Characterization of Three Indica Rice Multiparent Advanced Generation Intercross (MAGIC) Populations for Quantitative Trait Loci Identification

Lijun Meng, Longbiao Guo, Kimberly Ponce, Xiangqian Zhao, and Guoyou Ye*

Abstract

Three new rice (Oryza sativa L.) multiparent advanced generation intercross (MAGIC) populations were developed using eight elite indica rice varieties from different breeding programs. These three populations were two recombinant inbred line (RIL) populations derived from two 4-way crosses, DC1 and DC2, and one RIL population derived from an 8-way cross. These populations were genotyped using an Illumina Infinium rice 6K SNP chip. The potential of the three MAGIC populations in identifying marker–trait associations was demonstrated using the plant height (PH) and heading date (HD) measured in 2014. A population of 248 IRRI breeding lines and a population of 323 Chinese breeding lines were also included to compare genetic diversity and linkage disequilibrium (LD) pattern. Our study discovered that (i) the 8-way population had a higher gene diversity than the DC1, DC2, and IRRI populations; (ii) all three MAGIC populations showed no clear population structure; (iii) LD decayed to $r^2 < 0.2$ at about 2.5, 2.5, 1.25, 1.75, and 4.0 Mb for the DC1, DC2, 8-way, IRRI, and Chinese populations, respectively; and (iv) the 8-way population was more powerful than the DC1, DC2, and IRRI populations on QTL identification. The association analysis identified two and three QTL for PH and HD, respectively. Four of the five QTL had peak markers close to known genes. A novel QTL for PH was identified on chromosome 12 using the 8-way population. Therefore, our study suggests that the three new MAGIC populations are valuable resources for QTL identification.

Core Ideas

- Three rice MAGIC populations were developed to better integrate QTL identification and breeding.
- MAGIC populations are valuable for QTL identification, 8-way populations being more powerful.
- QTL (2 and 3) were identified for heading date and plant height, respectively.
- One novel QTL for PH was identified on chromosome 12 using the 8-way population.

The identification of QTL is a critical step for dissecting the molecular basis of agronomy traits in crops, including rice. The detection of the associations between traits of interest and molecular markers is also the prerequisite for most of the marker-assisted selection methods. Linkage mapping and association mapping (AM) are the two most commonly used approaches for QTL mapping in crops. The methods using biparental populations...
for linkage mapping are well established (Yue et al., 2015). The common population types are F2, backcross populations, RILs, backcross inbred lines, and double haploids. The major disadvantage of a biparental population is the reduction of genetic heterogeneity compared with the total genetic variations available for a species. Only two allelic variations are analyzed in a biparental population, which means that the useful and naturally occurring alleles from other genotypes may be missed (Guo and Ye, 2014). The linkage mapping using biparental population also has a low resolution because of the limited number of meioses. In contrast, AM can identify the marker–trait associations (MTAs) caused by LD, which is the nonrandom association of alleles at separate loci. Association mapping uses ancestral recombination events to identify marker–phenotype associations and can provide opportunities for fine mapping (Mackay and Powell, 2007). Usually, a large set of varieties, and sometimes their wild relatives, are used as the mapping populations (Huang et al., 2010, 2011b; Zhao et al., 2011). The advantage of this strategy is that association study can simultaneously detect many naturally occurring allelic variations. However, the breeding and natural populations of inbred crops are not strictly random-mating populations. The complex breeding history of breeding populations and the limited gene flow in most wild populations have created complex stratification within the germplasm. Associations are common between unlinked loci. It is difficult to distinguish the true and false-positive associations (Guo and Ye, 2014; Lipka et al., 2015).

Recently, the QTL mapping using multiparent populations has gained popularity. The Complex Trait Consortium outlines a strategy called collaborative cross to construct a very large set of recombinant inbred strains from a set of genetically diverse inbred mouse strains (Churchill et al., 2004). This strategy has been successfully used for fine mapping of the QTL in mice (Mott et al., 2000; Valdar et al., 2006; Collaborative Cross Consortium, 2012; Baud et al., 2013; Smallwood et al., 2014; Tsaih et al., 2014) and Drosophila (King et al., 2012; Macdonald and Long, 2007; Marriage et al., 2014). The term multiparent advanced generation intercross (MAGIC) was first coined by Mackay and Powell (2007) and was advocated by them and Cavanagh et al. (2008) for crops. The first MAGIC population in plant is developed for Arabidopsis thaliana (L.) Heynh.. It includes 527 MAGIC lines derived from 19 diverse founders (Kover et al., 2009). By simulating the characteristics of this MAGIC population, Kover et al. (2009) show that the QTL explaining 10% of the phenotypic variances can be detected in most situations with an average mapping error of ~300 kb. If the number of lines is doubled, the mapping error will be <200 kb. Using this population, several known QTL for different traits are mapped with high precision, and a few novel QTL are identified (Kover et al., 2009). The potential of identifying MTAs using the MAGIC population has also been demonstrated in Arabidopsis by Ehrenreich et al. (2009) and Gnan et al. (2014). Recently, MAGIC populations have been developed for various crops. There are already two MAGIC populations in wheat (Triticum aestivum L.). One consists of 1579 lines derived from four elite wheat cultivars (Huang et al., 2012a; Cavanagh et al., 2013; Rebetzke et al., 2014), and the other one consists of 1091 lines derived from eight elite winter-sown wheat cultivars (http://www.niab.com/pages/id/93/MAGIC_Populations_in_Wheat) (Mackay et al., 2014). A MAGIC population with 724 lines derived from six cultivars has been developed for sorghum [Sorghum bicolor (L.) Moench] (Higgins et al., 2014). A MAGIC population with 533 doubled-haploid lines derived from eight cultivars has been developed for barley (Hordeum vulgare L.) (Sannemann, 2013; Sannemann et al., 2015). A MAGIC population with 397 lines derived from eight cultivars has been developed for tomato (Solanum lycopersicum L.) (Pascual et al., 2015), and a MAGIC population with 648 lines derived from eight cultivars has been developed for dry bean (Phaseolus vulgaris L.) (Lobaton et al., 2015). A MAGIC population with 338 lines derived from four elite cultivars has been developed for durum wheat (Triticum turgidum ssp. durum) (Milner et al., 2015). As a part of the multigenotype breeding project, the MAGIC populations for upland cotton (Gossypium hirsutum L.), seed rape (Brassica napus L.), peanut (Arachis hypogaea L.), sweet potato [Ipomoea batatas (L.) Lam. var. batatas], cucumber (Cucumis sativus L.), and corn (Zea mays L.), etc. are being developed in China (Li et al., 2014b). In addition, other countries are also actively developing the MAGIC populations for various plant species (https://sites.google.com/site/ijmackay/work/magic). The IRRI has developed four MAGIC populations: indica MAGIC (with eight indica parents), MAGIC plus (with eight indica parents and two additional rounds of 8-way F1 intercrossing), japonica MAGIC (with eight japonica parents), and Global MAGIC (with 16 parents: eight indica and eight japonica). The AM and genotyping-by-sequencing on a subset (200) of the indica MAGIC population have identified many MTAs including the markers close to several known major genes, QTL, and the potential novel loci associated with the essential traits for rice improvement (Bandillo et al., 2013). Li et al. (2014b) have developed a rice MAGIC population consisting of 206 lines derived from 12 founder lines.

