Genetics and Molecular Research

Population structure analysis and association mapping of blast resistance in *indica* rice (*Oryza sativa* L.) landraces

Y.Y. Wu[1,3], J.B. He[1], A.H. Li[3], N.Y. Fang[1,2], W.W. He[1,2], L.L. Dang[1,2], G.Y. Zeng[1,2], J. Huang[1,2], Y.M. Bao[1,2] and H.S. Zhang[1,2]

[1]State Key Laboratory of Crop Genetics & Germplasm Enhancement, Nanjing Agricultural University, Nanjing, China
[2]Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing, China
[3]Lixiahe Agricultural Research Institute of Jiangsu Province, Yangzhou, China

Corresponding authors: Y.M. Bao / H.S. Zhang
E-mail: yongmeibao@njau.edu.cn / hszhang@njau.edu.cn

Genet. Mol. Res. 15 (3): gmr.15038254
Received December 11, 2015
Accepted March 21, 2016
Published August 12, 2016
DOI http://dx.doi.org/10.4238/gmr.15038254

ABSTRACT. Rice blast caused by *Magnaporthe oryzae* is one of the most devastating rice diseases worldwide. To understand the genetic diversity of *indica* landrace accessions and identify simple sequence repeat (SSR) markers that are associated with blast resistance, a population of 276 *indica* landraces from across the world was constructed. This population was then used to evaluate the blast-resistance phenotype through artificial inoculation under controlled conditions in 2012 and 2013. The genetic diversity and association of the population with resistance were analyzed by examining the phenotype for 160 SSR markers distributed on 12 rice chromosomes. The 276 accessions were classified into seven groups using model- and

distance-based cluster analyses. Associations between SSR markers and blast resistance showed that 26 SSR markers were significantly associated with blast resistance in 2012 and 2013 ($P < 0.01$) and that the phenotypic variation ranged from 2.68 to 13.11%. Nineteen of the markers associated with blast resistance were located in regions where genes or quantitative trait loci (QTLs) have been previously reported, and seven were newly identified in this study. These results indicate that marker-trait association has potential advantages over classical linkage analysis and QTL mapping, and that these markers could be used for marker-assisted selection in rice blast-resistance-breeding programs.

Key words: *Indica* landrace; *Magnaporthe oryzae*; SSR; Genetic diversity; Association analysis

INTRODUCTION

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating rice diseases. It occurs in all rice production regions and commonly reduces yield by 10 to 30% (Hossain and Fischer, 1995). Developing resistant cultivars by introducing resistance genes into elite rice varieties is the most economical method to combat the disease (Roy-Chowdhury et al., 2012). However, many resistant cultivars are only effective for a short period of time due to the pathogenic variability of *M. oryzae* (Dean et al., 2005). Partial resistance controlled by quantitative trait loci (QTLs) can be characterized by reduced pathogen reproduction in compatible combinations, and is usually considered to be broad-spectrum and durable (Wang et al., 2012). Therefore, it is important that new partial resistance genes or QTLs are identified and isolated from wild rice species and landraces for blast-resistance breeding.

In recent years, more than 300 QTLs have been identified on all 12 chromosomes of rice, using different mapping populations under greenhouse and field conditions through linkage analysis and QTL mapping (Ballini et al., 2008). A total of four QTLs were detected on chromosomes 4, 9, and 12 through QTL analysis, in which the phenotypic variation explained by each QTL ranged from 7.9 to 45.7%. In addition, the resistance gene, designated *pi21*, was mapped to chromosome 4 between restriction fragment length polymorphism (RFLP) marker loci G271 and G317 at a distance of 5.0 and 8.5 cM, respectively (Fukuoka and Okuno, 2001). In a segregation analysis of partial resistance in F_3 populations derived from Chubu32 x Norin29, Zenbayashi et al. (2002) identified and mapped *Pi34* between the RFLP markers C1172 and C189 at a distance of 4.8 cM on chromosome 11. Although substantial progress has been made in the identification and mapping of partial resistance genes and QTLs using molecular markers, there are some inherent limitations to QTL analyses. In linkage studies of any given cross, the genetic diversity sampled is limited, and the segregating population usually presents only two alleles per locus. In addition, it is difficult to detect recombination events between closely spaced (1 cM) loci when a limited number of meiosis cycles occur in the mapping population (Salvi and Tuberosa, 2005).

