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Identification of interspecific heterotic loci associated with agronomic traits in rice introgression lines carrying genomic fragments of *Oryza glaberrima*

Tondi Yacouba Nassirou · Wenchuang He · Caijin Chen · Adedze Y. M. Nevame · Athanase Nsabiyumva · Xilong Dong · Yilong Yin · Quanqin Rao · Wei Zhou · Han Shi · Wubin Zhao · Deming Jin

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Abstract In the present study, a set of 79 rice introgression lines (ILs) carrying variant introgressed segments of African rice (*Oryza glaberrima* Stued.) was used to identify quantitative trait loci (QTL) and heterotic loci (HL) associated with 7 agronomic traits. A total of 180 polymorphic markers between the donor and recurrent parents were found and 115 markers were used to identify the segregation of the introgression fragments. Based on the genotypic data of the ILs and test variety GZ63S as well as the phenotypic data of the IL and PIH, QTLs and HLs can be mapped on introgressed chromosome segments. One representative marker on each specific introgressed segment was defined as a QTL or a HL. 24 QTLs associated with six agronomic traits were mapped on 9 chromosomes and 23 interspecific HLs for seven agronomic traits were identified on 10 chromosomes in 2 years. Among them, 22 QTLs and 19 HLs were found to be associated with 5 yield-related traits respectively. The PIH (F1) testcross population showed superiority in most yield-related traits and was characterized by a high frequency of overdominant interspecific HLs. In addition, the pleiotropism was found in 5 marker loci for 11 QTLs associated with five agronomic traits and 4 marker loci for ten interspecific HLs for all the seven traits. This study is the first attempt for the identification of interspecific HLs between the two cultivated rice species, Asian rice (*Oryza sativa* L.) and African rice (*O. glaberrima* Steud.). Therefore, our results may help to lay the foundation for exploring the genetic mechanism of interspecific heterosis in rice.

Keywords Agronomic traits · Heterosis · Heterotic loci · Interspecific hybrid · *Oryza glaberrima* · *Oryza sativa*

Abbreviations

DH	Days to heading
FG	Filled grain per panicle
GD	Genetic diversity
GGT	Graphical genotypes
GW	Grains weight
HL	Heterotic loci
IL	Introgression line
LOD	Logarithm of odds ratio
MPH	Mid-parent heterosis
OD	Over-dominance
PN	Panicles per plant
PD	Partial-dominance
PIH	Partial interspecific hybrid

T. Y. Nassirou · W. He · C. Chen · A. Nsabiyumva · X. Dong · Y. Yin · Q. Rao · W. Zhou · H. Shi · W. Zhao · D. Jin (✉)
MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, 430070 Wuhan, China
e-mail: djin@mail.hzau.edu.cn

A. Y. M. Nevame
State Key Laboratory of Rice Biology, China National Rice Research Institute, 310006 Hangzhou, China

PH	Plant height
P/TGMS	Photo/thermo-sensitive genetic male sterility
PIC	Polymorphism information content
QTL	Quantitative trait loci
SN	Spikelets per panicle
SS	Seed setting rate
SSR	Simple sequence repeats

Introduction

African rice (*O. glaberrima* Steud.), an annual plant, is one of the two cultivated rice species. It is thought to have been domesticated 2000–3000 years ago in the inland delta of the Upper Niger River (Linares 2002). Now it is still sporadically grown by Saramaccan Maroons both for food and ritual uses (Van Andel 2010). Similar to the Asian rice or common rice (*O. sativa* L.), *O. glaberrima* belongs to the AA genome, having 12 chromosomes with an estimated size of 358 Mb, but can be clearly distinguished by its morphologic characteristics like much shorter, rounded ligules, spikelets that are pear-shaped with two prominent sterile lemmas at two sides, red bran, straight panicles with only primary branches (Linares 2002; Jin and Nassirou 2015). Because of the relatively lower yield potential of *O. glaberrima*, its cultivation is much less widespread than that of *O. sativa*; Nevertheless, it was characterized by traits that are unique for this rice species, such as significant weed competitiveness, drought tolerance and ability to respond to low input conditions (Ghesquière et al. 1997; Linares 2002; Sarla and Swamy 2005; Nassirou and He 2011). Scientists from the Africa Rice Center (ex. WARDA) have developed a group of interspecific cultivars called NERICA (New Rice for Africa) by crossing African rice and Asian rice varieties (Semagn et al. 2007). To develop interspecific lines between *O. sativa* and *O. glaberrima*, various methods have been used, including hybridization followed by several backcrosses with *O. sativa* as recurrent parent. In this way, many genetic populations termed with recombinant inbred line (RILs), introgression lines (ILs), and chromosome segment substituted lines (CSSLs) have been derived from the interspecific crosses (Lorieux et al. 2000; Aluko et al. 2004; Sarla and Swamy 2005; Ikeda et al. 2009; Efisue et al. 2009; Bimpong et al. 2010; Gutierrez et al. 2010; Adedze et al. 2012; Jin et al. 2012; Ndjiondjop et al. 2012). And *O. glaberrima* cytoplasm was also transferred into the genetic background of the *O. sativa* ssp *indica* rice to create a new CMS (Huang et al. 2012). All these results suggested that the *O. glaberrima* is a valuable germplasm for enriching genetic diversity in rice. However, the utilization of *O. glaberrima* in *O. sativa* breeding is usually hampered by reproductive isolation including sexual incompatibility and interspecific hybrid sterility. Advances made in large-scale genomic research (Wing et al. 2005; Piffanelli et al. 2007; Sasaki 2003; Wang et al. 2014) have provided extremely useful tools to overcome those barriers and the opportunity to rationalize and monitor introgressions between the two cultivated rice species by tagging and mapping both desirable and undesirable genes in *O. glaberrima* (Ghesquière et al. 1997), and also the opportunity for further distant heterosis exploitation. Attempts at exploiting distant heterosis have been made in rice heterosis breeding; and encouraging progress has been achieved in both intersubspecific heterosis between two subspecies of Asian rice (*O. sativa* L.), ssp. *indica* and ssp. *japonica*, and interspecific heterosis between two cultivated species, Asian rice and African rice (*O. glaberrima* Steud.) (Jin and Nassirou 2015).

The magnitude of heterosis of a hybrid mainly depends on the genetic diversity between its parents. Therefore, exploiting interspecific heterosis between African rice and Asian rice is a promising way for raising yield potential of rice crops. Jones (1926) was the first to report heterosis in rice, by observing marked increase in tiller number and grain yield in some F1 hybrids in comparison to their parents. Since then, hybrid vigor has been reported for various agronomic traits such as yield, grain weight, spikelets per panicle, panicles per plant, plant height, days to flowering, tiller number per plant, etc. (Virmani et al. 1981; Jones et al. 1998; Hu et al. 2002; Cheng et al. 2007; Jaikishan et al. 2010; Jin and Nassirou 2015). Determining the genetic basis of agronomic traits is regarded as a very important scientific problem for crop improvement (Pasam et al. 2012). The useful heterosis can be positive or negative depended on the breeding objectives. In general positive heterosis is required for grain yield while negative for earliness of growth period (Brandle and McVetty 1990). Heterosis is mainly attributed to genetic diversity. The detection of stable heterotic loci with positive effects on yield-related agronomic traits could be of great value in heterotic rice breeding

programs. Moreover, with the help of modern molecular technology and appropriate segregating populations, scientists can dissect the genetic basis of heterosis at the single-locus level. The term "heterotic loci" (HL) was put forward for the first time (Hua et al. 2003) and they developed a new method to dissect the genetic basis of heterosis directly. Since then, genetic dissection of heterosis based on HL has been reported in many crops. However, for the genetic analysis of heterosis, it is critical to have an appropriate experimental design and materials. Several experimental designs have been adopted to study the genetic basis of heterosis, such as F2, DH, RIL, and IF2 populations (Yu et al. 1997; Zhuang et al. 2001; Syed et al. 2005; Luo et al. 2009; Ma et al. 2009; Li et al. 2010; Hu et al. 2012). Introgression lines (ILs) and single segment substitution lines (SSSLs) have also been widely used to elucidate the molecular basis of interesting traits, QTL appraisal, fine mapping, QTL cloning, heterotic utilization and improvement of cultivated varieties in many crops such as rice (Xin et al. 2011; Zhao et al. 2011; Dai et al. 2012; Wang et al. 2013; Shen et al. 2014; Wang et al. 2015), maize (Wang et al. 2007; Feng et al. 2012; Wei et al. 2015), tomato (Monforte and Tanksley 2000) and cotton (Guo et al. 2013). Compared with other mapping populations, ILs has a simple genetic background, high efficiency in HL detection, and can be repeated conveniently. Using ILs, the heterotic loci can be analyzed directly in the Mendelian genetic model, so mapping results are more reliable.