The use of MAGIC populations for QTL analysis can bridge the gap between biparental mapping (with limited inference space) and AM (using natural or breeding populations and with complex population structure inherent). MAGIC has three major advantages: (i) more abundant genetic diversity than a biparental population and higher allele balanced frequency than the panels consisting of diverse accessions in AM because of controlled allelic inputs from multiple parents, (ii) negligible impact from population structure (Valdar et al., 2006; Mackay and Powell, 2007; Kover et al., 2009), and (iii) increased mapping resolution by taking the
advantages of both historical and synthetic recombination. Negligible population structure allows the use of simple association analysis with reduced false positive rate (Rakshit et al., 2012; Yamamoto et al., 2014). Accurate specification of the parental origin of alleles allows linkage information to have increased detection power (Huang et al., 2011a; Broman, 2012).

The highly recombined lines from a MAGIC population are the ideal materials for breeding if the parental lines are properly chosen. The development of MAGIC is a time-consuming process. It makes sense to simultaneously consider its applications in gene discovery and practical breeding. The 12-parent MAGIC population reported by Li et al. (2014b) has been used to develop the multilinete variety Duo-Ji-Xin 3, which is being commercialized in China (Li et al., 2013). The indica MAGIC population developed at IRRI (Bandillo et al., 2013) has been explored by IRRI breeders for targetting irrigated and rainfed environments. Four hundred lines selected from S2:4 bulks have been selected based on agronomic traits. According to yield and agronomic performances at three sites in the Philippines (Iloilo, Bukidnon, and IRRI), 130 bulked lines have been selected and included in a larger multi-environment trial in Philippines, Vietnam, Cambodia, Myanmar, China, Thailand, Tanzania, and Senegal. The breeders at these sites have selected the lines suited to their environments. In addition, the breeders from collaborating institutions have visited the field at IRRI and selected many MAGIC lines that are being further tested in their countries (Bandillo et al., 2013).

To further facilitate QTL discovery and variety development in rice, we developed a set of three MAGIC populations using eight diverse indica rice varieties from different breeding programs. Two populations were from two double crosses and one population from an eight-way cross. We characterized the genetic diversity and LD pattern of these three MAGIC populations and demonstrated their potentials in MTA identification using two important agronomic traits: HD and PH. A population of IRRI breeding lines and a population of Chinese breeding lines were used for comparison.

### Materials and Methods

#### Development of MAGIC Populations

Eight genetically diverse parental lines selected from different breeding programs were used to develop a set of RIL populations at IRRI. They were SAGC-08 (A), HHZ5-SAL9-Y3-Y1 (B), BP1976B-2-3-7-TB-1-1 (C), PR33282B-8-1-1-1-1-1 (D), FFZ1 (E), CT 16658-5-2-2SR2-3-6MP (F), IR 68 (G), and IR 02A127 (H) (Table 1). These eight founders were pairwise crossed to produce two-way hybrids. The two-way hybrids were intercrossed in a diallel fashion to generate four-way crosses. The four-way crosses with parental lines A, B, C, and D and the four-way crosses with parental lines E, F, G, and H were advanced by the single-seed decent method to develop the DC1 and DC2 RIL populations. The eight-way crosses were made by intercrossing 100 F1 plants of the four-way crosses ABCD and 100 F1 plants of the four-way crosses EFGH. One thousand eight-way cross F1 plants were advanced by the single-seed decent method to develop the eight-way RIL population (Fig. 1). In total, 495 DC1, 525 DC2, and 668 8-way lines were developed. This study used 271, 268, and 532 lines randomly chosen from the DC1, DC2, and 8-way populations, respectively.

Two hundred and forty-eight advanced breeding lines tested in the advanced yield trials of the irrigated rice breeding program at IRRI were used to represent the current breeding population. These lines were all derived from the pedigree breeding method. Three hundred and twenty-three indica varieties collected in China were used to represent the genetic diversity of the Chinese indica breeding programs (Supplemental Table S1). These lines have been sequenced by the 3K sequencing project (The 3,000 Rice Genomes Project, 2014).