Association analysis, also known as linkage disequilibrium analysis, can be used to detect and map QTLs based on the strength of the correlation between genetic markers and traits. This technique has been widely applied in a number of crop species, including maize, sugarcane, cotton, wheat, barley, potato, and soybean (Kraakman et al., 2004). In rice,

this approach has been applied to identify DNA markers associated with certain phenotypes, including grain quality traits, yield and its components, stigma and spikelet characteristics, eating and cooking qualities, and blast resistance (Tian et al., 2009; Kawasaki-Tanaka and Fukuta, 2014; Wang et al., 2014; Guo et al., 2015). The first attempt at high-density association mapping in rice was reported by Huang et al. (2010). In that study, a high-density rice haplotype map was constructed with 3.6 x 10^6 single nucleotide polymorphisms (SNPs) to perform genome-wide association studies (GWASs) for 14 agronomic traits. A total of 37 SNP marker-trait associations were identified in 507 rice landraces ($P < 5 \times 10^{-7}$), which indicated that GWASs of rice landraces could provide an effective approach for gene identification.

In this study, 276 *indica* rice landrace accessions were collected worldwide, and the blast-resistance phenotype was evaluated under artificial inoculation in 2012 and 2013. The aims of this study were to evaluate the genetic diversity and population structure of landrace accessions and to identify associations between simple sequence repeat (SSR) markers and rice blast resistance. The molecular markers identified as associated with the rice blast-resistance phenotype will be further applied to genetic improvement breeding.

MATERIAL AND METHODS

Plant materials and growth

A total of 276 *indica* rice accessions, including 165 landraces from different regions of China, 99 landraces from worldwide populations, and 12 varieties were used in this study (**Table S1**). Germinated rice seeds were sown in 60 x 30 x 5-cm plastic trays containing sieved garden soil, as described by Wang et al. (2002). In each tray, 38 experimental materials and two highly susceptible controls, such as Suyunuo and LTH, were planted. The seedlings were grown in a greenhouse at 24°-30°C with a light and dark cycle of 16 and 8 h, respectively. At the four-leaf stage, the seedlings were inoculated with blast isolates, as described by Wang et al. (2002). Before inoculation, two leaves of each line were sampled for DNA extraction and marker analysis.

Pathogen, inoculation, and disease evaluation

Seven blast isolates (*M. oryzae*), namely, 2009-70-2 (ZA_7), 2010-49-1 (ZB_{19}), 2010-28 (ZC_1), 2010-35 (ZD_{13}), 2010-55-1 (ZE_3), 2009-123 (ZF_1), and 2010-9 (ZG_1), all from China, were used to investigate resistance in all rice accessions. The four-leaf stage seedlings were placed in inoculation chambers and inoculated with a conidial suspension (5 x 10^4 conidia/mL). The inoculated plants were kept in the chambers at 26°C with 95% relative humidity and darkness for 24 h. They were then transferred to the greenhouse (28°-30°C/day and 20°-22°C/night) for disease incubation, at 100% relative humidity, by intermittently spraying water for 1-2 min every 2 h (Ashkani et al., 2011). Each line was inoculated in two independent experiments, and three replications were conducted for each experiment.

Disease reactions were evaluated 1 week after inoculation, and each line was scored according to a reference standard (Shi et al., 2010). Lesion scores from 0 to 5 were based on the lesion type and diseased area as described by Shi et al. (2010). Individuals with scores of 0, 1, and 2 were grouped into the resistant group, and those with scores of 3, 4, and 5 were grouped into the susceptible group.

DNA isolation and genotyping

Total DNA was extracted using the sodium dodecyl sulfate method. For SSR markers, the primer sets were adopted from those included in the International Rice Microsatellite Initiative (IRMI, http://www.gramene.org) (McCouch et al., 2002). The 160 SSR markers were distributed across all 12 rice chromosomes, with an average of 13 markers per chromosome (ranging from 10 to 20 markers per chromosome) (**Table S2**). Polymerase chain reaction (PCR) was used to perform amplifications in 10-μL reaction volumes, including 37.5 ng DNA (0.5 μL), 10X PCR buffer (1 μL), 0.025 U Taq DNA polymerase (0.5 μL, TaKaRa, China), 0.2 mM dNTPs (0.5 μL), 0.8 pM forward and reverse primers (0.5 μL), and 6.5 μL ddH_2O. The PCR program was run as follows: pre-denaturing at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 45 s, annealing at 55°-60°C for 45 s (depending on the primers), extension at 72°C for 60 s, and a final extension at 72°C for 10 min. The PCR products were analyzed on 8% non-denaturing polyacrylamide gels using the silver-staining method following the manufacturer recommendations (Promega, USA). SSR bands were scored with different letters according to their fragment size, and heterozygous loci were scored as missing data.