Here we present the first use of ILs, derived from crosses between the two cultivated rice species, to identify interspecific heterotic loci (HL), for seven agronomic traits, to explain the genetic basis of heterosis of *O. glaberrima* × *O. sativa* mode of inheritance, in addition to quantitative trait loci (QTL) mapping. This research provided an effective strategy to investigate heterosis directly, and will considerably influence ongoing research to explore the genetic basis of heterosis between the two cultivated rice.

Materials and methods

Material preparation

The seventy nine introgression lines (ILs) used in this study, carried variant genomic fragments of African rice in the genetic background of Asian rice. The ILs were developed by an interspecific crossing between an *O. glaberrima* variety, RAM3 as the donor parent and an *O. sativa indica* variety Jin23 (recurrent parent) followed by 1 or 2 backcrossing and generations of selfing (RAM3/Jin23//Jin23///Jin23F6 for IL1 to IL44 and RAM3/Jin23//Jin23F10 for IL45 to IL79). All the 79 ILs performed as morphologically stable inbred lines.

In our study, in the rice winter seasons of 2013 and 2014, crosses were made using the 79 ILs as pollen parents containing donor DNA fragments, and GZ63S, a Photo/Thermo-sensitive Genetic Male Sterility line (P/TGMS), *indica* variety as female parent, in Hainan Experimental Station of Huazhong Agricultural University (Latitude 18°33′12″, Longitude 110°3′23″, Lingshui site, Hainan province, P. R. China). A total of 79 ILs/GZ63S partial interspecific hybrids (PIHs) were obtained.

Evaluation of agronomic traits and mid-parent heterosis

All the 79 ILs, GZ63S and their correspondent 79 PIHs were grown in Wuhan, Huazhong Agricultural University (HZAU), [30°28′17″N, 114°21′56″W, Wuhan, Hubei province, P.R. China], in the summer rice seasons of 2013 and 2014. A randomized complete block design with two replications was used, each plot two rows of 11 plants with 15 cm × 20 cm spacing. Normal agricultural practices were applied for field management.

Seven agronomic traits, i.e. panicles per plant (PN), spikelets per panicle (SN), filled grain per panicle (FG), 1000 grains weight (GW), plant height (PH), seed setting rate (SS) and days to heading (DH) were evaluated. The middle five plants of each plot were sampled for estimating panicle/plant, spikelet/panicle, and grain number/panicle, the average value of the total panicles, spikelets, and filled grains of the five representative plants collected from each IL, GZ63S and PIH lines, respectively. Only full grains were counted as filled grains. Seed setting rate was calculated following this formula: (SS (%) = FG/SN × 100). GW was measured by weighing 1000 seeds. DH is the time from seed sowing up to 50% of the flowered plants.

Mid-parent heterosis (MPH%) was calculated using the equation (MPH% = (F1 − MP)/MP × 100%), where F1 is the value of each testcross hybrid of ILs

and GZ63S and MP is the mean value of corresponding parents (IL and GZ63S) for the same measured trait.

Correlation and ANOVA analyses were conducted using Analysis Of Variance (AOV), a new option in the January 2016 updated version of QTL-Icimapping.

Genotyping and polymorphism analysis

The extracted DNA was used to perform polymerase chain reactions (PCR) in a total reaction volume of 20 µl that consisted of 2 µl (20 ng) DNA, 2 µl 10× PCR buffer (50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 50% glycerol and 10 mM Tris–HCl, pH 8.3), 0.4 µl of each of the forward and reverse primers, 0.4 µl dNTP and 0.2 µl Taq DNA polymerase and complete with ddH_2O. All primers were amplified using a Touchdown protocol with amplification conditions as follows: 95 °C for 5 min, 15 cycles of 94 °C for 45 s, 65 °C for 45 s with a reduction of 0.5 °C per cycle, 72 °C for 1 min, followed by 25 cycles of 95 °C for 5 min, 50 °C for 45 s, 72 °C for 1 min, with a final step of 72 °C for 10 min. Amplified products were then separated on 6% denaturing poly-Acrylamide gels and stained with silver nitrate following the silver staining protocol.

The software package Graphical GenoTypes.2 = GGT.2 (http://www.dpw.wau.nl/pv/pub/ggt) (Van Barloo 1999) was used to perform a molecular profiling statistical analysis, to determine the extent of the *O. glaberrima* genomic fragments introgressed in the ILs. This software analyses assumed distances among markers in such a way that a chromosomal segment flanked by two markers derived from the donor parent (D'D") is considered to contain 100% donor DNA across the region, while a chromosomal segment flanked by two markers of recipient type (R'R") is considered as 100% recurrent parent DNA, and a chromosomal segment flanked by one marker of donor type and one marker of recipient type (DR) is considered to be a recombinant, with donor DNA extending approximately half way across the interval and recurrent parent DNA across the other half (Young and Tanksley 1989). The average number of alleles per locus was calculated using PowerMarker V3.25 (Liu and Muse 2005). The linkage map was constructed using MapDraw V2.1 (Liu and Meng 2003).

The genotypes of 79 ILs and their parents Jin23 and RAM3 as well as testing variety GZ63S were directly evaluated. SSR markers were screened for polymorphism among the parents RAM3, Jin23 and GZ63. The specific genetic band patterns of the African rice accession RAM3 were scored as R and the specific band patterns of Jin23 were scored as J, while the specific bands of GZ63S were scored as G, respectively.

Identification of QTLs and interspecific HLs

The standard *t* test is not suitable for non-idealized ILs population carrying several introgression segments from the donor parent. A likelihood ratio test based on stepwise regression (RSTEP-LRT) is a suitable method to detect HL of non-idealized ILs (Guo et al. 2013; Wang et al. 2015). Thus, the RSTEP-LRT method was used in this study to detect QTL and HL with the software QTL-Icimapping 4.1 (Wang et al. 2016) (http://www.isbreeding.net) (Institute of Crop Science, Academy of Agricultural Sciences, China). A logarithm of odds ratio (LOD) threshold of 2.0 was used to declare significant loci. On the basis of the genotype structure of the IL population, QTLs or HLs can be mapped on introgressed chromosome segments.

For the heterotic loci (HL), it is assumed that homozygous loci do not contribute to heterosis at the single locus level, and that heterosis arises due to the effect of heterozygous loci. Thus, only loci showing a significant difference between the F1 (heterozygote) and the mean of the two parents (homozygotes) were considered to be HLs (i.e., HL is the QTL for heterosis) (Hua et al. 2003; Xin et al. 2011; Guo et al. 2013). And the mid-parent heterosis of the traits was used as basis for phenotypic data input.

In order to isolate the interspecific heterosis from the mixed heterosis including both inter- and intra-heterosis in PIHs, a specific dataset of SSR alleles was created with 'A' stands for African rice allele in RAM3 scored as R, 'B' for Asian rice allele in Jin23 scored as J. In this case, each 'A' detected indicated a homozygous locus RR in ILs and could represent an interspecific heterozygous locus in the PIH IL/GZ63S (R/G), while each 'B' indicated a homozygous locus JJ in ILs and could be homozygous locus or an intraspecific heterozygous (J/G) in the PIH. Therefore, only if a'A' has significantly higher effects than that of the 'B' on the heterosis of a trait can be detected as a interspecific HL. Thus we can estimate the effects of interspecific heterozygous loci associated with the heterosis of PIHs.

According to the experimental design of the test-cross hybrid population, each HL theoretically had

only dominance effect (D). The additive effect (A) of the same locus was computed from partial interspecific hybrid (PIH) testcross F1 s performance (A + D). The testcross PIH F1 trait measurements were used to identify the loci affecting PIH performance. Additive and dominance effects calculated using the performance data at the exact genomic location of the HL. The ratio of D/A for a given HL reflects the degree of dominance, a ratio greater than 1 indicates over-dominance (OD) of the HL, a ratio less than 1 indicates partial-dominance (PD), of the HL, and equal to 1 indicates complete dominant effects, respectively (Tang et al. 2010; Wei et al. 2015).

