PH ($P < 0.01$), PL ($P < 0.01$) and L/W ($P < 0.05$). Year had a significant effect for PL ($P < 0.05$). Genotype \times location had a significant effect for PH, PL and TGW (all $P < 0.01$). Genotype \times year had no significant effects.

One nutritional and three cooking and eating quality characteristics were investigated at Sanming location in 2014 (Fig. 1). There was a wide variation in the four quality traits, with variable coefficients in the range of 12.19–35.53 %. Genotype had a highly significant effect for the four grain traits ($P < 0.01$) (Table S3).

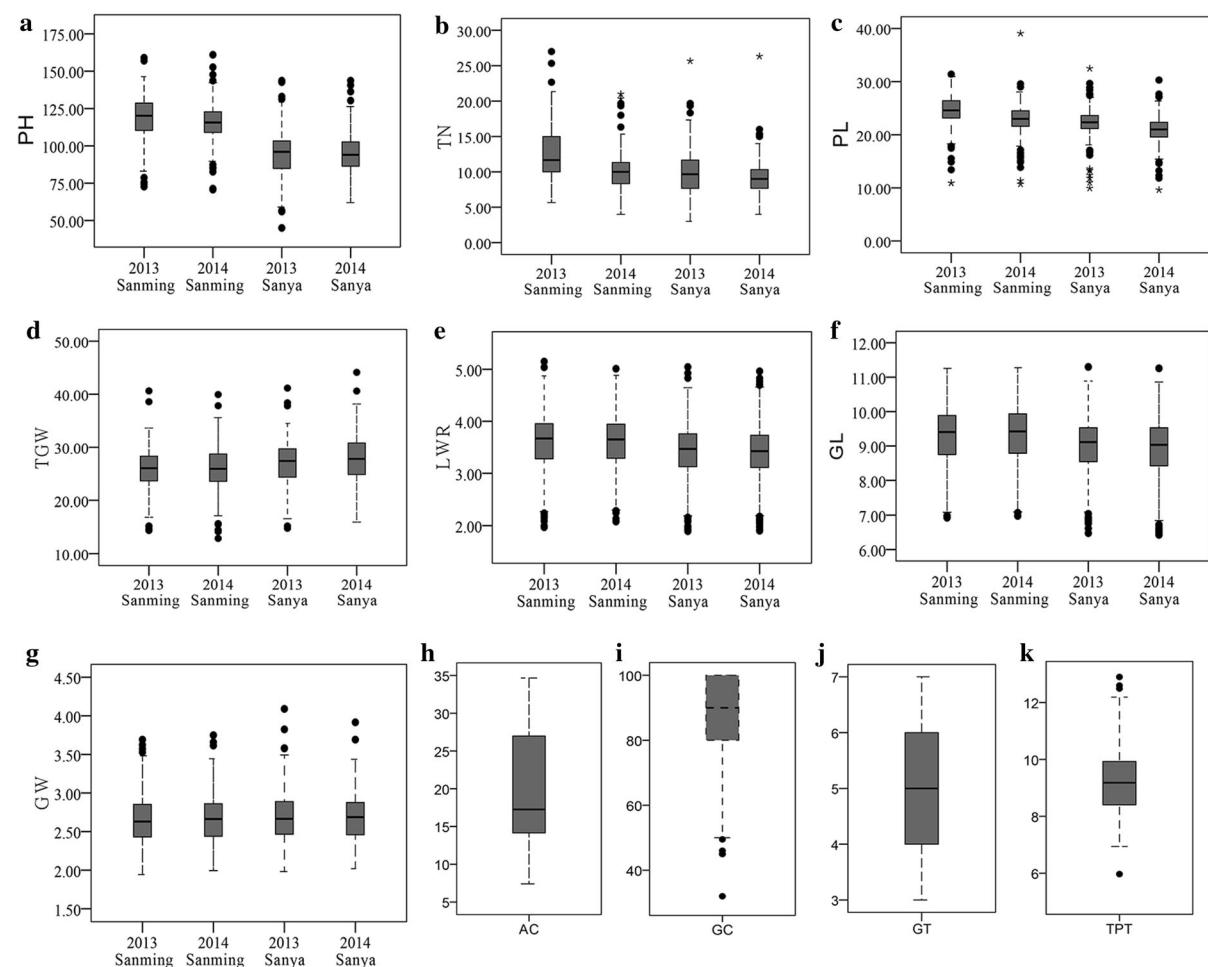


Fig. 1 Box-plot for yield and quality traits. **a–g** PH, TN, PL, TGW, L/W, GL and GW investigated at Sanming and Sanya in 2013 and 2014. **h–k** AC, GC, GT and TPC investigated at Sanming in 2014