#### Phenotyping

The field trials were conducted at the headquarters of IRRI (Los Baños, Laguna, Philippines) in the 2014 dry season. Seeds were sown in seedling nursery. Seedlings were transplanted into three blocks of six-by-six plots with 36 plants in each plot at 20 d after germination. The spacing was 20 by 20 cm. For the MAGIC populations, the augmented randomized complete block design was adopted to layout the trial. One hundred and twenty checked genotypes (eight parental lines and 112 genotypes from a biparental RIL population) were replicated.
three times. For the IRRI population, a row–column design with two complete replicates was used to layout the trial. Randomizations were generated using the PBTools software (bbi.irri.org) developed by IRRI. Heading date was recorded as the number of days from sowing to 50% of the plants flowering. Three representative plants in the middle of each plot were sampled for measuring PH (cm).

**Genotyping**

The DNA for single-nucleotide polymorphism (SNP) genotyping was extracted from the leaf sample of 5-wk-old seedlings using TissueLyser (Qiagen) according to the protocol used by IRRI’s Genotyping Services Laboratory (GSL) (http://gsl.irri.org). The leaf tissue was lyophilized in plastic cartridges before being transferred into a deep 96-well plate using the PlantTrak Sx (Brooks Life Science Systems) benchtop unit, which also provided a plate layout with the identity of each sample (barcodes). Steel balls were added to each well and the tissue was grounded using TissueLyser II (Qiagen) or Geno/Grinder (SPEX SamplePrep). DNA extraction was performed using the sbeadex magnetic bead kit (LGC Ltd.) that uses a two-step binding mechanism to provide high quality DNA for downstream SNP protocols. After incubation with lysis buffer, the lysate was transferred to a KingFisher Flex 96 plate (Thermo Scientific) for automated DNA extraction. DNA samples were then checked with the PicoGreen double-stranded DNA quantitation assay (Thermo Scientific) before SNP genotyping, which was performed at GSL. Automated calling of genotypes from the raw data produced by the Illumina GoldenGate Assay was performed by a novel method (Wright et al., 2010) to handle inbred sample collections as well as to overcome the limitations of more traditional clustering-based approaches. The calling algorithm did not depend on ad hoc or generalized clustering methods and could accept a priori specified inbreeding coefficients, which allowed this method to make adjustments depending on the expectation of heterozygosity. It also provided a posteriori quality scores on a per-SNP basis so that the reliability of specific SNPs.

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**Fig. 1.** Crossing schemes for developing three MAGIC populations (4-way and 8-way populations). A, SAGC-08; B, HHZ5-SAL9-Y3-Y1; C, BP1976B-2-3-7TB-1-1; D, PR33282-B-8-1-1-1-1-1; E, FFZ1; F, CT 16658-5-2-2SR-2-3-6MP; G, IR 68; H, IR 02A127.
could be evaluated. Approximately 4695 markers yielded good quality genotypic calls.

Besides the standard SNP calling quality checks conducted by the GSL, a three-step filtering strategy was applied to choose high-quality SNPs for QTL mapping. First, the markers that are nonpolymorphic among the founders and RILs of a population were removed. Second, all heterozygous genotypes were set to missing. The markers with >10% missing values or <3% minor allele frequencies (MAFs) were removed. Finally, highly correlated markers (0.95) were excluded by removing one marker from each mark pair. Custom R scripts were developed for this purpose (R Development Core Team, 2013). The number of remained markers was 907, 892, 1329, and 1208 for the DC1, DC2, 8-way, and IRRI populations, respectively.

For the Chinese population, genotypes were identified by sequencing with ~16× base depth using the Hiseq 2000 next-generation sequencing platform. The next-generation sequencing reads were mapped to the reference IRGSP-1.0 using BWA (Version 0.7.10) with bwa mem-M. Single-nucleotide polymorphism calling was conducted using Picardtools (Version 1.119) with SortSam, FixMateInformation, MarkDuplicates, GATK (Version 3.2), IndelRealigner, and UnifiedGenotyper. Highly confident genomic positions were extracted from vcf files using the following criteria: (i) The quality of SNP position $\geq 50$, (ii) The quality of non-SNP position $\geq 30$, (iii) reads mapping quality $\geq 20$, (iv) read depth $\geq 2$ and $\leq 200$, and (v) SNP genotyping quality $\geq 20$. The SNP markers corresponding to the Illumina Infinium rice 6K SNP chip were identified using marker position information. The closest SNP marker was used to replace the requested one if there was no SNP at the exact position.

**Statistical Analysis**

**Analysis of Genetic Diversity and Population Structure**

Genetic diversity was measured by the number of alleles per locus, gene diversity, and polymorphism information content using the program Powermarker V3.25 (Liu and Muse, 2005). The STRUCTURE software (Pritchard et al., 2000) was used to infer the number of subgroups in a population. STRUCTURE used a model based on Bayesian clustering to infer population structure. It placed the individuals of a population into $k$ clusters and assigned each genotype to a subpopulation. The $k$-value was chosen in advance but could be varied for independent runs. Ten independent runs with $k$-value from 1 to 10 were performed based on an admixture model with correlated allele frequency. Both the length of burn-in period and the number of iterations were set to 100,000. The estimated normal logarithm of the probability of fit (averaged for the 10 runs) provided in the STRUCTURE output was plotted against the $k$-value. This value reached a plateau when the minimal number of groups best describing the population substructure had been attained (Pritchard et al., 2000). An ad hoc quantity statistic ($\Delta k$) based on the rate of change in the log probability of data between successive $k$-values (Evanno et al., 2005) was used to determine the number of subpopulations. We also examined population structure via the multidimensional scaling and hierarchical cluster analysis implemented in the AWclust R package (Gao and Starmer, 2007, 2008).