Results

Performance of the seven agronomic traits in the ILs, GZ63S and PIHs

There were large variations in the performances of the seven agronomic traits among the ILs, GZ63S and the PIHs in 2 years (Table 1; Fig. 1). The mean value of SN were 243.35 and 233.31 spikelets per panicle for the ILs and 201.67 and 193.00 spikelets per panicle for GZ63S, in 2013 and 2014, respectively, with ranges of 130.33–402.00 and 135.00–389.17 spikelets per panicle, respectively for the ILs. While, the mean values of SN were 308.05 (ranging 193.67–526.00) and 290.83 (ranging 218.83–415.33) spikelets per panicle for the PIH population in 2013 and 2014, respectively, apparently higher than both the parents of the ILs and GZ63S. The mean values of PH were also much higher for the PIH (125.37 and 123.84 cm) than the ILs (111.03 and 110.41 cm) and GZ63S (86.33 and 86.63 cm) in the two years. The mean values of SS of the PIHs are similar to those of the ILs but much higher than the female parent (GZ63S) which had very low SS value due to the expression of male sterility in summer season. The mean values of GW of the PIHs were higher than those of the ILs but lower than those of GZ63S. However, the mean values of PN are similar for the PIHs and the ILs but lower than those of GZ63S.

Heterosis performance of the PIHs

The heterosis performances of the seven agronomic traits of the PIHs (ILs/GZ63S) population showed variations between the two years (Table 2). The PIHs performed positive heterosis in SN, SS, FG and PH but negative heterosis in PN and DH. Among all traits, FG showed the highest MPH of 96.10 and 109.74%, ranging from −32.19 to 216.70% and from 44.41 to 233.05%, in 2013 and 2014, respectively; followed by SS (51.19 and 61.04%), SN (25.95 and 36.02%) and PH (26.38 and 26.09%) in the 2 years respectively. Negative MPH was detected for PN (−32.62 and −33.21%), ranging from −58.49 to 12.57% and from −46.39 to −7.57%, in 2013 and 2014, respectively. While no apparent MPH was observed on GW, with mean values of 0.51 and −0.71% in the two years, respectively.

Correlations between the agronomic traits and genotype-environment interaction

To determine whether there are relationships among agronomic traits and if they are also maintained at the heterosis level, correlation analyses on both phenotypic data and MPH of the PIH in the 2 years were performed (Table 3). For example, significant positive correlations between SN and FG ($r = 0.8343, 0.6957$; $P < 0.001$), FG and SS ($r = 0.4328$, 0.4267; $P < 0.001$), SN and PH ($r = 0.2370$, 0.5263; $P < 0.05$, 0.001), and DH and PH ($r = 0.2989$, 0.3840; $P < 0.01$, 0.001) was detected in the 2 years, respectively. Similar correlations were also detected between the MPH of these traits. Significant positive correlations among the MPH of SN and FG ($r = 0.7933$, 0.6776; $P < 0.001$), SN and PH ($r = 0.3523$, 0.3991; $P < 0.01$, 0.001), and DH and PH ($r = 0.3274$, 0.3714; $P < 0.01$, 0.001) were detected in the two years, respectively. Significant negative correlation was found between SN and SS ($r = -0.4789, -0.4905$; $P < 0.001$). Different correlations could be detected between the same pairs of traits in different years.

Variance analysis (ANOVA) was also performed a procedure in the statistical software IciMapping (Table 4) for detecting the effects of genotype (G) and environment (E) factors and the interaction effects between genotype and environment (G × E) in the ILs/GZ63S PIH population for six traits. Significant effects of both genotypes and environments were detected for most of traits. For example, the F-values of genotypes were significant at $P < 0.01$ level for SN, SS and PH, and significant at $P < 0.05$ for FG and PN.

Table 1 Performance statistics of parents and F1 partial interspecific hybrids

Traits*	ILs		GZ63S	ILs/GZ63S (PIH)	
	Range	Mean ± SD	Mean	Range	Mean ± SD
SN	130.33–402	243.35 ± 60.52	201.67	193.67–526	308.05 ± 70.55
	135–389.17	233.31 ± 51.69	193	218.83–415.33	290.93 ± 41.6
SS (%)	52.01–95.17	85 ± 8.41	13.64	56.38–94.91	75.73 ± 9.35
	52.96–96.33	84.64 ± 7.94	8.98	56.7–92.82	76.83 ± 8.52
FG	116.33–356.98	205.32 ± 49.73	27.5	109.19–433.49	232.03 ± 55.43
	124–336	196.25 ± 41.96	17.33	159.5–319.33	221.61 ± 29.05
PN	7.6–22.2	12.37 ± 2.77	20.67	7.33–20	11.98 ± 2.34
	7.33–14.33	10.16 ± 1.53	23.33	8.33–15	10.73 ± 1.36
PH (cm)	80.67–140.8	111.03 ± 10.83	86.33	105–141	125.37 ± 7.71
	80.67–139	110.41 ± 10.82	86.33	104–135.67	123.84 ± 7.04
GW (g)	18–35	23.55 ± 2.91	27.7	22–28.8	25.52 ± 1.48
	18.95–30	23.4 ± 2.27	28.4	23–28.9	25.56 ± 1.36
DH (d)	63–75	71.65 ± 3.27	75	68–75	70.46 ± 3.11
	63–75	71.68 ± 3.29	75	68–75	70.46 ± 3.19

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight, *DH* days to heading

Min–Max minimum and maximum, *SD* standard deviation, *cm* centimeter, *g* gram, *d* day, *%* seed setting percentage

* Each trait values correspond to data recorded in 2013 (upper) and 2014 (lower), respectively

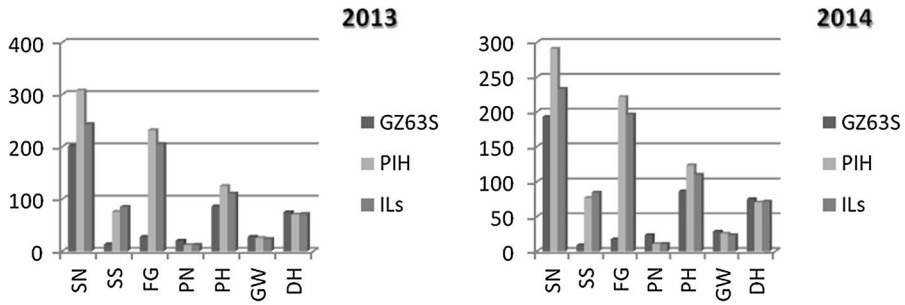


Fig. 1 The mean values of the seven agronomic traits in ILs, GZ63S and the F1 partial interspecific hybrids. *PIH* partial interspecific hybrids, *SN* spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight and *DH* days to heading

Table 2 Mid-parent heterosis of the F1 partial interspecific hybrids

2014			2015	
Traits	Range	Mean ± SD	Range	Mean ± SD
SN	−33.64–88.64	25.95 ± 27.34	5.04–125.03	36.02 ± 18.4
SS (%)	9.78–107.46	51.19 ± 19.71	24.13–126.06	61.04 ± 18.09
FG	−32.19–216.7	96.1 ± 52.82	44.41–233.05	−6.51 ± 8.41
PN	−58.49–12.57	−32.62 ± 13.64	−46.39 to −7.57	−33.21 ± 7.97
PH (cm)	5.81–51.68	26.38 ± 7.98	8.52–52.89	26.09 ± 7.54
GW (g)	−23.72–18.14	0.51 ± 8.39	−13–10.12	−0.71 ± 4.1
DH (d)	−15.56–15.38	−6.47 ± 8.45	−15.56–15.38	109.74 ± 41.46

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight, *DH* days to heading

The F-values of environment were significant at $P < 0.01$ level for SN, FG and PH, and significant at $P < 0.05$ for PN. No significant G × E interaction was detected for the traits except GW ($P < 0.05$). The results showed although both the genotype and environment components had an important effect on phenotypic variation of these traits, their interactions had relatively little contribution.