Allele diversity, phylogenetic analysis, and population structure

Summary statistics, including the number of alleles per locus, major allele frequency, gene diversity, and polymorphism information content (PIC), were calculated at 160 SSR marker loci in 276 accessions using PowerMarker version 3.25 (http://www.powermarker.net) (Liu and Muse, 2005).

Nei's distance (Nei et al., 1983) was computed among these genotypes, and cluster analysis was performed on the unrooted phylogeny reconstruction using the neighbor-joining method, as implemented in PowerMarker. Finally, the tree was viewed with MEGA 4.0 (http://www.megasoftware.net).

The population structure that creates genome-wide linkage disequilibrium between unlinked loci is required to avoid false-positive associations between genetic polymorphisms and traits in association mapping. To analyze the population structure, the Q matrix was used to describe the percent subpopulation parentage for each line among *indica* accessions, and a model-based cluster analysis was performed based on 60 unlinked markers from 12 different chromosomes by the package STRUCTURE 2.2 software (http://pritch.bsd.uchicago.edu/software.html) (Pritchard et al., 2000). The optimum number of populations (K) was selected after five independent runs of a burn-in of 500,000 iterations followed by 500,000 iterations for each value of K (set from K = 2 to 10) (Falush et al., 2003).

Association analysis

Associations between markers and blast resistance were analyzed using the TASSEL software with a mixed linear model (MLM) according to the method described by Yu et al. (2006). In the MLM, the subpopulation data (Q matrix from the STRUCTURE program) were used as the fixed effects, and the marker-based pairwise kinship matrix was used to model the variance-covariance matrix of the genetic background random effect. The P value of the marker determined whether the marker (as a QTL) was associated with the trait, and the R^2 (marker) indicated the fraction of the total variation explained by the reported markers.

RESULTS

Phenotypic analysis of rice blast resistance

According to the referred standard, each accession was evaluated for its resistance to seven *M. oryzae* isolates in each of 2 years (2012 and 2013). In 2012, 74 of the 230 tested accessions (32.2% of tested accessions) were found to be resistant to seven races of *M. oryzae*, 52 accessions (22.6% of tested accessions) showed resistance to six races, and only 11 accessions (4.8% of tested accessions) were susceptible to seven races. In 2013, 76 of the 271 tested accessions (28.0% of tested accessions) were found to be resistant to seven races, 76 accessions (28.0% of tested accessions) showed resistance to six races, and only four accessions (1.5% of tested accessions) were susceptible to seven races (**Table S1**). These results indicate that most of the *indica* rice landraces showed some resistance to rice blast.

Genetic diversity

A total of 160 SSR markers, which were randomly distributed across 12 chromosomes, were used to evaluate the genetic diversity of the rice population. In total, 689 alleles were produced by 160 SSR markers among the 276 accessions assayed (**Table S2**). The average number of alleles per locus was 4.31 and ranged from 2 (RM13538) to 8 (RM10920). The average gene diversity was 0.49 and ranged from 0.08 (RM18861) to 0.76 (RM16763). The average PIC per locus was 0.43 and ranged from 0.08 (RM18861) to 0.72 (RM16763).

Model- and distance-based analyses of the population structure

To eliminate the effects of population structure and to ensure the accuracy of the association between genetic polymorphisms and traits, 60 unlinked SSR alleles were used to check the structure of the genetic variation of the *indica* landraces used in this study. The model-based method was performed to check the genetic structure among 276 *indica* landrace accessions. Five independent calculations were performed for each K value from K = 2 to 10, and the results showed that the likelihood was maximized and minimized when the number of populations was set at seven. This suggested that these *indica* landrace accessions could be grouped into seven subpopulations, which were named P1, P2, P3, P4, P5, P6, and P7 (Figure 1).

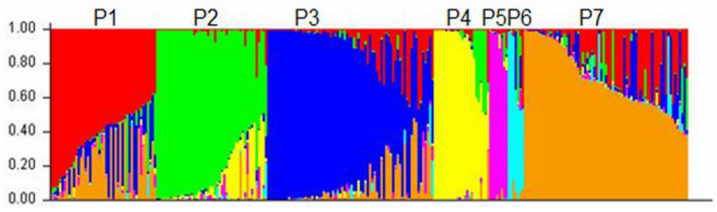

Figure 1. Population structure of *indica* accessions based on 60 simple sequence repeat loci.

Cluster analysis of the 276 accessions was performed on the unrooted phylogeny reconstruction using the neighbor-joining method. The results showed that seven major clusters were detected based on Nei's genetic distance (Figure 2), which coincided well with

the model-based membership assignment used for most of the accessions. In addition, the results of cluster analysis revealed high coherence with the geographical distribution of the materials tested. For example, Population 1 contained accessions that mainly came from the middle and lower regions of the Yangtze River of China, Population 2 included accessions mainly collected from southwest China, and Population 3 was mainly composed of accessions from South China. However, Population 4 was found to include accessions mainly from North America (Figure 2).