**Analysis of Linkage Disequilibrium**

The LD patterns of the five populations were evaluated by computing the $r^2$ values between SNP marker pairs on all chromosomes using TASSEL v5.0 (Bradbury et al., 2007). Statistically significant LD marker pairs were selected ($p < 0.001$), and the remaining $r^2$ values were not considered as informative. Two approaches were used to estimate the range of LD: (i) LD decayed to 50% of its initial value, according to Zhao et al. (2011), and (ii) the critical value for $r^2$ was estimated as the parametric 95th percentile of the distribution of square root-transformed unlinked $r^2$ values (Breseghello and Sorrells, 2006). Unlinked $r^2$ referred to the $r^2$ between the marker loci with a physical distance longer than 30 Mb or on independent chromosomes. Statistically significant $r^2$ values of intrachromosomal LD were plotted against physical distance, and a second-degree loess curve (Cleveland, 1979) was drawn using the R software package ggplot2 (Wickham, 2009). The intersection of the critical value baseline with the loess curve was considered to be the estimated extent of LD (Breseghello and Sorrells, 2006).

**Phenotypic Analysis**

Phenotypic analysis was conducted using the PBTools (bbi.irri.org) to obtain the adjusted trait value for each line. For the MAGIC populations, the broad-sense heritability of two traits was calculated from the estimates between RIL (genotypic) variance and RIL-by-block (error) variance: Heritability = genotypic variance/(genotypic variance + error variance/1.2), where 1.2 is the number of replicates.

**Association Analysis**

Association analyses were conducted based on a mixed linear model (MLM). The relative kinship estimated as identity by state was used for correcting the unequal relationships between genotypes, and the first three principal components were used to correct population structure using the software TASSEL 5.0 (Bradbury et al., 2007). The $p$-values were adjusted with the Benjamini and Hochberg (1995) method to obtain $q$-values (false discovery rate). The $q$-values were calculated with the R package QVALUE using the smoother method of Storey and Tibshirani (2003). The $q$-value cut off 0.05 was used as the threshold to detect whether there was a statistically significant association. When QTL profile displayed more than one peak exceeding the significance threshold on a chromosome, and these peaks were separated by >2.5 Mb, multiple QTL were declared. The qman package was used for creating the Manhattan plots.
Results

Genetic Diversity

A total of 4695 SNP markers were used to assess the MAF and genetic diversity of the DC1, DC2, 8-way, MPP, DC1_parents, DC2_parents, IRRI, and Chinese populations. The MAGIC populations had much better balance in allele frequencies. Approximately 32.1% of the markers in the IRRI population had a MAF <0.1 while only 6.9, 15.7, and 12.3% in the three MAGIC populations (Fig. 2). The Chinese and IRRI populations had much larger number of polymorphic markers than the three MAGIC populations (Table 2). The Chinese population had much more polymorphic markers than the IRRI population. As expected, there were more polymorphic markers in the 8-way population than in the 4-way populations. The genetic diversity of the Chinese population was higher than all the other populations (Table 2). Interestingly, the average gene diversity indexes of the three MAGIC populations were slightly higher than IRRI population. The two 4-way populations had similar gene diversity indexes and polymorphism information contents, which were slightly lower than those in 8-way population. The average gene diversity indexes of the eight founders (eight-way parents) and the 8-way population were very similar, suggesting that the 8-way population was created with good quality. Similarly, the genetic diversity indexes of the two 4-way populations were very similar to those of their parent populations (DC1_parents and DC2_parents), indicating the good quality of these two 4-way MAGIC populations.

Table 2. Genetic diversity statistics of the DC1, DC2, 8-way, International Rice Research Institute (IRRI), Chinese, and parental line populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Gene diversity</th>
<th>PIC</th>
<th>Major allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC1</td>
<td>271</td>
<td>0.1445</td>
<td>0.1162</td>
<td>0.8924</td>
</tr>
<tr>
<td>DC2</td>
<td>268</td>
<td>0.1429</td>
<td>0.1157</td>
<td>0.8946</td>
</tr>
<tr>
<td>8-way</td>
<td>531</td>
<td>0.1543</td>
<td>0.1257</td>
<td>0.8886</td>
</tr>
<tr>
<td>IRRI</td>
<td>248</td>
<td>0.1407</td>
<td>0.1145</td>
<td>0.8975</td>
</tr>
<tr>
<td>China</td>
<td>323</td>
<td>0.1900</td>
<td>0.1574</td>
<td>0.8659</td>
</tr>
<tr>
<td>8-way parents</td>
<td>8</td>
<td>0.1523</td>
<td>0.1235</td>
<td>0.8876</td>
</tr>
<tr>
<td>DC1_parents</td>
<td>4</td>
<td>0.1376</td>
<td>0.1096</td>
<td>0.8925</td>
</tr>
<tr>
<td>DC2_parents</td>
<td>4</td>
<td>0.1288</td>
<td>0.1032</td>
<td>0.9001</td>
</tr>
</tbody>
</table>

Fig. 2. Minor allele frequency distribution in the DC1, DC2, 8-way, International Rice Research Institute (IRRI), Chinese, and Molecular Plant Pathology (MPP) populations.
Population Structure
Cluster analysis and multidimensional scaling using AWclust showed that the DC1, DC2, and 8-way populations had no clear population structure, while the IRRI and Chinese populations had two and six subgroups, respectively (Fig. 3a). For all five populations, the log likelihood revealed by STRUCTURE gradually increased from $K = 1$ to $K = 10$ and showed no obvious optimum (data not shown). In contrast, the maximum of $D_k$ was observed at $k = 2$ (Fig. 3b), indicating that all populations can be divided into two subgroups. With the membership probabilities >0.50, 24, 36, and 66 lines were assigned to a separate group for the DC1, DC2, and 8-way populations, respectively. Surprisingly, the IRRI and Chinese populations also only had a small number of lines forming a separate subpopulation. For the three MAGIC populations, there were some lines with exceptional high kinship values (Supplemental Fig. S1). After discarding the lines with very high kinship, STRUCTURE analysis did not identify any population structure for all populations.