Table 3 Correlation coefficients for phenotype and heterosis of the seven agronomic traits of the F1 partial interspecific hybrids

Traits	SN	SS	FG	PN	PH	GW	DH
SN		−0.0165	0.8343***	0.0754	0.2370*	−0.1671	0.1472
		−0.2299*	0.6957***	−0.0526	0.5263***	−0.3000**	0.2794*
SS	−0.4789***		0.4328***	−0.0186	−0.2836*	0.2054	−0.0325
	−0.4905***		0.4267***	0.0668	−0.1607	0.0041	0.0581
FG	0.7933***	0.1394		0.0082	0.1656	−0.1123	0.0838
	0.6776***	0.3014**		−0.0865	0.4003***	−0.1701	0.1862
PN	−0.0141	0.1016	0.043		0.0624	0.0138	0.137
	0.0584	0.1341	0.1734		−0.0654	−0.0756	0.2594*
PH	0.3523**	−0.1856	0.2751*	0.1733		−0.0022	0.2989**
	0.3991***	−0.0646	0.3779***	0.2472*		−0.0039	0.3840***
GW	−0.127	0.2233*	−0.004	−0.0422	0.0593		−0.172
	−0.4346***	−0.0502	−0.5007***	−0.1784	−0.1537		−0.3740***
DH	0.2653*	−0.1607	0.2166	0.1563	0.3274**	−0.0819	
	0.3350**	−0.1496	0.2639*	0.1225	0.3714***	−0.3024**	

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight, *DH* days to heading

*, **, *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Correlation coefficients for the seven agronomic traits and mid-parent heterosis of each trait are listed down and above of the diagonal, respectively, and values correspond to coefficients in 2013 (upper) and 2014 (lower), respectively

Table 4 Two-way analysis of variance (ANOVA) of F1 PIH population (F-value)

SOD	SN	SS	FG	PN	PH	GW
Genotype (G)	5.005**	5.3259**	2.2028*	2.7028*	16.5375**	1.4177
Environment (E)	8.3789**	0.051	5.2483**	3.127*	7.1581**	0.2642
G × E	0.5956	0.3409	0.4316	0.2724	1.8415	1.7603*

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight and *DH* days to heading

*, ** Significance at 0.05 and 0.01 respectively; G × E = environment-genotype interaction

Detecting the *O. glaberrima* genomic fragments in the ILs

Initially a polymorphism analysis was conducted between the two parents (Jin23 and RAM3) of the ILs and GZ63S. One hundred and eighty out of the 420 simple sequence repeats (SSR) primers screened were polymorphic. The number of polymorphic markers per chromosome varied from 8 on chromosomes 11 to 22 on chromosome 6, with an average of 15 per chromosome. The population of ILs was then genotyped with the 180 polymorphic SSR markers, in which 115 markers (63.9%) showed distinct readable bands. Genetic band patterns from African rice (RAM3) and Asian rice (Jin23) showed introgressions over 12 chromosomes in almost all ILs. Jin23 alleles were detected at all 115 marker loci in three or more ILs but only 91 loci (79.13%) showed introgressions from RAM3 in at least one IL.

A graphical representation of the introgression lines was drawn using Graphical GenoType 2.0 software and showed the consensus alleles on each of the 12 chromosomes and variant distributions were observed (Fig. 2). The extent of *O. glaberrima* introgressions on each chromosome was estimated with map distances between markers in centi-Morgan (cM). More than 2 alleles were detected in 22 SSR loci, in which 3, 4 and 5 alleles were detected in 19, 1 and 2 loci, respectively. The average number of alleles per locus was 2.25.

A graphical representation of the introgression lines was drawn using Graphical GenoType 2.0 software and showed the consensus alleles on each of the 12 chromosomes and variant distributions were observed (Fig. 2). The extent of *O. glaberrima* introgressions on each chromosome was estimated with map distances between markers in centi-Morgan (cM). The coverage rate of *O. glaberrima* genome is 79.13% (1288.00 cM/1627.7 cM) in the ILs. The average proportion of *O. glaberrima* alleles varied from 14.19% on chromosome 8 to 43.2% on chromosome 9. The average proportion of the genome containing *O. glaberrima* fragments in the ILs was 19.31% (314.31 cM/1627.7 cM), varying from 12 to 29.1%, and that of the *O. sativa* genome was 74.28% (1209.05 cM/1627.7 cM) varying from 59.5 to 85.8%. The frequency of heterozygosity ranged from 0.3 to 5.9% per line, with an average of about 0.52% per line (8.5 cM/1627.7 cM). One or more non-parental alleles were detected at 66 ILs in a total of 22 SSR loci. The frequency of non-parental alleles varied from 0.4 to 9.2% per line, with an average of 3.98% per line (64.8 cM/1627.7 cM).

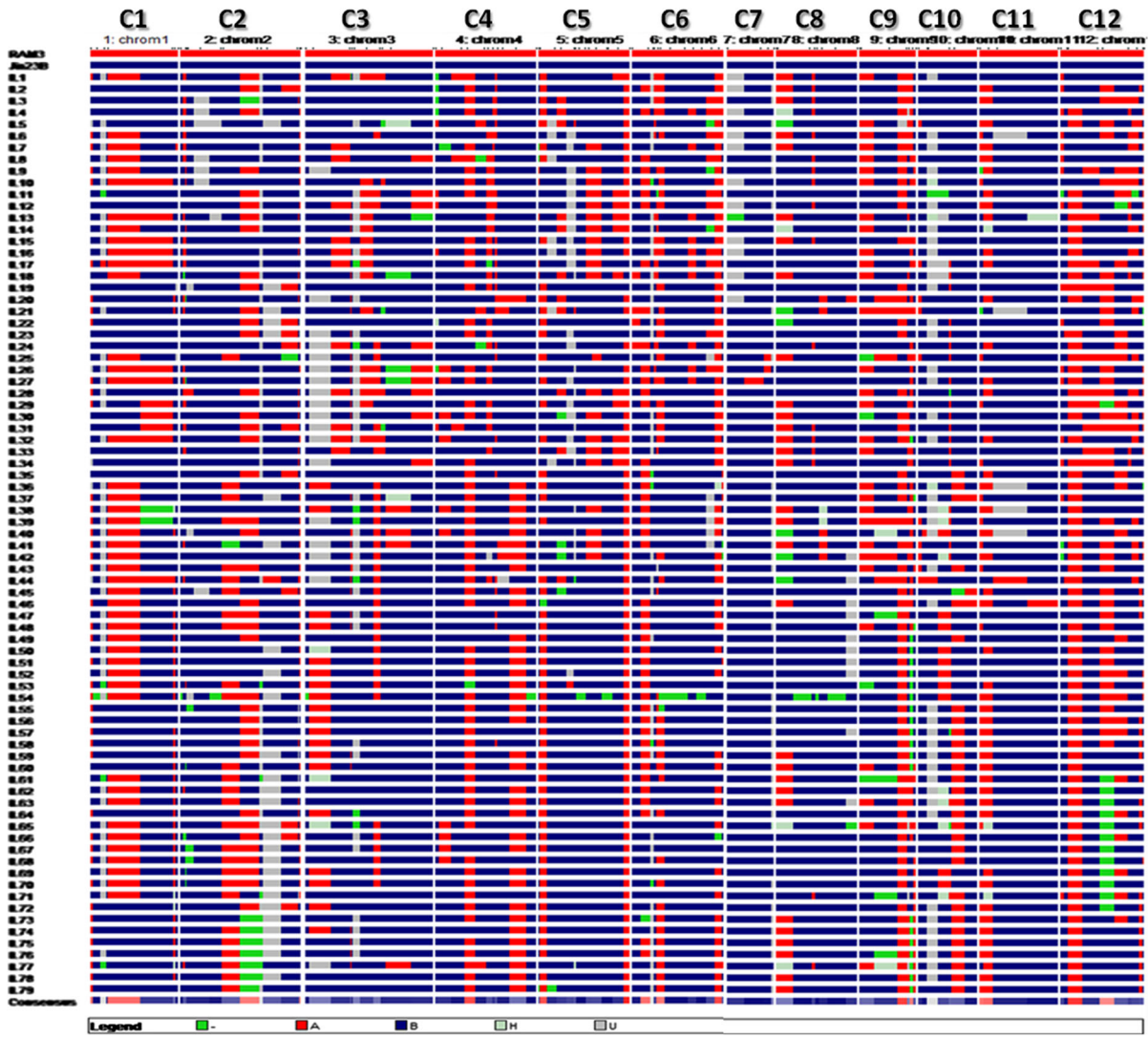


Fig. 2 Graphical representation of the 12 chromosomes in the 79 introgression lines showing the genomic proportion inherited from each of the parents.*A *Red color* represents the donor parent RAM3 (*O. glaberrima*) genomic portion, *B Blue color* represents the recurrent parent Jin23B (*O. sativa*) genomic portion; the light ones are *H* the heterozygote portions, -missing data, *U* unknown allele portions are represented by *green and gray color* respectively. *C1–C12* chromosome1 to chromosome12. (Color figure online)