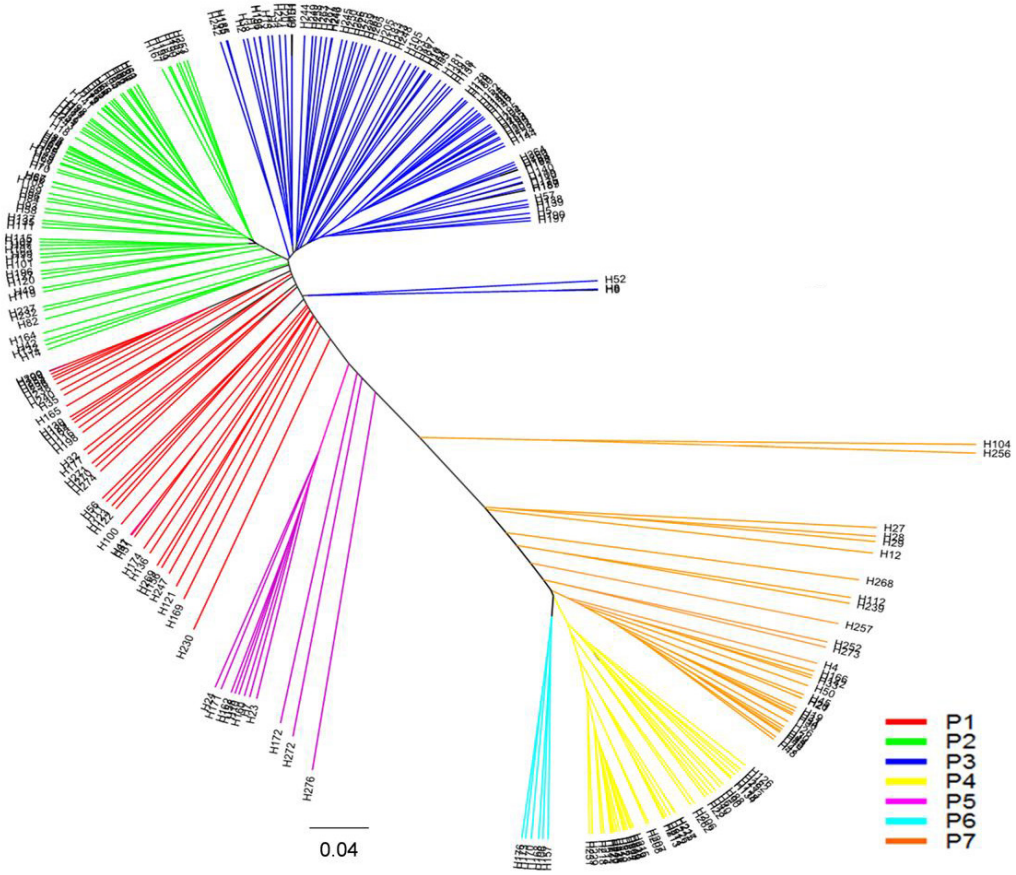

Figure 2. Unrooted neighbor-joining trees of 276 accessions based on Nei's genetic distance.

Association analysis

We performed association mapping of SSR markers with blast resistance to seven races (ZA_7, ZB_{19}, ZC_1, ZD_{13}, ZE_3, ZF_1, ZG_1) in 2012 and 2013 using the MLM implemented in TASSEL. A total of 31 marker-trait associations were identified by 26 different SSR markers ($P < 0.01$; Figure 3), and the phenotypic variation ranged from 2.68 to 13.11% (Table 1). In 2012, four SSR markers were associated with resistance to ZA_7, three SSR markers were associated with resistance to ZF_1, and one SSR marker was associated with resistance to ZB_{19},

ZC_1, ZD_{13}, ZE_3, and ZG_1, respectively. In 2013, two markers were associated with resistance to ZA_7, four markers were associated with resistance to ZD_{13}, seven markers were associated with resistance to ZE_3, three markers were associated with resistance to ZF_1, and one marker was associated with resistance to ZB_{19}, ZC_1, and ZG_1, respectively. Despite the significant associations between markers and traits, the only consistent association over the 2 years was between RM27289 and resistance to ZA_7.