Linkage Disequilibrium
Extensive variability in the magnitude of $r^2$ at a given physical distance was detected, reflecting the wide local variation in LD extents across chromosomes. The scatter diagram of $r^2$ against physical distance (Supplemental Fig. S2) showed a clean pattern of LD decay in all five populations. Linkage disequilibrium decayed more rapidly and cleanly in the 8-way population than in the DC1, DC2, IRRI, and Chinese populations (Supplemental Fig. S2). A critical value of $r^2$ or basal LD, beyond which LD was likely to be caused by genetic linkage in the absence of population structure, was estimated at 0.14, 0.2, 0.08, 0.14, and 0.48 for the DC1, DC2, 8-way, IRRI, and Chinese populations, respectively (Fig. 4). An $r^2 = 0.2$ was determined to be the appropriate threshold for LD because of the physical linkage for all five populations. In addition, LD declined to 50% of its initial value at about 2.5, 2.5, 1.25, 1.75, and 4.0 Mb in the DC1, DC2, 8-way, IRRI, and Chinese populations, respectively (Fig. 4). Given that the average intermarker distance was ~100 kb (4695 markers), we expected to have reasonable power to identify the large-effect common variants associated with our traits of interest. The distance of most marker pairs with $r^2$ above the critical value of 0.2 were within 2.5 Mb in all populations. Similarly, most of the marker pairs in complete LD ($r^2 > 0.9$) were spaced by a physical distance <1.25 Mb for the MAGIC populations.

Marker pairs could be classified into four groups based on the intermarker physical distance: 0 to 2.5 Mb (tightly linked markers), 2.5 to 5.0 Mb (moderately linked markers), 5.0 to 7.5 Mb (loosely linked markers), and >7.5 Mb (independent markers). The differences in
the percentage of significant loci pairs, the proportion of \( r^2 \) values exceeding the basal LD level of 0.2, and mean \( r^2 \) values between groups could be clearly seen in the MAGIC populations (Table 3). For the 8-way population, the proportion of significant marker pairs reduced from 74.5% in tightly linked group to 23.8% in unlinked group. The proportion of \( r^2 \) values exceeding the basal LD level decreased from 34.3% in tightly linked group to <3.0% in unlinked group. Mean \( r^2 \) values decreased from 0.21 in tightly linked group to 0.06 in unlinked group. Similar patterns were also seen in the DC1 and DC2 populations (Table 3). The proportion of significant marker pairs decreased from 77.6% (71.7%) in tightly linked group to 12.1% (14.2%) in unlinked group of the DC1 (DC2) population. The proportion of \( r^2 \) values exceeding the basal LD level decreased from 53.0% (48.8%) in tightly linked group to less than 11.5% (12.9%) in the unlinked group of the DC1 (DC2) population.

**Phenotypic Variation**

Among the eight parental lines, the parental lines of the DC2 population had the lowest (E, 87.0 d) and highest (G, 99.7 d) HD (Table 4). The DC1 population had a larger HD variation than the DC2 and 8-way populations. The lines with the shortest HD were observed in the DC1 population, while the lines with the longest HD were in the 8-way population. The 8-way population had a slightly higher

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**Table 3. Linkage disequilibrium (LD) overview for the DC1, DC2, and 8-way MAGIC populations**

<table>
<thead>
<tr>
<th>Population Group</th>
<th>Total pairs</th>
<th>Significant pairs†</th>
<th>Percent- age</th>
<th>Mean ( r^2 )</th>
<th>Pairs in ( r^2 &gt; 0.2 )</th>
<th>Percent- age</th>
<th>Mean ( r^2 )</th>
<th>Pairs in complete LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC1 0.0–2.5</td>
<td>6,149</td>
<td>77.6</td>
<td>0.31</td>
<td>53.0</td>
<td>0.48</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5–5.0</td>
<td>4,888</td>
<td>55.1</td>
<td>0.16</td>
<td>25.0</td>
<td>0.35</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0–7.5</td>
<td>4,121</td>
<td>34.7</td>
<td>0.14</td>
<td>16.9</td>
<td>0.37</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7.5</td>
<td>20,691</td>
<td>12.1</td>
<td>0.11</td>
<td>11.5</td>
<td>0.34</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC2 0.0–2.5</td>
<td>5,611</td>
<td>71.7</td>
<td>0.29</td>
<td>48.8</td>
<td>0.48</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5–5.0</td>
<td>4,471</td>
<td>45.8</td>
<td>0.18</td>
<td>27.6</td>
<td>0.40</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0–7.5</td>
<td>3,976</td>
<td>32.8</td>
<td>0.16</td>
<td>26.4</td>
<td>0.37</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7.5</td>
<td>20,536</td>
<td>14.2</td>
<td>0.11</td>
<td>12.0</td>
<td>0.32</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-way 0.0–2.5</td>
<td>12,412</td>
<td>74.5</td>
<td>0.21</td>
<td>34.3</td>
<td>0.46</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5–5.0</td>
<td>10,191</td>
<td>52.5</td>
<td>0.11</td>
<td>14.6</td>
<td>0.37</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0–7.5</td>
<td>8,671</td>
<td>38.7</td>
<td>0.09</td>
<td>10.5</td>
<td>0.34</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7.5</td>
<td>45,720</td>
<td>23.8</td>
<td>0.06</td>
<td>3.0</td>
<td>0.29</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Marker pairs with \( r^2 \) being statistically significant at \( p \leq 0.05 \) level.