Differentiation of *indica-japonica* rice

Among the 277 hybrid rice primal parental lines, 256 lines were classified as *indica* rice (92.42 %), 20 were *japonica* (7.22 %) and one was intermediate. The *indica* lines were further classified into 192 typical *indica*, 62 *indica* and two *indica*-clined based on the method (Lu et al. 2009). Similarly, the 20 *japonica* were classified into six *japonica*-clined, 10 *japonica*, and four typical *japonica* (Fig. S1 and Table S4).

Genetic diversity

By using the 193 SSR markers, 835 alleles were detected in the 277 rice lines in total. There were 2–15 alleles per locus, with an average of 4.326. The He was in the range of 0.014–0.881 with an average of 0.415. PIC was 0.014–0.869 with an average of 0.373. There were 792 and 478 alleles for *indica* and *japonica*, respectively (Table 1, Table S5).

Population structure

The model-based STRUCTURE 2.2 program was used to infer population structure. The 277 lines could be divided into two subgroups (Fig. 2a and b). With $Q = 0.80$, 253 *indica* lines were assigned to the subgroup POP 1, 20 *japonica* lines were assigned to subgroup POP 2 and four lines were assigned to AD (Fig. 2c). The genetic structure pattern was similar to the InDel marker classification (Table S4) where POP 1 included 192 typical *indica* and 61 *indica* lines. POP 2 included six *japonica*-clined, 10 *japonica*, and four typical *japonica* lines. The AD subgroup included one *indica*, two *indica*-clined and one intermediate line.

A neighbor-joining tree based on Nei's distance was constructed (Fig. 3). The 277 rice lines were assigned to cluster I and II, which corresponded to POP 1 and POP 2, respectively. Clusters I and II

contained *indica* and *japonica* rice, respectively. There were four sub-clusters in the *indica* cluster (POP 1A-D), which were related to landrace, maintainer, restorer, and lines from IRRI, respectively (Figs. 2d, 3).

PCoA based on the marker genotypes revealed two distinct clusters for the 277 lines (Fig. 4). The first and second principal components explained 13.22 and 7.11 % of the molecular variance, respectively.

These results indicated that the whole population included *indica* and *japonica* subgroups and that the *indica* subgroup was split into four different sub-subgroups.

LD and decay

The levels of LD between pairs of loci were inferred both in the entire population and the *indica* population. In the entire population, 64.57 % of the loci pairs were significant ($P < 0.01$) for LD, and 6.14 % of the loci pairs were both significant and syntenic. In the *indica* population, only 19.67 % of the loci pairs were significant ($P < 0.01$) for LD, but 7.91 % of the loci pairs were both significant and syntenic (Table S6). The minimum distance for LD decay was deduced via regression equation. LD decay distance of the entire and the *indica* population were ~2415 and ~118 kb, respectively (Fig. 5a, b), where the average pairwise correlation coefficient (r^2) dropped to 0.1.

Marker-trait association

Association analysis between SSR markers and 11 agronomic traits, including seven yield and four grain quality traits was carried out using MLM model over two years across two locations in the entire population and the 256 *indica* rice lines. However, 516 markers were significantly associated with 11 agronomic traits at the FDR correction level, or 353 were significant at the Benferroni correction ($P < 0.05/n$) level, in the entire population. The reasons for the many significant association markers were mainly a strong population structure and that LD decayed slowly in the entire population. Hence, we did not do more analysis across the entire population.