Table 5 Detected QTLs associated with agronomic traits in the ILs

Traits	2013					
	QTL*	Marker	Chr	LOD	PV%	ADD
SN	*qSN-3-1*[a]	RM5626	3	2.95	15.79	23.94
SS	*qSS-4*	RM241	4	2.59	11.69	−6.51
	qSS-7	RM47	7	3.76	17.58	−11.15
FG	*qFGa-2*[b]	RM110	2	2.49	10.75	−17.70
	qFG-3-1	RM5626	3	2.07	9.91	15.58
	qFG-4[a]	RM1812	4	2.23	10.63	24.09
PN	*qPN-1*	RM212	1	4.65	25.27	1.40
PH	*qPH-2*[a,b]	RM3763	2	2.24	10.21	−15.38
	qPH-4[a]	RM1018	4	2.05	9.82	−3.75
GW	*qGW-1-1*[b]	RM265	1	2.79	11.91	4.47
	qGW-4-1	RM252	4	3.11	13.41	1.53
Traits	2014					
	QTL	Marker	Chr	LOD	PV%	ADD
SN	*qSN-3-2*	RM168	3	2.04	10.14	34.08
	qSN-3-1[a]	RM5626	3	2.01	9.50	20.41
	qSN-3-3	RM81A	3	2.22	10.37	55.65
	qSN-4	RM1812	4	2.20	12.33	34.69
SS	*qSS-5*	RM5818	5	3.32	15.27	6.85
	qSS-10	RM7492	10	3.63	16.87	3.82
FG	*qFG-3-2*	RM81A	3	2.02	10.07	45.74
	qFG-4[a]	RM1812	4	2.37	13.07	22.21
PN	*qPN-9*	RM3249	9	3.06	16.53	0.86
PH	*qPH-2*[a,b]	RM3763	2	2.03	9.81	−15.06
	qPH-4[a]	RM1018	4	2.28	10.14	−3.81
GW	*qGW-1-2*[b]	RM212	1	2.14	10.05	−1.13
	qGW-3	RM5626	3	2.31	10.39	−0.78
	qGW-4-2	RM1018	4	3.20	17.36	1.12
	qGW-5[b]	RM7293	5	2.24	10.53	1.62
	qGW-6	RM3498	6	2.42	10.74	−1.36
	qGW-9[b]	RM278	9	2.02	9.06	0.73

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight, *DH* days to heading, *LOD* logarithm of odds, *PV* percentage of phenotypic variance explained by the QTL, *chr* chromosome

[a] Loci associated with quantitative trait locus (QTL) identified in 2 years

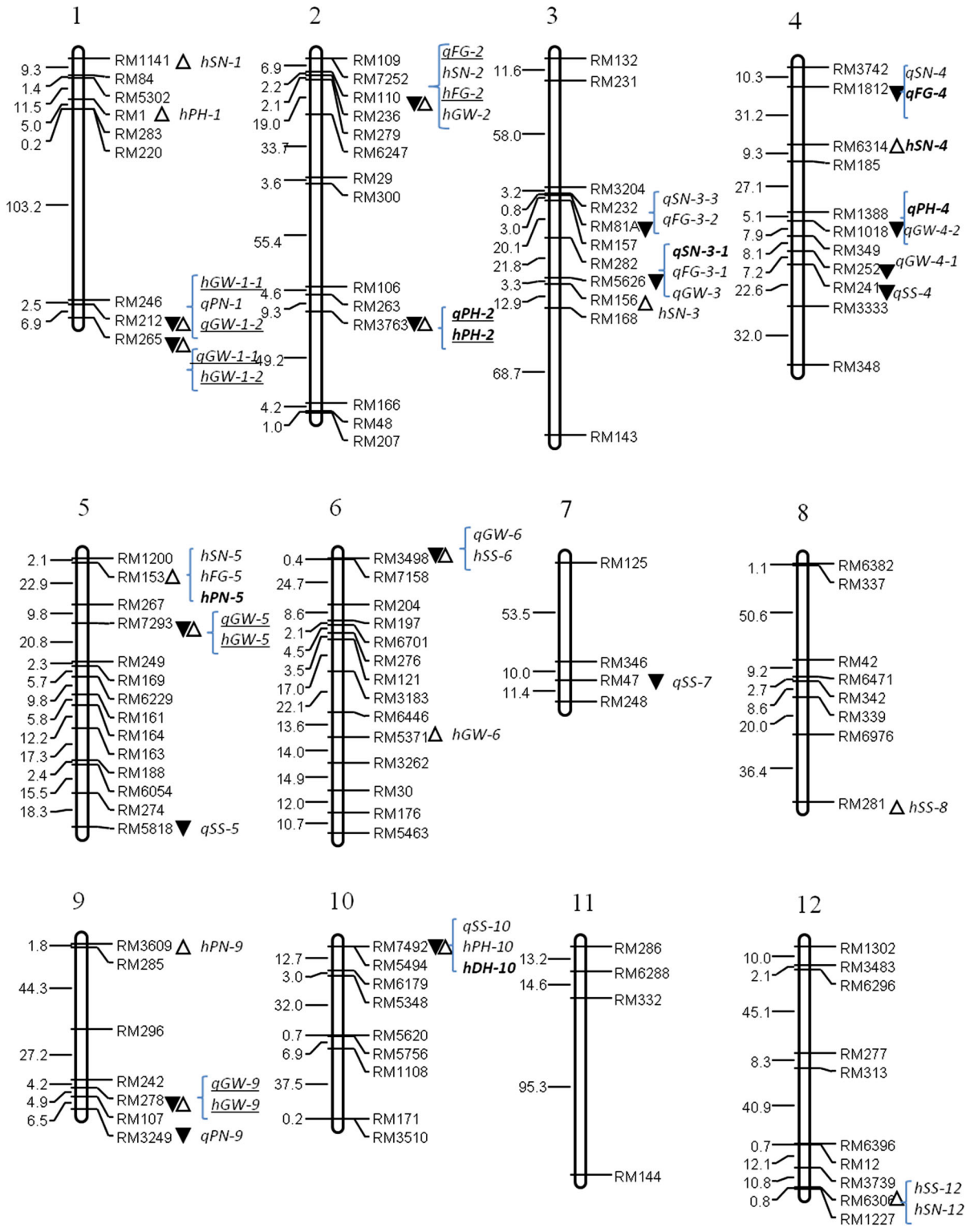
[b] Loci which coincided with HL at the same position and in same trait analysis

* QTL nomenclature: *q* QTL, trait name followed by chromosome number (1, 2… if multiples on same chromosome)

Mapping QTLs associated with the agronomic traits in the ILs

A total of 24 QTLs associated with six agronomic traits were mapped on 9 chromosomes in the two years (Table 5; Fig. 3). Among them, 22 QTLs were found to be associated with five yield-related traits. Four QTLs (*qSN-3-1,qFG-4,qPH-2 and qPH-4*) were identified in both years.

Four QTLs associated with SN were detected. One QTL *qSN-3-1* was detected on chromosome 3 in both years accounting for 15.79 and 9.50% of phenotypic variation with positive additive effects in the two years, respectively. The other 3 QTLs were all found


1
9.3 RM1141 △ hSN-1
1.4 RM84
11.5 RM5302
5.0 RM1 △ hPH-1
0.2 RM283
RM220
103.2
2.5 RM246 hGW-1-1 qPN-1 qGW-1-2
6.9 RM212 ▼△
RM265 ▼△ qGW-1-1 hGW-1-2
2
6.9 RM109 qFG-2 hSN-2
2.2 RM7252
2.1 RM110 ▼△ hFG-2 hGW-2
19.0 RM236
RM279
33.7 RM6247
3.6 RM29
RM300
55.4
4.6 RM106
9.3 RM263
RM3763 ▼△ qPH-2 hPH-2
49.2
4.2 RM166
1.0 RM48
RM207
3
11.6 RM132
RM231
58.0
3.2 RM3204 qSN-3-3
0.8 RM232 qFG-3-2
3.0 RM81A ▼
20.1 RM157 qSN-3-1
21.8 RM282 qFG-3-1
3.3 RM5626 ▼ qGW-3
12.9 RM156 △ hSN-3
RM168
68.7
RM143
4
10.3 RM3742 qSN-4
RM1812 ▼ qFG-4
31.2
9.3 RM6314 △ hSN-4
RM185
27.1
5.1 RM1388 qPH-4
7.9 RM1018 ▼ qGW-4-2
8.1 RM349
7.2 RM252 ▼ qGW-4-1
22.6 RM241 ▼ qSS-4
RM3333
32.0
RM348
5
2.1 RM1200 hSN-5
22.9 RM153 △ hFG-5 hPN-5
9.8 RM267
RM7293 ▼△ qGW-5 hGW-5
20.8
2.3 RM249
5.7 RM169
9.8 RM6229
5.8 RM161
12.2 RM164
17.3 RM163
2.4 RM188
15.5 RM6054
18.3 RM274
RM5818 ▼ qSS-5
6
0.4 RM3498 ▼△ qGW-6 hSS-6
24.7 RM7158
8.6 RM204
2.1 RM197
4.5 RM6701
3.5 RM276
17.0 RM121
22.1 RM3183
13.6 RM6446
RM5371 △ hGW-6
14.0 RM3262
14.9 RM30
12.0 RM176
10.7 RM5463
7
RM125
53.5
RM346
10.0 RM47 ▼ qSS-7
11.4 RM248
8
1.1 RM6382
RM337
50.6
9.2 RM42
2.7 RM6471
8.6 RM342
20.0 RM339
RM6976
36.4
RM281 △ hSS-8
9
1.8 RM3609 △ hPN-9
RM285
44.3
RM296
27.2
4.2 RM242 qGW-9
4.9 RM278 ▼△ hGW-9
6.5 RM107
RM3249 ▼ qPN-9
10
12.7 RM7492 ▼△ qSS-10 hPH-10 hDH-10
3.0 RM5494
RM6179
32.0
0.7 RM5620
6.9 RM5756
RM1108
37.5
0.2 RM171
RM3510
11
13.2 RM286
14.6 RM6288
RM332
95.3
RM144
12
10.0 RM1302
2.1 RM3483
RM6296
45.1
8.3 RM277
RM313
40.9
0.7 RM6396
12.1 RM12
10.8 RM3739 hSS-12
0.8 RM6306 △ hSN-12
RM1227