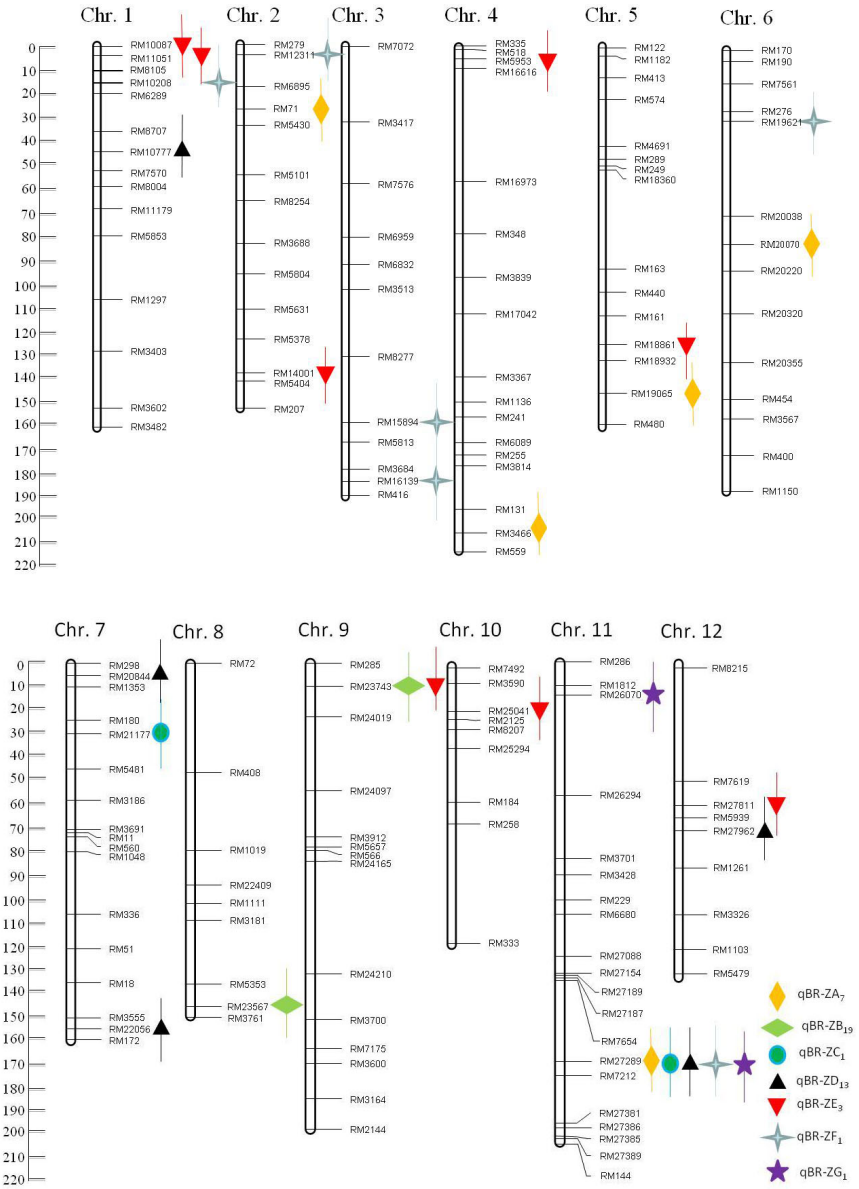

Figure 3. Chromosome locations of QTLs conferring resistance to *Magnaporthe oryzae* in *indica* landraces.

Table 1. Association of simple sequence repeat markers with blast resistance in 2012 and 2013.