In the *indica* rice group, a total of 24 SSR markers were identified as significantly associated with six agronomic traits at the FDR correction level,

Table 1 Genetic diversity

Germplasm type	N	Allele no	Gene diversity	PIC
Entire collection	277	4.3264	0.4145	0.3733
<i>Indica</i> rice	256	4.1036	0.3650	0.3254
<i>Japonica</i> rice	20	2.4767	0.3157	0.2758

explaining 1.03–7.21 % of the phenotypic variance. No significantly associated marker was detected for PH, PL, AC, GT and TPC at the FDR correction level. Therefore, we discarded all but the most significant markers for the five agronomic traits. In total, we identified 29 SSR markers associated with 11 agronomic traits (Table 2). Among the associated markers, 16 were located close to loci that had previously been identified.

RGS1 and RM6283 were co-associated with three grain shape traits, and they were located close to the known -loci *GS3* and *GL3.2/qGW-3*. Moreover, four other markers (RM7187, RM6645, RM351 and RM1345) were co-associated with two grain shape

traits. RM7187 was located close to *d11*, and RM351 close to *GL7/GW7*, while no known -locus was close to RM6645 and RM1345. More study on these genomic areas will be useful, as this could further our understanding of grain shape traits.

Identification of superior alleles and their carrier lines

The phenotypic effects of significant association marker alleles on agronomic traits and their carrier lines were analyzed (Table 3). For grain shape traits, including GL, GW, L/W and TGW, the alleles with the most positive effect were RM6283-93 (+0.19 mm),