◀**Fig. 3** Chromosomal location of the agronomic traits related heterotic loci (HL) and quantitative trait loci (QTL). The name of marker is given on the *left side* of each chromosome. *Open traingle* stands for HL. *Filled inverted triangle* stands for QTL. For the traits abbreviations see Table 1; In *Bold* Loci associated with quantitative trait locus (QTL) or Heterotic loci (HL) identified in 2 years; *Underline* Loci which coincided with HL and QTL at the same position and for same trait analyzed. 1–12 chromosome 1 to chromosome 12

only in 1 year (2014), 2 QTLs on chromosome 3 and 1 QTL on chromosome 4, accounted for 10.14, 10.37, and 12.33% of phenotypic variation with positive additive effects, respectively.

Four QTLs were detected for SS in one of the two years, 2 QTLs detected in 2013 on chromosomes 4 and 7 explaining 11.60 and 17.58% of phenotypic variation with negative additive effects, respectively; and

Table 6 Heterotic loci (HL) for the seven agronomic traits using the mid-parent heterosis data

Traits	HL*	Marker	Chr	Year	LOD	PV %	D	PIH F1(A + D)	D/A	Mode of effect
SN	*hSN-1*	RM1141	1	2013	2.06	11.30	−9.17	−7.52	−5.56	OD
	hSN-2	RM110	2	2013	2.17	12.44	10.47	2.03	1.24	OD
	hSN-3	RM156	3	2013	2.02	11.88	18.88	38.79	0.95	D
	hSN-4[a]	RM6314[a]	4	2013	2.00	9.99	22.46	31.54	2.47	OD
			4	2014	2.05	10.65	0.09	29.77	0.003	
	hSN-5	RM153	5	2014	2.05	10.46	0.05	3.61	0.01	PD
	hSN-12	RM6306	12	2014	2.12	11.17	-0.04	−2.02	−0.002	
SS	*hSS-8*	RM281	8	2013	2.09	10.67	28.43	3.01	1.12	OD
	hSS-6	RM3498	6	2014	2.38	14.04	0.05	0.42	0.12	PD
	hSS-12	RM6306	12	2014	2.09	10.42	0.04	−0.28	0.12	PD
FG	*hFG-2*[b]	RM110[b]	2	2013	2.21	12.58	20.33	6.46	1.47	OD
	hFG-5	RM153	5	2014	3.24	17.60	−0.07	5.20	−0.01	PD
GW	*hGW-1-1*[b]	RM212[b]	1	2013	2.03	10.42	2.72	0.23	1.09	OD
	hGW-1-2[b]	RM265[b]	1	2013	2.06	10.82	−12.27	−0.87	−1.08	OD
	hGW-2	RM110	2	2013	3.90	19.11	−3.98	−0.49	−1.14	OD
	hGW-5[b]	RM7293[b]	5	2013	2.04	10.68	−3.85	−0.33	−1.09	OD
	hGW-6	RM5371	6	2013	2.01	10.41	−4.05	−0.32	−1.09	OD
	hGW-9[b]	RM278[b]	9	2013	2.05	10.74	−2.74	0.11	−0.96	D
PN	*hPN-5*[a]	RM153[a]	5	2013	2.13	11.95	5.14	1.15	1.29	OD
			5	2014	2.14	11.94	0.10	0.84	0.14	PD
	hPN-9	RM3609	9	2014	2.12	11.22	−0.09	−0.20	0.84	PD
PH	*hPH-2*[a,b]	RM3763[a,b]	2	2013	2.40	13.05	12.81	1.16	1.10	OD
			2	2014	2.39	12.98	0.07	1.94	0.04	PD
	hPH-1	RM1	1	2014	2.01	10.08	−0.06	−10.07	−0.01	PD
	hPH-10	RM7492	10	2014	2.34	12.75	−0.02	−1.83	−0.01	PD
DH	*hDH-10*[a]	RM7492[a]	10	2013	2.01	9.48	−2.59	−1.14	−1.79	OD
			10	2014	2.01	9.69	−0.03	−1.14	−0.02	PD

SN spikelets per panicle, *SS* seed setting rate, *FG* filled grain per panicle, *PN* panicles per plant, *PH* plant height, *GW* 1000 grains weight and *DH* days to heading

[a] Loci associated with Heterotic Loci (HL) identified in two years

[b] Loci which coincided with QTL at the same position and in same trait analysis

* HL nomenclature: *h* HL, trait name followed by chromosome number (1, 2… if multiples on same chromosome); *D* dominance effect from MPH values; *PIH F1(A + D)* additive and dominance effects from the performance values of the testcross partial interspecific hybrids F1; *D/A* Ratio of dominance to the absolute value of additive effect; *LOD* logarithm of odds; *PV* percentage of phenotypic variance explained by the HL; *chr* chromosome; *OD* overdominance; *D* dominance; *PD* partial-dominance

the other 2 QTLs in 2014 on chromosomes 5 and 10 accounting for 15.27 and 16.87% of phenotypic variation with positive additive effects, respectively.

There were four QTLs identified for FG in which one (*qFG-4*) was detected in both years on chromosome 4 explaining 10.63 and 13.07% of phenotypic variation with positive additive effects in the 2 years, respectively. Two QTLs were found in 2013 on chromosomes 2 and 3, accounting for 10.75% of phenotypic variation with negative additive effects and 9.91% of phenotypic variation with positive additive effects, respectively. And one QTL found in 2014 on chromosome 3 with 10.07% of phenotypic variations with positive additive effects.

Two QTLs associated with PN were detected in each of the two years, one in 2013 on chromosome 1 and one in 2014 on chromosome 9, accounting for 25.27 and 16.53% of phenotypic variations with positive additive effects, respectively.

Two QTLs (*qPH-2* and *qPH-4*) associated with PH were identified in both years on chromosomes 2 and 4 accounting for 10.21 and 9.82% of phenotypic variations in 2013 and 9.81 and 10.14% of phenotypic variations in 2014, respectively. Both QTLs had negative additive effects.

There were eight QTLs identified for GW. Two QTLs detected in 2013 on chromosomes 1 and 4 accounted for 11.91 and 13.41% phenotypic variation with positive additive effects, respectively. Six QTLs found in 2014 on chromosomes 1, 3, 4, 5, 6 and 9 accounting for 10.05, 10.39, 17.36, 10.53, 10.34, and 9.06% of phenotypic variation, respectively. The three QTLs on chromosomes 1, 3 and 6 had negative additive effects, while the others three QTLs had positive additive effects.

Identification of interspecific HLs in the PIHs

A total of 23 interspecific HLs for the seven agronomic traits were identified on 10 chromosomes in the 2 years (Table 6; Fig. 3). Among them, 19 HLs were found for five yield-related traits, respectively and 2 HLs (*hSN-4* and *hPN-5*) were identified in both years.

There were six HLs for SN found in the two years. One stable HL (*hSN-4*) was detected on chromosome 4 in both years, accounting for 9.99 and 10.65% of phenotypic contribution of MPH with overdominant effect, respectively. Three HLs were detected on chromosomes 1, 2, and 3 in 2013 accounting for 10.30, 12.44 and 11.88% of phenotypic contribution with overdominant or dominant effects. Two HLs were detected on chromosomes 5 and 12 in 2014 accounting for 10.46 and 11.17% of phenotypic variation with partial dominant effect.