Race	Year	Locus	Chr.	Physical position (Mb)	P	R^2	References for linkage mapping in adjacent regions
ZA_7	2012	RM71	2	8.7	0.00993	0.08071	(Pan et al., 1998)
		RM3466	4	33.8	0.009146	0.054088	(Talukder et al., 2004)
		RM19065	5	27.2	0.002542	0.056356	(Sallaud et al., 2003)
		RM27289	11	26.8	0.001413	0.072803	(Wang et al., 2009)
	2013	RM20070	6	16.4	0.005569	0.058135	(Jeung et al., 2007)
		RM27289	11	26.8	7.94E-07	0.131078	(Wang et al., 2009)
ZB_{19}	2012	RM23567	8	27.4	0.005069	0.059453	(Liu et al., 2005)
	2013	RM23743	9	2.7	0.007707	0.028055	(Kinoshita and Kiyosawa, 1997)
ZC_1	2012	RM27289	11	26.8	5.33E-04	0.082068	(Wang et al., 2009)
	2013	RM21177	7	5.7	0.002193	0.077224	★
ZD_{13}	2012	RM27289	11	26.8	0.007278	0.057407	(Wang et al., 2009)
	2013	RM10777	1	12.3	0.009051	0.063797	★
		RM20844	7	0.7	9.90E-04	0.082848	★
		RM22056	7	26.7	0.009231	0.038764	(Talukder et al., 2004)
		RM27962	12	12.1	4.38E-04	0.073929	(Nakamura et al., 1997)
ZE_3	2012	RM10087	1	1.6	0.001161	0.049574	(Hayashi et al., 2010)
	2013	RM11051	1	19	1.53E-04	0.110789	★
		RM14001	2	35.5	0.003928	0.043128	(Chen et al., 2003; Xu et al., 2004)
		RM5953	4	9.4	0.007851	0.047688	(Tabien et al., 2002)
		RM18861	5	23.1	0.009601	0.02681	(Bagali et al., 2000)
		RM23743	9	2.7	9.40E-04	0.041865	(Kinoshita and Kiyosawa, 1997)
		RM25041	10	3.9	3.47E-04	0.078908	★
		RM27811	12	7.4	4.45E-04	0.087661	(Imbe and Matsumoto, 1985)
ZF_1	2012	RM10208	1	3.9	0.006192	0.075989	(Xu et al., 2004)
		RM19621	6	6.2	0.001654	0.085127	(Sallaud et al., 2003; Xu et al., 2004)
		RM27289	11	26.8	1.07E-04	0.096955	(Wang et al., 2009)
	2013	RM12311	2	0.1	0.007007	0.040316	(Pan et al., 1999)
		RM15894	3	30.2	0.007055	0.039772	(Bagali et al., 2000)
		RM16139	3	34.4	0.008985	0.037118	★
ZG_1	2012	RM27289	11	26.8	0.008792	0.054732	(Wang et al., 2009)
	2013	RM26070	11	2.4	0.006735	0.062987	★

★New loci were identified in this study.

The number of SSR markers associated with each type of blast resistance varied from one to three in most cases; however, four markers were associated with resistance to ZA_7 blast in 2012 and seven with resistance to ZE_3 blast in 2013. In contrast, one marker could be associated with resistance to several races of blast. For example, RM23743 on chromosome 9 was associated with both ZB_{19} and ZE_3 resistance in 2013, and RM27289 on chromosome 11 was associated with resistance to five races of blast (ZA_7, ZC_1, ZD_{13}, ZF_1, and ZG_1) in 2012. Among the 26 marker-trait associations, 19 markers were located in the same regions as previously identified resistance on QTLs (Table 1), and seven markers (RM10777, RM11051, RM16139, RM21177, RM20844, RM25041, and RM26070) were identified in this study.

DISCUSSION

With advantages including co-dominance, high polymorphism, and high relative abundance with uniform genome coverage, SSR markers have been widely applied to explore the origin, evolution, and genetic variation within living beings. In this study, a total of 689 alleles were detected with 160 SSR markers, which were randomly distributed across the genome of a panel of 276 accessions, with the average number of alleles per locus being 4.31, ranging from 2 (RM13538) to 8 (RM10920). This is consistent with the 3.9 alleles per

locus previously identified in 416 rice accessions, most of which were collected in China, with 100 SSR markers (Jin et al., 2010), and 5 alleles per locus, which were detected in 170 rice accessions from a putative mini-core collection of Chinese germplasms with 132 SSR and InDel markers (Wen et al., 2009). However, the value obtained in the present study was lower than the 7.7 alleles per locus detected in 247 rice accessions from a mini-core collection of Japanese rice landraces using 32 SSR markers, the 11.9 alleles per locus detected in 236 rice accessions from 23 countries with 60 SSR markers, and the 13.47 alleles per locus identified by 70 SSR markers in 217 rice accessions selected from the United States Department of Agriculture (USDA) Rice Genebank (Agrama et al., 2009). To reduce the impact of population structure on phenotypic variation, only *indica* landraces were selected in the present study, which may explain why the number of alleles per locus was lower than that found in other studies. *Indica* and *japonica* subspecies have been shown to exist independently of the genetic structure, and these two subspecies represent most rice cultivars produced worldwide. Differentiation of two subspecies is the main form of genetic differentiation among Asian cultivated rice.

The main constraint of crop association mapping is unidentified population substructure and admixture. Genetic loci that do not have an effect on the trait may demonstrate statistical significance for their co-segregation with a trait of interest when the population structure is affected by factors such as adaptation or domestication (Flint-Garcia et al., 2003). Some false associations between phenotypes and RAPD markers in rice have been detected in uninspected population structures (Virk et al., 1996). Populations are assumed to be in Hardy-Weinberg equilibrium in model-based inferences of population structure. Therefore, to eliminate the effects of population structure and to ensure the accuracy of the association between genetic polymorphisms and traits in the present study, a model-based method was adopted, using data on 60 unlinked SSR alleles in order to determine the genetic structure among all 276 samples. The results showed that the 276 samples could be divided into seven subpopulations, which is consistent with clustering based on genetic distance. The *indica* landrace accessions used in this study originated from broad geographical regions and had a complex breeding history, which involved intercrossing and introgression between germplasms from diverse backgrounds. This may explain why the results of the present study coincided fairly well with the rice genetic structure as previously documented (Jin et al., 2010).