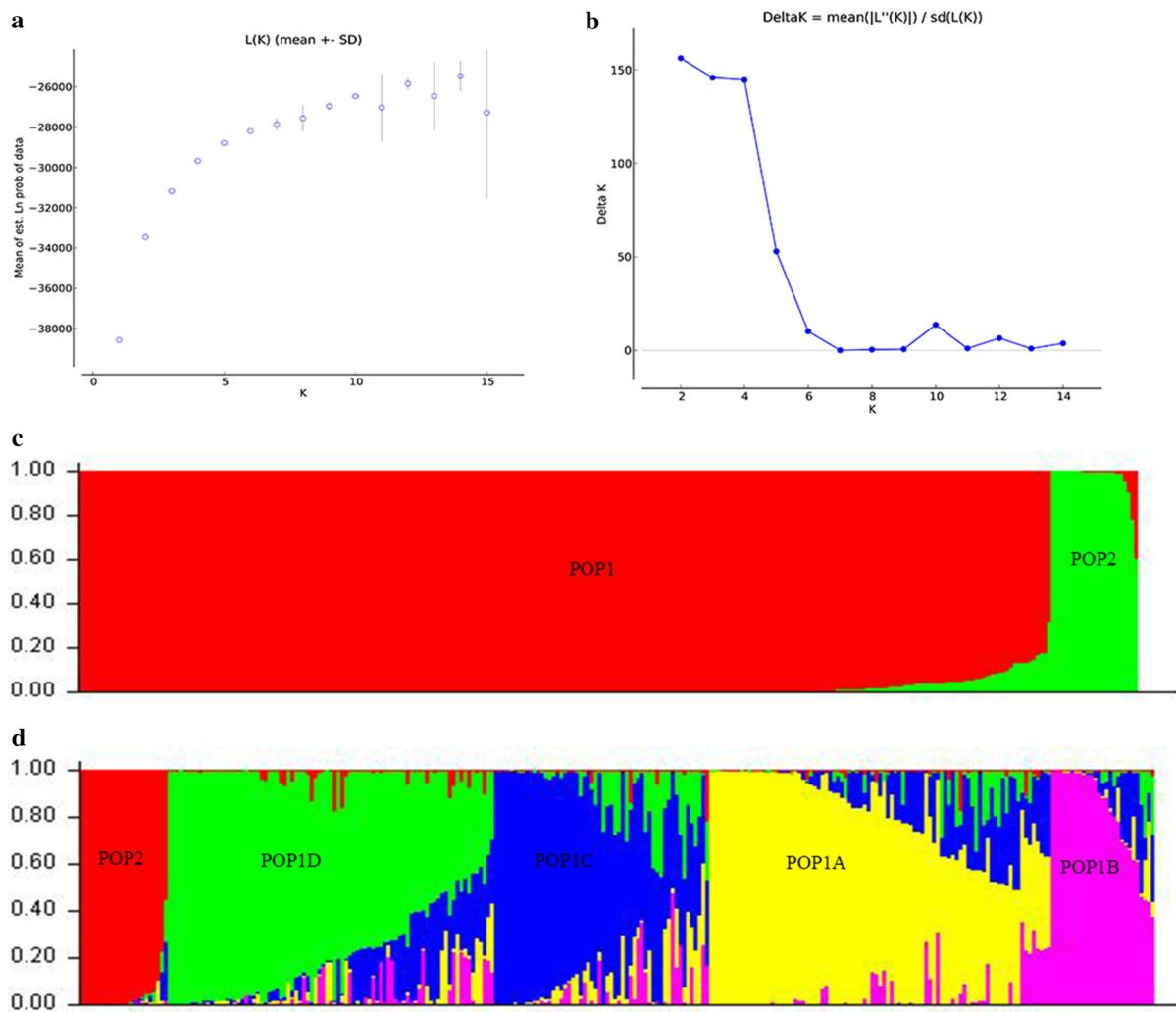


Fig. 2 Estimation of K and population structure. **a** Changes in the log-likelihood function value with the number of subpopulations. **b** Changes in ΔK value with the number of

subpopulations. **(c and d)** Population structure predefined by STRUCTURE (c, $K = 2$; d, $K = 5$)

Fig. 3 Neighbor-joining tree for the 277 lines based on Nei's genetic distance

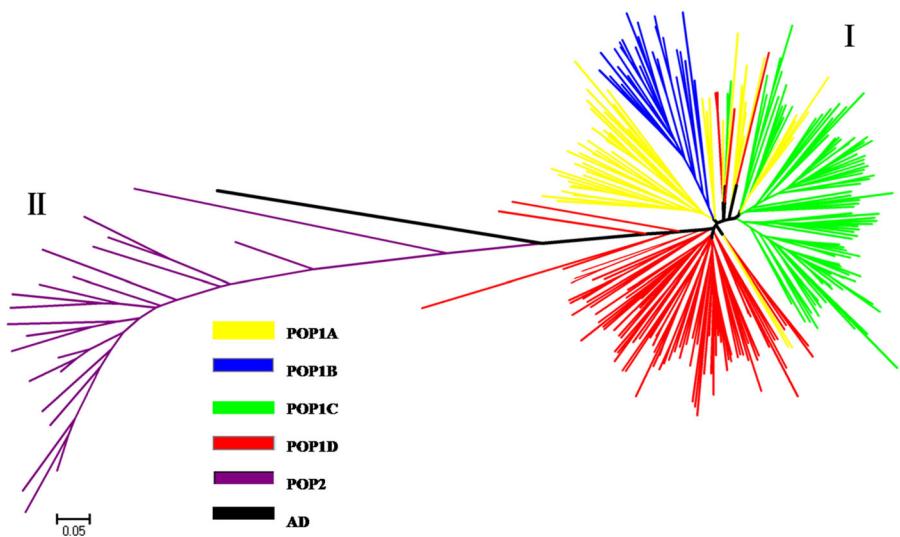
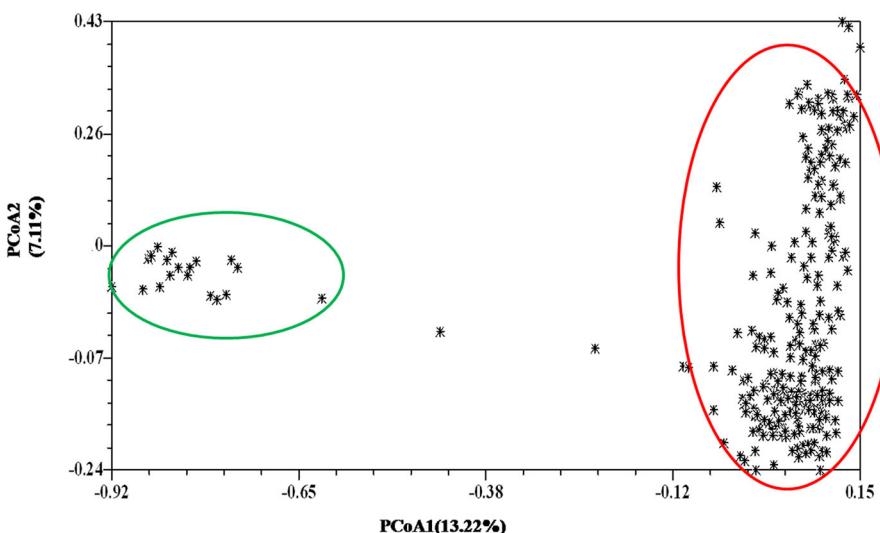