Three HLs were identified for SS, one HL on chromosome 8 in 2013 and two HLs in 2014 on chromosomes 6 and 12, explaining 10.67, 14.04 and 10.42% of phenotypic contribution of MPH with overdominant or partial dominant effect, respectively.

Two HLs were found to be associated with FG. One HL was detected on chromosome 2 in 2013 and another HL on chromosome 5 in 2014, explaining 12.58 and 17.60% of phenotypic variation with overdominant or partial dominant effect respectively.

Six HLs associated with GW were detected in 2013. Two HLs were on chromosome 1 and the rest HLs were located on chromosomes 2, 5, 6 and 9, accounting for 10.42, 10.82, 19.11, 10.68, 10.41, and 10.74% of phenotypic variations with overdominant or dominant effect, respectively.

There was also two HLs identified for PN. One stable HL (*hPN-5*) were detected on the chromosome 5 in both years, accounting for 11.95 and 11.94% of phenotypic contribution of MPH with overdominant and partial dominant effect, respectively. Another HL was found in 2014 on chromosome 9 with 11.22% of contribution to the phenotypic variation with partial dominant effect.

There are also HLs found for agronomic traits not directly related to yield. Three HLs were detected for PH in the two years. A stable HL (*hPH-2*) on chromosome 2 was found in both years, accounting for 12.1 and 12.98% of phenotypic variation with overdominant and partial dominant effect, respectively. The other two HLs were detected in 2014 on chromosomes 1 and 10 explaining 10.08 and 12.75% of phenotypic contribution to MPH with partial dominant effects. One stable HL (*hDH-10*) for DH was detected in both years on chromosome 10, explaining 9.48 and 9.69% of phenotypic contribution to MPH with overdominant and partial dominant effect, respectively. In general, the PIH (F1) testcross population showed superiority in most yield-related traits and was characterized by a high frequency of overdominant interspecific HLs.

Because the test variety GZ63S expressed male sterility and had a very low seed setting rate resulted from open pollination, the MPH of seed setting rate (SS) and filled grain number (FG) in the PIHs could be overestimated. The overestimation of the MPH of SS and FG could affect the 5 HLs concerning the two

traits. However, the overestimation of the MPH data was a systematic error which could be minimized by the statistic method employed.

Pleiotropism of the QTLs and the HLs

The phenomenon of pleiotropism was found in 5 marker loci for 11 QTLs associated with five agronomic traits and 4 marker loci for 10 interspecific HLs for all the seven traits. The pleiotropism effect was observed in QTLs. For example, RM5626 on the chromosome 3 was simultaneously associated with SN (*qSN-3-1*), FG (*qFG-3-1*), and GW (*qGW-3*). And four markers, RM212, RM81A, RM1812 and RM1018 on chromosomes 1, 3, 4 and 4 were each associated with QTLs for two traits respectively. Pleiotropism was also found in the HLs. There were 2 markers associated with HLs for 3 traits and 2 markers associated with HLs for 2 traits, respectively. For example, RM110 on chromosome 2 was found to be associated with the MPH of SN (*hSN-2*), FG (*hFG-2*), GW (*hGW-2*) and RM153 on chromosome 5 associated with the MPH of SN (*hSN-5*), FG (*hFG-5*) and PN (*hPN-5*), respectively (Table 6).

In addition, the same marker locus may account for both QTL and HL associated with the same trait. Six QTLs were found to be consistent with corresponding HLs for the same trait, i.e. RM110 associated with FG (*qFG-2* and *hFG-2*), four markers such as RM212 etc. associated with GW (*qGW-1-2* and *hGW-1-1* etc.). And the RM3763 associated with PH (*qPH-2* and *hPH-2*) was detected in both years of 2013 and 2014.

Discussion

Potential of distant heterosis in rice

The use of heterosis plays a major role in strategies for increasing the productivity of crops. Since the heterosis of hybrids is generally positively correlated to the genetic diversity of the parents, the heterosis of distant crosses including intersubspecific and interspecific crosses is theoretically higher than that of the intrasubspecific crosses (Jin and Nassirou 2015).

Although the observed vegetative vigor of the hybrids is generally positively correlated to the genetic diversity between the parents, the grain yield of distant crosses is usually not satisfactory due to the low seed set rate caused by reproductive isolation. For example, the typical intersubspecific hybrids between indica and japonica rice are semi-sterile, while the interspecific hybrids between Asian rice (*O. sativa* L.) and African rice (*O. glaberrima* Steud.) are almost completely sterile. Apparently, hybrid sterility is a major obstacle for exploiting the distant heterosis in rice.

Remarkable progress has been achieved in the intersubspecific hybrid rice breeding in China in recent years. Chinese rice breeders have adopted a more effective approach to overcome the reproductive barrier by producing partial intersubspecific hybrid rice such as Yongyou6, Yongyou12 and Yongyou15 released by the Ningbo Academy of Agricultural Sciences, rather than simply depending on the wide compatible genes (Ikehashi and Araki 1986; Qiu et al. 2005).

A more intensive and complex reproductive isolation including both prezygotic and postzygotic barriers exists in the crosses between *O. sativa* and *O. glaberrima* (Sano et al. 1986). Several major genes responsible for the interspecific hybrid sterility between the two species have been detected from *O. glaberrima* (Heuer and Miezan 2003; Hu et al. 2006; Li et al. 2011). No effective wide-compatibility gene has been found to overcome the interspecific reproductive isolation. The hybrid sterility is a major constraint for exploiting the interspecific heterosis in two cultivated rice species (Adedze et al. 2012).

The interspecific reproductive isolation also caused a problem for this study, i.e. it affected the free segregation of the offsprings of the interspecific cross and resulted in a non-random distribution of the introgressions in the ILs population. This leads to the appearance of some similar introgressions in a few ILs, such as IL6, IL7 and IL8. To cope with the non-randomized IL population, we used a statistical method with a likelihood ratio based on stepwise regression (RSTEP-LRT) that can be used more effectively for QTL mapping in non-idealized populations (Guo et al. 2013; Wang et al. 2015, 2016). This method could achieve similar results equivalent to the standard t-test with idealized population such as a randomized population of CSS lines containing a single chromosome segment from the donor parent.

The mechanism of the interspecific heterosis

Heterozygosity is the basis of heterosis. A locus showing significant difference in heterosis between

the heterozygote and the mean of the two homozygotes was considered to be a HL (Hua et al. 2003). Many genetic analysis of crop heterosis have been assessed to identify heterotic loci (HL) previously in cotton (Shen et al. 2014), maize (Feng et al. 2012; Wei et al. 2015) and rice (Luo et al. 2011; Xin et al. 2011; Wang et al. 2015). Several different types of populations have been developed to investigate heterotic loci, such as recombinant inbred lines (RILs), near-isogenic lines (NILs), introgression lines (ILs), and chromosome segment substitution lines (CSSLs), and immortalized F2 (Hua et al. 2003), etc. For example, Wang et al. (2013) reported 53 heterosis loci with significant effects identified in rice, using 66 CSSLs and their 66 corresponding F1 plants with the recurrent parent; Xin et al. (2011) reported 41 HLs contributing to heterosis of six yield-related traits by comparing performance among 70 ILs and their testcross hybrids population.

Exploiting the interspecific heterosis between *O. sativa* and *O. glaberrima* is a new approach for breeding high yielding hybrid rice (Jin and Nassirou 2015). The present study is the first attempt for the identification of interspecific heterotic loci between the two cultivated rice species. A locus showing a significant difference between the interspecific heterozygote locus and the mean of its two corresponding homozygote loci is considered to be an interspecific HL. Twenty three HLs associated with six agronomic traits were identified in the 2 years of 2013 and 2014, using the MPH of PIHs and the genotype data of the IL population as entry data for the analysis, respectively. Four HLs, *hPH-2*, *hSN*-4, *hPN-5* and *hDH-10* were repeatedly detected in both 2013 and 2014 (Table 6), on chromosomes 2, 4, 5 and 10, respectively, and all the four HLs had positive dominant effect on their associated traits.

Classical genetic theories of heterosis are divided into three categories: dominance hypothesis (Davenport 1908): overdominance hypothesis (Hull 1945) and epistatic hypothesis (Powers 1944). In this study, each HL's gene action type was characterized, and the 23 HL revealed three different genetic effects, namely, partial-dominant, dominant and overdominant. Some HLs showed different effects in the two years. Among them 13 loci (56.52%) concerning all these traits showed overdominant effects, indicating that overdominance resulted from intra-locus interaction could be a major type of effects contributed to the interspecific heterosis besides epistasis. These results are consistent with many previous studies (Semel et al. 2006; Shen et al. 2014; Wang et al. 2015; Wei et al. 2015).