Association analysis is a new approach that can be used to identify genes that control important traits. This approach has been successfully applied to identify genes related to quantitative traits in crop species and is more powerful than linkage analysis and QTL-mapping methods (Myles et al., 2009). In an association analysis of seed protein content in 48 soybean germplasm accessions with 150 SSR markers, 11 putative QTLs were identified based on highly significant markers, and nine of these QTLs were in regions that had been previously reported (Jun et al., 2008). Using 218 SSR markers in a set of 48 rice germplasms from the Chinese core collection, 20 associations were identified between SSR markers and the concentrations of 29 metabolites (Lou et al., 2011). In this study, the association between SSR markers and blast resistance was analyzed, and the results showed that 26 SSR markers were significantly associated with blast resistance in both 2012 and 2013 ($P < 0.01$). Furthermore, 19 markers that were associated with blast resistance in this study coincided with previously reported trait-associated SSR markers from linkage analysis and QTL mapping studies in various experimental populations, which is useful when interpreting the association results (Kraakman et al., 2004). For example, RM27289 on chromosome 11 was located near the region of *Pik* clustering (including *Pi1*, *Pi7*, *Pi18*, *Pi44*, *Pik*, *Piks*, *Pikm*, *Pikh*, *Pikg*, and *Pikp*),

which was found to be associated with resistance to five blast isolates (ZA_7, ZC_1, ZD_{13}, ZF_1, ZG_1) in 2012 and only one consistent association with ZA_7 resistance over the 2 years of this experiment. Seven markers associated with blast resistance were newly identified in this study. Once these markers have been shown to be effective across different genetic backgrounds under different environmental conditions, they may help breeders to pyramid QTLs from diverse sources through MAS in rice blast-resistance-breeding programs.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by grants from the National Key Project for Transgenic Crops (#2016ZX08009-003-001, #2014ZX08009-001B), the Natural Science Foundation of China (#31171516), the Fundamental Research Funds for the Central Universities (#KYZ201302), and the Jiangsu Agriculture Science and Technology Innovation Fund (#CX(12)1003-4, #CX(12)1003-6, #CX(15)1054). We would like to thank Prof. Chen Zhi-Yi and Prof. Liu Yong-Feng for kindly providing all the blast isolates.

REFERENCES

Agrama H, Yan WG, Lee F, Fjellstrom R, et al. (2009). Genetic assessment of a mini-core subset developed from the USDA Rice Genebank. *Crop Sci.* 49: 1336-1346. http://dx.doi.org/10.2135/cropsci2008.06.0551

Ashkani S, Rafii MY, Sariah M, Siti Nor Akmar A, et al. (2011). Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genet. Mol. Res.* 10: 1345-1355. http://dx.doi.org/10.4238/vol10-3gmr1331

Ballini E, Morel JB, Droc G, Price A, et al. (2008). A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant Microbe Interact.* 21: 859-868. http://dx.doi.org/10.1094/MPMI-21-7-0859

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, et al. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434: 980-986. http://dx.doi.org/10.1038/nature03449

Falush D, Stephens M and Pritchard JK (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

Flint-Garcia SA, Thornsberry JM and Buckler ES, 4th (2003). Structure of linkage disequilibrium in plants. *Annu. Rev. Plant Biol.* 54: 357-374. http://dx.doi.org/10.1146/annurev.arplant.54.031902.134907

Fukuoka S and Okuno K (2001). QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice. *Theor. Appl. Genet.* 103: 185-190. http://dx.doi.org/10.1007/s001220100611

Guo L, Guo W, Zhao H, Wang J, et al. (2015). Association mapping and resistant alleles' analysis for japonica rice blast resistance. *Plant Breed.* 134: 646-652. http://dx.doi.org/10.1111/pbr.12310

Hossain M and Fischer K (1995). Rice research for food security and sustainable agricultural development in Asia: achievements and future challenges. *GeoJournal* 35: 286-298. http://dx.doi.org/10.1007/BF00989136

Huang X, Wei X, Sang T, Zhao Q, et al. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nat. Genet.* 42: 961-967. http://dx.doi.org/10.1038/ng.695

Jin L, Lu Y, Xiao P, Sun M, et al. (2010). Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theor. Appl. Genet.* 121: 475-487. http://dx.doi.org/10.1007/s00122-010-1324-7