Fig. 4 PCoA of the SSR genotypes in the whole population. PCoA1 and PCoA2 refer to the first and the second principal components, and explain 13.22 and 7.11 % of the molecular variance, respectively



RM6283-96 (+0.40 mm), RM1345-128 (+0.63) and RM1370-173 (+0.67 g), from the carrier lines Minghui63, II-32B, Jinhangsimiao and Minghui86, respectively. The alleles RGS1-193 and RM6283-93 increased GL (+0.18 and +0.19 mm, respectively) and L/W (+0.13 and +0.11, respectively) but simultaneously reduced GW (−0.05 and −0.04 mm, respectively). The allele with the greatest negative effect for GW was RM1345-128 (−0.56 mm) from line Jinhangsimiao. The allele with the greatest negative effect for TN was RM3425-136 (−1.99) from Minghui86. For grain quality traits, the allele with the most positive effect for GC was RM331-167 (+5.59 mm) from R659. The allele RM411-110

reduced AC (−0.57 %), RM1022-148 increased GT (0.97) and RM495-165 increased TPC (0.06 %). The superior alleles identified here could be applied in marker assisted breeding practice to pyramid multiple favorable alleles into one plant.

Discussion

The differentiation of *indica-japonica* rice and the population structure of rice lines are important for parent matching in rice breeding. Population structure analysis is also necessary for association analysis. In previous studies, various rice population have been

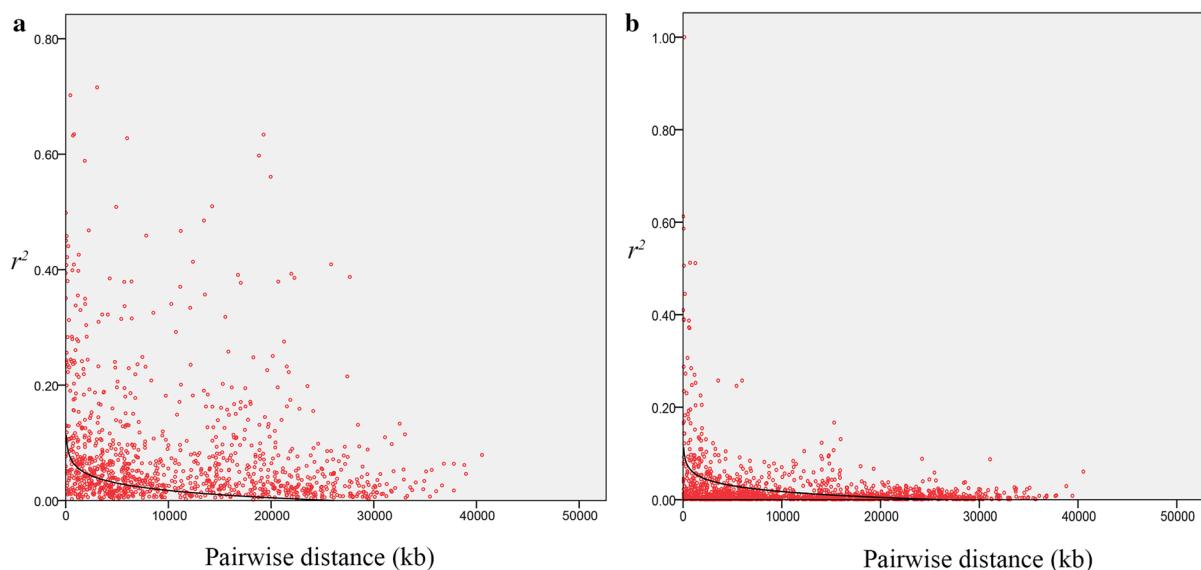


Fig. 5 Linkage disequilibrium (LD) decay plot. **a** Entire population. **b** *Indica* population