Both dominant or overdominant effects of intra-locus interactions (Tang et al. 2010) and epistatic effects of inter-locus interactions (Hua et al. 2003) have been reported as the main cause for intra-specific heterosis in rice. In this study, 6 HLs were located in the same position as a corresponding QTL for the same trait. However, 17 HLs were not found at the same location with QTL for the same trait. It might show that trait phenotypes and their heterosis could be governed by different sets of loci. We can assume that the 6 HLs located at the same position as corresponding QTLs associated with the same agronomic traits mainly have dominant or overdominant effects of intra-locus interactions. The remaining 17 HLs located at different regions to the QTLs associated with the same agronomic traits mainly have epistatic effects of inter-locus interactions. And both intra- and inter-locus interactions could contribute to interspecific heterosis.

The interspecific heterosis could have a very complex mechanism. Its expression may be further conditioned by various factors including genetic background and environments. It is a new challenge to identify favorable interspecific HLs between the two cultivated rice. The 23 HLs, especially the 4 stable HLs detected in this study could be useful as candidate loci contributing to the interspecific heterosis for the future study on the genetic mechanism. Further fine mapping and functional study on HLs for agronomic traits will facilitate interspecific heterosis breeding programs between the two cultivated rice.

The extent of the proportion of *O. glaberrima* genome fragments in the ILs

Development of introgression lines is a promising approach to distinguish *O. glaberrima* favorable alleles from unfavorable ones in genetic background of *O. sativa.* Knowledge about the proportions and contribution of the parent genome within the introgression lines (ILs) by the way of the molecular markers provide useful information on the selection and development of the varieties. The microsatellite markers used in this study are representative and have a good distribution along the 12 rice chromosomes.

These SSR markers used are considered to be very appropriate in molecular genetics studies because they are co-dominant with multiple alleles and very polymorphic even among very closely linked subjects.

In this study, we attempted to target the introgression of the parental genome within the ILs. It would be more interesting if the useful genes of the *O. glaberrima* (resistance to local constraints) were introgressed as it's a donor parent with poor agronomic performance and only used to introduce a particular trait to the *O. sativa* parent (high yielding ability). In general in crosses between the two cultivated rice species (African and Asian rice), a small fragments of *O. glaberrima* in the genetic pool of *O. sativa* seems more favorable for the assessment of genetic diversity. Previous efforts deployed by various studies to transfer the useful genes of *O. sativa* into the cytoplasm of *O. glaberrima* achieved limited success due to the sterility barriers between the two species (Agnoun et al. 2012; Fukuta et al. 2012).

Recurrent backcrossing programs are planned on the assumption that the proportion of recurrent parent genome is recovered at a rate of $1-(1/2)^{t+1}$ for each of t generations of backcrossing.

In order to expend the range of the genomic composition of the donor parent in the IL population, we added one more backcross for some ILs lines of F4 generation, which further reduced the average donor genomic composition of these lines. The average proportion of introduced *O. glaberrima* fragments in the ILs was 19.31%, higher than the theoretical proportion expected (12.5%), according to the breeding of two times' backcross with the Asian rice variety. Unlike the CSSLs which requires many generations of backcrosses, the ILs may be developed by few generations of backcrosses. Even more generations of backcross would further reduce the genomic composition of the donor in the ILs. Our previous study indicated that an optimal proportion of glaberrima genome is 10–20% for breeding purpose (Adedze et al. 2012; Chen et al. 2016).

The detection of non-parental alleles

Although the alleles of the ILs population are supposed to be inherited from their parents, non-parental alleles are not uncommon in molecular profiling of different segregation populations and have been observed by many previous studies. Non-parental alleles were detected in 83% of the progenies derived from an interspecific cross between WAB56-104 and CG14, contributed an average of 38 cM per line (~2.2% of genomic DNA) and the inbreeding lines in the field contained more non-parental alleles (2.7%) compared to the DH lines (1.3%) Semagn et al. (2007). The proportions of non-parental alleles in descendants between TOG5681 and TOG5674 (*O. glaberrima*) and IR64 (*O. sativa*) vary from 0 to 11.7% with an average of 2.4% (Agnoun et al. 2012). Three types of non-parental banding patterns were detected in a recombinant inbred line maize population developed from 2 inbred lines. 20 SSR loci (3.96%) showed non-parental inheritance among the 80 inbred lines of the F7 population and the non-parental allele frequency varied from 23.8 to 53.8% in 20 SSR loci with an average of 41.6%. There are a variety of potential causes for the formation of non-parental bands, including recombination or mutation in the simple-sequence repeat region, residual heterozygosity in parental lines, or chromosomal aberrations resulting from rearrangements and transposons. In addition, for non parent alleles of interspecific hybridization between *O. sativa* and *O. glaberrima*, outcrossing from other parents might be a reason too. I.e. an arom gene of progenies between *O. sativa* and *O. glaberrima* occurs most likely from the *O. sativa* not from the O. *glaberrima* parent.

In the present study, the average proportions of non-parental alleles are estimated at 3.98% which was relatively lower comparing with previous studies. Most of the non-parental alleles in the present study may be caused by interspecific genetic recombination between genomes of the two species, particularly unequal crossover, e.g. many of the non-parental bands were observed either independently from any parental bands or co-existed with heterozygous bands. It has been suggested that recombination enzymes show high affinity towards dinucleotide repeat sequences (Biet et al. 1999). In this study, 21 SSR loci were dinucleotide repeat motifs among the 28 SSR loci with non-parental alleles. It suggested that the SSR regions, particularly dinucleotide repeats may induce the formation of non-parental alleles. If the non-parental alleles were caused by pollen contamination, a high frequency of about 50% could be observed in ILs affected. However, the non-parental alleles detected in 11 chromosomes except

chromosome 12 showed a related low frequency (<9.2%) in 66 ILs affected, which is even lower than the average frequency of the alleles from the original donor parent RAM3 (20.94%).

The presence of non-parental alleles in the ILs would not affect our final results including the detection of QTLs or HLs. To determine the extent of the genomic composition of the ILs, we assumed that only the DNA band patterns belonging to RAM3 and Jin23 were considered to be parental alleles, respectively. For the purpose of detecting QTLs and HLs, a new data set of the ILs genotypes was generated, i.e., only alleles that are consistent with RAM3 were scored as 'A' for *O. glaberrima* alleles while all remained DNA alleles including non-parental alleles were considered as *O. sativa* alleles (scored as 'B'). Only each 'A' indicated a homozygous locus *O. glaberrima/O. glaberrima* in ILs and could represent an interspecific heterozygous locus in the PIH IL/GZ63S (R/G), while the each 'B' indicated a homozygous locus *O. sativa/O. sativa* in ILs and could be homozygous locus or an intraspecific heterozygous (J/G) in the PIH. Therefore, the statistics employed in this study has excluded effectively the effects of non-parent alleles on the detection of interspecific heterotic loci associated with the heterosis.

Conclusion

The introduction of genomic fragments from close related species is an effective way to enrich genetic diversity and create new germplasm in crops. And exploiting of distant heterosis is a promising way to further raise the yield potential of crops. A total of 79 ILs carry African rice (*O. glaberrima*.) genomic fragments were used to produce the 79 PIHs for studying the genetic basis for the interspecific heterosis of 7 agronomic traits.

A total of 24 QTLs associated to six agronomic traits were mapped on 9 chromosomes and 23 interspecific HLs for seven agronomic traits were identified on 10 chromosomes in 2 years. And 22 QTLs and 19 HLs were found to be associated with 5 yield-related traits respectively. Both intra-locus and inter-locus interactions were found to contribute to the interspecific heterosis. At intra-locus interaction level, 13 HLs had overdominant effects while the remaining 10 HLs had dominant or partial dominant effects. And 12 HLs had positive effects and 11 HLs had negative effects on the traits. Out of all 23 HLs, 17 HLs had different location to their corresponding QTLs associated to the same traits, indicating the epistasis effects of inter-locus interaction could also play an important role in the interspecific heterosis. In addition, the pleiotropism was found in 5 marker loci for 11 QTLs associated to five agronomic traits and 4 marker loci for 10 interspecific HLs for all the seven traits. This study is the first attempt to detect the interspecific HLs in rice. Therefore, our results could help to lay the foundation for exploring the genetic mechanism of interspecific heterosis in rice.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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