**Table 4. Heading date (HD) and plant height (PH) of the eight parental lines and three MAGIC populations measured in International Rice Research Institute headquarters (Los Baños, Philippines) in the dry season of 2014.**

<table>
<thead>
<tr>
<th>Line or population</th>
<th>HD Mean ± SD Range</th>
<th>PH Mean ± SD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC1</td>
<td>92.0 ± 6.7 73.7–115.7</td>
<td>109.9 ± 18.2 76.7–163.7</td>
</tr>
<tr>
<td>DC2</td>
<td>91.1 ± 6.7 78.4–111.4</td>
<td>107.1 ± 18.2 75.7–163.5</td>
</tr>
<tr>
<td>8-way</td>
<td>94.8 ± 6.7 78.0–116.4</td>
<td>109.7 ± 12.7 76.7–151.5</td>
</tr>
</tbody>
</table>
average HD than the DC1 and DC1 populations. All three MAGIC populations showed transgressive segregation in both directions (Table 4). Among the eight parental lines, those of the DC1 population had the lowest (A, 106.7 cm) and highest (C, 133.0 cm) PH (Table 4). The average PH was similar for all three MAGIC populations, while the average PH in the DC2 population was ~3 cm shorter than those of the DC1 and 8-way populations. The DC1 and DC2 populations had slightly larger variations in PH than 8-way population. The lines with the shortest PH were found in all three MAGIC populations, while the lines with the highest PH were in the DC1 and DC2 populations. The transgressive segregation of PH in both directions was seen in all three MAGIC populations (Table 4).

The estimated heritability of HD and PH using all MAGIC lines were 0.57 and 0.86, respectively, which were lower than most of the previously reported estimates.

Association Analysis

Heading Date

Using the DC1, DC2, 8-way, and IRRI populations, zero, one, two, and three significant associations were detected, respectively (Fig. 5). By delineating the significant markers within a physical distance of 2.5 Mb into a single QTL, a total of two QTL for HD were identified on chromosomes 3 and 6, respectively (Table 5). The qHD6 signal peaked at a 2.9-Mb region on chromosome 6.
6, where the known gene for HD, *Hd3a*, locates. The allele carried by the parental line D and H was associated with increased HD. The signal peaked at a 1.3-Mb region on chromosome 3, where the known gene *DTH3/Hd9* locates. *DTH3* was identified in the 8-way, DC2, and IRRI populations. The allele carried by the parental lines A, B, C, G, and H was associated with increased HD in these three populations. The impact of the HD-increasing allele varied among different populations. It was the largest in the IRRI population and the lowest in the DC2 population. The phenotypic variance associated by this QTL showed a similar trend to the allelic effect but varied more greatly (Table 4).

**Plant Height**

Six, zero, four, and one significant MTA were detected using the DC1, DC2, 8-way, and IRRI populations, respectively (Fig. 5). By delineating the significant markers within a physical distance of 2.5 Mb into a single QTL, three QTL were identified for PH on chromosomes 1 and 12, respectively (Table 5). The *PH1.1* signals peaked at a 37.0-Mb region on chromosome 1, where the known gene *Psd1* locates. *PH1.1* was identified in the 8-way and DC1 populations. The *PH1.1* allele carried by the parental line C was associated with increased PH. The *PH1.2* signal peaked at a 38.3- to 38.8-Mb region on chromosome 1, where the known gene *sd1* locates. *PH1.2* was identified in the DC1, 8-way, and IRRI populations. The *PH1.2* allele carried by the parental line C was associated with increased PH in all these three populations and had the largest impact on DC1 population. *PH1.2* explained more phenotypic variation in the DC1 population than in the 8-way and IRRI populations. The *PH12* signal peaked at a 22.5-Mb region on chromosome 12. *PH12* was identified in the 8-way population and explained 3.2% of the phenotypic variance. The *PH12* allele carried by the parental line C was associated with increased PH (by 5.7 cm on average).