divided into different groups, for example the 203 USDA rice mini-core (URMC) subset was divided into five groups, AUS, IND, TEJ, TRJ, and ARO (Li et al. 2012); 905 worldwide rice cultivars were divided into five groups, IND, AUS, TEJ, TRJ, and INT (Huang et al. 2011); and 150 rice core local germplasm lines were divided into *indica* and *japonica*, with four subgroups in the *indica* group (Zhang et al. 2011b). In the present study, we used InDels and SSRs markers to assess *indica-japonica* properties and population structure in 277 hybrid primal parental lines. Our results assigned the entire population into *japonica* and *indica* groups, with *indica* further divided into four subgroups, representing landrace, maintainer, restorer and lines from IRRI. Our study and previous study (Zhang et al. 2011b) all indicated that the *indica-japonica* differentiation caused the observed population structure, and geographical distribution further promoted subgroup formation. The *indica-japonica* hybrid sterility is a common problem that prevents the use of heterosis between *indica* and *japonica*. According to our study, rice breeders could choose parental lines with appropriate gene frequencies F_i or F_j to avoid this disadvantage. Moreover, they could improve the restorer or maintainer lines by crossing them with landraces or rice lines from IRRI, or even with *japonica*, to extend heterosis and genetic diversity in future rice breeding programs. The clustering of heterotic groups has been successfully

applied to maize breeding (Duvick 2005), so the elucidation of rice population structure will be of great importance to rice parent matching and to increase rice genetic diversity.

In the entire population, 64.57 % of the loci pairs were significant, but only 6.14 % loci pairs were both significant and syntenic. However, the percentages in the *indica* group were 19.67 and 7.91 %, respectively. Moreover, r^2 of the entire population was much higher than that of the *indica* population, especially for the loci pairs both significant and syntenic (Table S6). All of this indicated a strong population structure in the entire population lead higher LD level and much longer LD decay distance (Fig. 5a, b). Our results were similar with previous studies, 63 % of the SSR pairs in the 416 accessions were in LD, since the number of locus pairs in LD was reduced sharply within each subpopulation, with the SSR pairs in LD ranging from 5.9 to 22.9 % (Jin et al. 2010). Another study (Zhang et al. 2011b) found the LD decay distance were in the region of 40–50 cM in the entire germplasm set, <5 cM in the subgroups and ≤ 3 cM in the sub-subgroups.

Since a strong population structure in the entire population lead higher LD level and much longer LD decay distance, and most of the loci pairs (58.43 %) significant in LD but not on the same chromosome, there were so many markers significantly associated with 11 agronomic traits in the entire population, we

Table 2 SSR markers significantly associated with agronomic traits

Trait	Locus	Chr	SSR start	SSR end	P value	R2/ %	Known-loci
GL	RGS1	3	16731445	16731637	2.39E-16	7.21	<i>GS3</i>
	RM6283	3	16775927	16775950	3.63E-06	2.75	<i>GL3.2/qGW-3</i>
	RM7187	4	27623896	27623923	2.54E-05	2.64	<i>d11</i>
GW	RGS1	3	16731445	16731637	2.72E-06	2.30	<i>GS3</i>
	RM6283	3	16775927	16775950	2.80E-05	1.90	<i>GL3.2/qGW-3</i>
	RM6645	5	14944802	14944825	1.97E-05	2.21	
	RM3777	5	4113575	4113610	6.95E-04	1.34	<i>GW5</i>
	RM1089	5	5299836	5299901	5.16E-04	1.62	<i>GW5</i>
	RMw-513	5	5379351	5379521	0.001	1.68	<i>GW5</i>
	RM351	7	23872425	23872451	8.61E-04	1.03	<i>GL7/GW7</i>
	RM1345	8	26140424	26140469	5.30E-04	1.81	
L/W	RGS1	3	16731445	16731637	8.95E-16	6.83	<i>GS3</i>
	RM6283	3	16775927	16775950	6.57E-06	2.57	<i>GL3.2/qGW-3</i>
	RM7187	4	27623896	27623923	0.0016	1.68	<i>d11</i>
	RM6645	5	14944802	14944825	6.87E-04	1.87	
	RM204	6	3168425	3168514	5.57E-04	2.79	
	RM351	7	23872425	23872451	1.08E-05	2.10	<i>GL7/GW7</i>
	RM1345	8	26140424	26140469	6.62E-04	2.11	
	RM3700	9	15373650	15373679	6.91E-04	2.10	<i>SG1</i>
PH	RM6466	1	9191223	9191246	0.0011	1.89	
PL	RM3425	1	5116190	5116225	0.0049	1.73	
TGW	RM1370	6	28149879	28149934	6.77E-04	2.29	<i>TGW6</i>
TN	RM3425	1	5116190	5116225	1.25E-05	4.13	
	RM6144	10	15159052	15159078	4.60E-04	2.32	<i>OsCesA7</i>
AC	RM411	3	21231694	21231717	4.40E-04	2.06	
GC	RM5310	1	41190755	41190784	9.83E-05	2.85	
	RM331	10	9487243	9487406	3.76E-04	3.33	
GT	RM1022	3	7201374	7201399	0.0053	2.24	
TPC	RM495	1	216000	216020	0.0019	2.05	