Jun TH, Van K, Kim MY, Lee SH, et al. (2008). Association analysis using SSR markers to find QTL for seed protein content in soybean. *Euphytica* 162: 179-191. http://dx.doi.org/10.1007/s10681-007-9491-6

Kawasaki-Tanaka A and Fukuta Y (2014). Genetic variation in resistance to blast disease (*Pyricularia oryzae* Cavara) in Japanese rice (*Oryza sativa* L.), as determined using a differential system. *Breed. Sci.* 64: 183-192. http://dx.doi.org/10.1270/jsbbs.64.183

Kraakman AT, Niks RE, Van den Berg PM, Stam P, et al. (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics* 168: 435-446. http://dx.doi.org/10.1534/genetics.104.026831

Liu K and Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128-2129. http://dx.doi.org/10.1093/bioinformatics/bti282

Lou Q, Ma C, Wen W, Zhou J, et al. (2011). Profiling and association mapping of grain metabolites in a subset of the core collection of Chinese rice germplasm (*Oryza sativa* L.). *J. Agric. Food Chem.* 59: 9257-9264. http://dx.doi.org/10.1021/jf201602g

McCouch SR, Teytelman L, Xu Y, Lobos KB, et al. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res.* 9: 199-207. http://dx.doi.org/10.1093/dnares/9.6.199

Myles S, Peiffer J, Brown PJ, Ersoz ES, et al. (2009). Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell* 21: 2194-2202. http://dx.doi.org/10.1105/tpc.109.068437

Nei M, Tajima F and Tateno Y (1983). Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J. Mol. Evol.* 19: 153-170. http://dx.doi.org/10.1007/BF02300753

Pritchard JK, Stephens M and Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Roy-Chowdhury M, Jia Y, Jackson A, Jia MH, et al. (2012). Analysis of rice blast resistance gene *Pi-z* in rice germplasm using pathogenicity assays and DNA markers. *Euphytica* 184: 35-47. http://dx.doi.org/10.1007/s10681-011-0481-3

Salvi S and Tuberosa R (2005). To clone or not to clone plant QTLs: present and future challenges. *Trends Plant Sci.* 10: 297-304. http://dx.doi.org/10.1016/j.tplants.2005.04.008

Shi X, Wang J, Bao Y, Li P, et al. (2010). Identification of the quantitative trait loci in japonica rice landrace Heikezijing responsible for broad-spectrum resistance to rice blast. *Phytopathology* 100: 822-829. http://dx.doi.org/10.1094/PHYTO-100-8-0822

Tian Z, Qian Q, Liu Q, Yan M, et al. (2009). Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. *Proc. Natl. Acad. Sci. USA* 106: 21760-21765. http://dx.doi.org/10.1073/pnas.0912396106

Virk PS, Ford-Lloyd BV, Jackson MT, Pooni HS, et al. (1996). Predicting quantitative variation within rice germplasm using molecular markers. *Heredity (Edinb)* 76: 296-304. http://dx.doi.org/10.1038/hdy.1996.43

Wang C, Yang Y, Yuan X, Xu Q, et al. (2014). Genome-wide association study of blast resistance in indica rice. *BMC Plant Biol.* 14: 311. http://dx.doi.org/10.1186/s12870-014-0311-6

Wang JF, He XJ, Zhang HS and Chen ZY (2002). Genetic analysis of blast resistance in *japonica* rice landrace heikezijing from Taihu region. *Yi Chuan Xue Bao* 29: 803-807.

Wang Y, Wang D, Deng X, Liu J, et al. (2012). Molecular mapping of the blast resistance genes *Pi2-1* and *Pi51(t)* in the durably resistant rice 'Tianjingyeshengdao'. *Phytopathology* 102: 779-786. http://dx.doi.org/10.1094/PHYTO-03-12-0042-R

Wen W, Mei H, Feng F, Yu S, et al. (2009). Population structure and association mapping on chromosome 7 using a diverse panel of Chinese germplasm of rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 119: 459-470. http://dx.doi.org/10.1007/s00122-009-1052-z

Yu J, Pressoir G, Briggs WH, Vroh Bi I, et al. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* 38: 203-208. http://dx.doi.org/10.1038/ng1702

Zenbayashi K, Ashizawa T, Tani T and Koizumi S (2002). Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. *Theor. Appl. Genet.* 104: 547-552. http://dx.doi.org/10.1007/s00122-001-0779-y

Supplementary material

Table S1. Phenotypic data and basic information of 276 rice accessions used in the present study.

Table S2. Summary statistics for the 160 SSR markers used in the present study.