**Table 5. Quantitative trait loci (QTL) identified for HD and PH using the DC1, DC2, 8-way and IRRI populations**

<table>
<thead>
<tr>
<th>QTL</th>
<th>Population</th>
<th>Marker</th>
<th>Chromosome</th>
<th>Position</th>
<th>Allele†</th>
<th>No. of lines‡</th>
<th>Effect</th>
<th>q-value</th>
<th>R²</th>
<th>Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qHD6</td>
<td>8-way</td>
<td>5904398</td>
<td>6</td>
<td>2,924,222</td>
<td>C/T</td>
<td>81/445</td>
<td>3.5</td>
<td>0.0481</td>
<td>0.031</td>
<td><em>Hd3a</em></td>
<td>Taoka et al., 2011; Huang et al., 2012b</td>
</tr>
<tr>
<td>qHD3</td>
<td>8-way</td>
<td>fd9</td>
<td>3</td>
<td>1,270,943</td>
<td>A/G</td>
<td>343/183</td>
<td>3.5</td>
<td>0.0481</td>
<td>0.031</td>
<td><em>DTH3/Hd9</em></td>
<td>Bian et al., 2011; Lin et al., 2002</td>
</tr>
<tr>
<td>IRRI</td>
<td>fd9</td>
<td>1,270,943</td>
<td>A/G</td>
<td>148/99</td>
<td>4.1</td>
<td>0.0000</td>
<td>0.137</td>
<td></td>
<td></td>
<td><em>Psd1</em></td>
<td>Lin et al., 2002</td>
</tr>
<tr>
<td>DC2</td>
<td>8-way</td>
<td>1,270,943</td>
<td>A/G</td>
<td>61/207</td>
<td>4.6</td>
<td>0.0105</td>
<td>0.075</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPH1.1</td>
<td>8-way</td>
<td>id1023347</td>
<td>1</td>
<td>37,030,733</td>
<td>A/G</td>
<td>67/463</td>
<td>10.2</td>
<td>0.0066</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC1</td>
<td>id1023347</td>
<td>37,030,733</td>
<td>A/G</td>
<td>78/193</td>
<td>15.7</td>
<td>0.0016</td>
<td>0.080</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPH1.2</td>
<td>8-way</td>
<td>12373000</td>
<td>1</td>
<td>38,652,270</td>
<td>T/C</td>
<td>64/466</td>
<td>13.8</td>
<td>0.0001</td>
<td>0.059</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRRI</td>
<td>1243398</td>
<td>38,847,770</td>
<td>T/C</td>
<td>21/226</td>
<td>11.3</td>
<td>0.0090</td>
<td>0.083</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPH12</td>
<td>8-way</td>
<td>12926167</td>
<td>12</td>
<td>22,544,640</td>
<td>C/T</td>
<td>149/381</td>
<td>5.7</td>
<td>0.0112</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Allele before slash increases trait value.‡ Number of lines carrying the corresponding allele.

**Discussion**

**Genetic Diversity**

In the present study, the Chinese population had the highest diversity. China has many *indica* rice breeding programs. Although germplasm exchange happens between programs, different programs serve different target production environments. Therefore, genetic diversity is maintained across different programs. The IRRI only has one large-scale breeding program—the irrigated breeding program. A few small programs of IRRI all depend on this large program to obtain parental lines, which arguably provides less genetic diversity to IRRI’s breeding germplasm. As expected, the 8-way population had a higher gene diversity than the DC1 and DC2 populations, since more parental lines were involved. It was unexpected that the 8-way population had a slightly higher diversity than the IRRI population. This might be caused by the following reasons: (i) all the lines of the IRRI population were from the current breeding population of the irrigated breeding program; (ii) many more markers had 1 to 5% MAF in the IRRI population; and (iii) five of the founder lines for the 8-way population were from other breeding programs, which might bring in new genes not available in the IRRI population. It should be pointed out that many more polymorphic markers existed in the IRRI population than in the 8-way population, indicating that the genetic diversity available in the IRRI population was not well captured by the 8-way MAGIC population. This was expected, since only three IRRI lines were used in creating the 8-way MAGIC population. We deliberately used the parental lines from a few breeding programs to bring favorable genes together, which seemed to have increased the diversity of the 8-way population.

**Population Structure and Linkage Disequilibrium**

In the present study, no structure was found in the three MAGIC populations by cluster analysis and multidimensional scaling. STRUCTURE analysis revealed two subpopulations for each of the three MAGIC populations.
After discarding the lines with exceptionally high kinship, no subpopulation was identified in any of the three MAGIC populations by STRUCTURE. The *indica* rice MAGIC population reported by Bandillo et al. (2013) also has negligible structure. Similarly, the tomato MAGIC population reported by Pascual et al. (2015), the wheat MAGIC populations reported by Huang et al. (2012a), and the barley MAGIC population reported by Sannemann et al. (2015) all have no obvious substructure. The winter-sown wheat MAGIC population has some remaining structure (Mackay et al., 2014).

The resolution of association studies depends on the decay of LD within population. As expected, the LD decay was slower in the DC1 and DC2 populations than in the 8-way population. The average LD in the 8-way, DC1, and DC2 populations decayed below a critical level ($r^2 = 0.2$) within a distance of 1.25 and 2.5 Mb, respectively. The LD decays in our MAGIC populations were much slower than those reported previously in the panels of diverse *indica* rice germplasm. The estimated LD extends to ~200 kb in the *indica* group consisting of 20 landraces according to McNally et al. (2009). In an *indica* group consisting of 413 accessions (including landraces and cultivars), Zhao et al. (2011) discovers that LD almost drops to background levels and is ~500 kb to 1 Mb, reaching half of its initial value at ~100 kb. In the study of Huang et al. (2010), the LD decay rate of the *indica* group of 517 landraces and cultivars is estimated at ~123 kb, and the $r^2$ drops to 0.25 (Huang et al., 2010). This was not unexpected given the relatively narrow base of our populations. It was unexpected that LD decayed more rapidly in the 8-way population than in the IRRI population. This might be caused by the fact that the elite lines from other breeding programs are recently used as the parents in IRRI’s irrigated breeding program. The much slower LD decay in the Chinese population is very surprising. Since all the lines in the Chinese population are advanced lines from breeding programs instead of landraces, the selection by breeders for adapting different production environments may contribute to the observed long-range LD.

**Association Power and Resolution**

Using a MLM, two and three QTL were detected for HD and PH in the three MAGIC populations and the IRRI population, respectively. In our study, a stringent threshold ($q < 0.05$) was used, which caused reduced detection power and thus increased false negatives. It can be one of the reasons accounting for the small number of detected QTL. Many studies use a $q$-value of 0.1 or $p$-value of 0.01 to declare significant MTA. Among these five QTL, two, one, five, and two of them were detected in the DC1, DC2, 8-way, and IRRI populations, respectively. The differences in detection power between the 4-way and 8-way populations were due partially to their differences in population size and the number of parental lines involved.