regarded lots of the markers were as false positive. Moreover, the *indica* group represented 92.41 % of the entire population, and so we conducted association only in *indica* population.

Rice yield is a complex trait, determined by the number of panicles, number of grains per panicle, and grain weight (Xing and Zhang 2010). The number of panicles depends on the TN; the number of grains per panicle is related to PL, panicle branch number and setting percentage; and grain weight depends on GL, GW, and TGW. Grain milling quality, appearance quality, cooking and eating quality, and nutritional quality comprise overall grain quality, which is also a complex trait. In this study, the MLM model was used to analyze seven yield traits and four grain quality

traits over 2 years at two locations. In total, 29 SSR markers were identified as associate with 11 traits. Among them, 16 were near known QTLs or genes. RGS1 and RM6283 were simultaneously associated with GL, GW and L/W. RGS1 was designed based on the *GS3* sequence (Wang et al. 2011) and RM6283 is linked with *GL3.2*. *GS3* is the major-effect QTL controller of grain weight and GL, but it is the minor-effect QTL controller of GW and grain plumpness (Mao et al. 2010). *GL3.2* is a homolog of *CYP78A13* and is associated with GL, GW, grain thickness, and TGW (Xu et al. 2014). RMw-513, RM3777 and RM1089 were associated with GW and were linked to *GW5*, which controls GW and grain weight (Yan et al. 2011). RM351 was associated with L/W and was

Table 3 Marker alleles at each locus for the loci significantly associated with agronomic traits and their carrier lines

Trait	Locus-allele	Phenotypic effect	Carrier variety	Trait	Locus-allele	Phenotypic effect	Carrier variety
GL (mm)	RGS1-179	-0.99	II-32B	L/W	RGS1-179	-0.68	II-32B
	RGS1-193	0.18	Minghui63		RGS1-193	0.13	Minghui63
	RM6283-96	-0.97	II-32B		RM6283-96	-0.79	II-32B
	RM6283-80	-0.57	TaifengB		RM6283-80	-0.25	TaifengB
	RM6283-93	0.19	Minghui63		RM6283-93	0.11	Minghui63
	RM7187-165	-1.52	OM576		RM7187-165	-0.70	OM576
	RM7187-157	-0.14	Jing23B		RM7187-157	-0.10	Jing23B
	RM7187-161	0.13	Minghui86		RM7187-161	0.09	Minghui86
GW (mm)	RGS1-193	-0.05	Minghui63	RM6645-144	RM6645-144	-0.50	Teqing
	RGS1-179	0.27	II-32B		RM6645-141	-0.12	Minghui63
	RM6283-93	-0.04	Minghui63		RM6645-126	0.17	TaifengB
	RM6283-80	0.03	TaifengB		RM6645-138	0.25	Jinhangsimiao
	RM6283-96	0.40	II-32B		RM351-130	-0.06	GG8
	RM6645-138	-0.20	Jinhangsimiao		RM351-134	0.43	Han526
	RM6645-126	-0.08	TaifengB		RM1345-124	-0.23	Zhixi
	RM6645-141	0.10	Minghui63		RM1345-126	-0.20	II-32B
	RM6645-144	0.26	Teqing		RM1345-102	0.02	R527
	RM3777-192	-0.05	ShengtaiB		RM1345-128	0.63	Jinhangsimiao
	RM3777-188	0.01	Duoxi 1		RM204-127	-0.59	CB06505
	RM3777-168	0.29	CBB23		RM204-123	-0.13	9311
	RM1089-239	-0.03	Minhui3301		RM204-137	-0.07	Caitang
	RM1089-272	0.08	Gumei 2		RM204-185	-0.05	Minghui86
	RM1089-206	0.11	Han526		RM204-125	0.05	Guangh721
TN (N)	RM1089-221	0.32	GufengB	TGW (g)	RM204-169	0.06	Yipindao
	RMw-513-171	-0.04	IR661		RM204-157	0.46	IRRI134
	RMw-513-163	-0.02	Tungro		RM3700-145	-0.31	Minghui 86
	RMw-513-175	-0.02	A164		RM3700-141	-0.07	N175
	RMw-513-169	0.00	BINDESHWARI		RM3700-143	0.05	Fuhui7018
	RMw-513-167	0.20	Yipindao		RM1370-174	-8.42	Shuh128
	RM351-134	-0.22	Han526		RM1370-167	-4.27	IR661
	RM351-130	0.03	GG8		RM1370-173	0.67	Minghui 86
	RM1345-128	-0.56	Jinhangsimiao		RM5310-162	-19.18	Gang46B
	RM1345-102	0.00	R527		RM5310-154	1.35	Fuhui7022
	RM1345-126	0.09	II-32B		RM331-176	-0.35	Chenghui178
	RM1345-124	0.10	Zhixi		RM331-167	5.59	R659
	RM3425-136	-1.99	Minghui86	AC (%)	RM411-116	3.24	TianfengB
	RM3425-128	0.06	Duoxi1		RM411-110	-0.57	Minghui 63
	RM3425-132	0.42	Wunong1		RM1022-152	-0.68	Fuhui 7018
	RM3425-126	0.99	IR28Z		RM1022-150	0.04	Minghui86
TPC (%)	RM6144-144	-1.86	Gui99	GT	RM1022-148	0.97	Huahangsimiao
	RM6144-138	0.09	Minghui63		RM495-165	0.06	Minghui 63
	RM6144-132	0.58	IRRI134		RM495-159	0.02	Minghui 86

linked to *GL7/GW7*, which was the main QTL for GL and GW. RM7187 was associated with GL and L/W and was linked to *D11*, which controlled PH; and over-expression of *D11* increased GL, GW and TGW (Zhu et al. 2015). RM3700 was associated with L/W and was close to *SG1*. Over-expression of *SG1* resulted in short grain and a dwarf phenotype (Zhu et al. 2015). RM6144 was associated with TN and was linked to *OsCesA7*. *OsCesA7* participates in cellulose biosynthesis, and the *cesa7* mutant exhibited brittle culms and other pleiotropic phenotypes including reduced TN (Wang et al. 2016). RM1307 was associated with TGW and was linked to *TGW6*, which encodes *IAA-glucose hydrolase*, affecting grain weight and enhancing rice yield (Ishimaru et al. 2013). The other 13 SSR markers have not been reported to be linked with any agronomic trait, and could be new loci for important agronomic trait.

Rice yield and grain quality are complex traits, and yield is significantly correlated with the number of superior alleles (Huang et al. 2015). Our study utilized the hybrid rice primal parental lines and intermediate materials collected from China and abroad, which included many important parental lines and intermediate materials for China's hybrid rice such as Minghui 63, Minghui 86, Duoxi 1, Shuhui527 and II -32B. Our research grouped the primal parental lines of hybrid rice into different subgroups, identified significant loci associated with yield and grain quality traits, and analyzed the allele effect on phenotype. This information can be applied to parent selection for restorer or maintainer line improvement in three-line hybrid rice breeding. Furthermore, it will aid in marker assisted selection and molecular design breeding.

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