In the present study, HD QTL were only detected in the DC2 population, while PH QTL were only detected in the DC1 population. The parental lines of DC1 QTL have the same allele for the HD QTL detected in other populations. Similarly, the parental lines of DC2 QTL have the same allele for the PH QTL detected in other populations. It can explain why the QTL for HD (PH) could not be detected in DC1 (DC2) population. However, all five QTL were detected in the 8-way population. Therefore, the multiparent population derived from the 8-way cross is better than those derived from the 4-way crosses. This is consistent with the expectation that generating the progenies from multiple parental lines offers an advantage that more QTL can be detected (Pascual et al., 2015). Similar to the QTL mapping using biparental populations, the selection of founders will be one of the most important design considerations. Although the 8-way population had a bigger population size than the two 4-way populations, it did not have the lowest $p$-values for some of the identified QTL. For instance, the 12.7-Mb region on chromosome 3 containing *DTH3/Hd9* revealed a stronger association in the DC2 population ($p = 1.2 \times 10^{-5}$) than in the 8-way population ($p = 7.3 \times 10^{-3}$). The 38.4- to 38.8-Mb region containing *sd1* on chromosome 1 revealed a stronger association in the DC1 population ($p = 3.0 \times 10^{-5}$) than in the 8-way population ($p = 4.1 \times 10^{-5}$). In simulating the power of MAGIC population designs for *Arabidopsis*, Klasen et al. (2012) discovers that a higher number of combined genomes per individual may result in a higher power, since the combination of one QTL allele with more diverse genetic background. In addition, the involvement of more parents in developing a population usually means that the allele frequencies are lower and uneven across alleles, which may result in reduced power for detecting the QTL with small effects. If the objective is to identify the QTL with relatively larger effects and to be directly used in breeding, the use of more parental lines is advantageous. For detecting the QTL with small effects, it is beneficial to map the populations with fewer parental lines.

Incorporating multiple parents in crossing design will instantaneously increase recombination frequency (Huang et al., 2011a; Kover et al., 2009). In the present study, LD analysis suggests that the 8-way population has a higher resolution. We compared mapping resolution using the distance between the peak positions of the identified QTL from the locations of corresponding known genes. *qHd6* was detected only in the 8-way population with the peak marker (SNP 5904398 at 2,924,222 bp) 1.9 kb away from the known gene *Hd3a* (Os06 g0157700). *qHD3* had the same peak marker (Id9 at1270943) in the 8-way, IRRI, and DC2 populations and was 623 kb away from the known gene *DTH3/Hd9* (Os03 g0122600). *qPH1.1* had the same peak marker (SNP 1023347 at 37,030,733 kb) in the 8-way and DC1 populations and was 1.9 kb away from the known gene *Psd1* (Os01 g0829200). For *qPH1.2*, the peak markers were 19.5, 271, and 466 kb away from the known gene *sd1* (Os01 g0883800) in the DC1, 8-way, and IRRI populations, respectively. The lower marker density used in the present study might be one of the reasons why the
theoretically higher resolution of the 8-way population was not clearly shown for the detected QTL. Another possible reason is that only one of the eight parental lines, line E, has the PH-decreasing qPH1.2 allele.

Conclusion and Prospective

Identification of the markers associated with HD and PH in the chromosome regions of previously cloned genes (sd1/OsGA20ox2, Psd1, Hd3a, and DTH3/Hd9) can provide direct support to our expectation that the three new MAGIC populations are valuable resources for QTL identification. The usefulness of the MAGIC population can be further improved. First, the number of polymorphic markers can be significantly increased. There are already rice SNP chips with higher marker density such as the 44K Affymetrix array (McCouch et al., 2010) and 50K Infinium array (RiceSNP50) (Chen et al., 2014). Genotyping-by-sequencing has also been demonstrated to be a reliable method for rapidly generating tens of thousands of SNPs at a low cost (Elshire et al., 2011; Thomson, 2014) in rice and has been used to genotype the rice MAGIC populations reported by Bandillo et al. (2013). Second, the population size can be increased. We did not use all the lines of the MAGIC populations in the present study. The numbers of lines available for the DC1, DC2 and 8-way populations are 495, 525, and 668, respectively. It is well known that both mapping power and resolution can be improved by increasing population size.

Cavanagh et al. (2008) suggest that the population size of a MAGIC population needs to be much larger than 50 individuals per founder (Cavanagh et al., 2008). Kover et al. (2009) report that their 12-parent Arabidopsis MAGIC population can directly identify causal SNPs if there are more than 1000 lines. Third, more advanced methods and multiple methods for QTL identification can be applied. In the present study, we only adopt the MLM implemented in the TASSEL software, which is basically a method for association analysis, because it is simple and computationally straightforward and requires fewer degrees of freedom than other methods. There are a few potentially more effective methods based on estimating the founder haplotype probabilities and exploiting linkage disequilibrium information, including HAPPY (Mott et al., 2000), R/mpMap (Huang and George, 2011), and MPWGAIM (Verbyla et al., 2014). Although it has been shown that the MLM-based association analysis is effective (Ehrenreich et al., 2009; Bandillo et al., 2013; Higgins et al., 2014; Li et al., 2014b; Lobaton et al., 2015), different mapping results are shown during analyzing empirical datasets. The MAGIC populations may be better explored by using the complementary characteristics of various mapping methods. Finally, our three MAGIC populations are interconnected. The eight founders for the 8-way population include the founders of the DC1 and DC2 populations, which will allow the use of multiple-population combined analysis to increase resolution and power.

We are currently phenotyping the MAGIC populations for a number of new traits. In addition, we will compare the mapping results of different statistical procedure.

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References


