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Identification of interspecific grain yield heterosis between two cultivated rice species *Oryza sativa* L. and *Oryza glaberrima* Steud.

Adedze Y.M. Nevame[1], Efisue Andrew[2], Zhang Sisong[3], Samoura Demba[1], Huang Feng[1], He Wenchuang[1], Xie Guosheng[1], Jin Deming[1 *]

[1]College of Plant Sciences and Technology, Huazhong Agricultural University (HZAU), Wuhan 430070, China
[2]Crops & Soil Science Department, University of Port Harcourt, Port Harcourt, Nigeria
[3]Hubei Agricultural Extension Station, Wuhan, China

[*]Corresponding author: djin@mail.hzau.edu.cn

Abstract

Distant heterosis is a promising genetic phenomenon to achieve the yield ceiling of hybrid rice. Heterosis and agronomic performance of indica rice (*Oryza sativa* L. ssp. indica) hybrids were studied using a partial diallel cross among 8 indica male sterile lines and 8 pollen parents. The pollen parents comprised of 4 indica restorer lines and 4 lines derived from interspecific crosses between indica rice (*Oryza sativa* L. ssp. indica) and African rice (*Oryza glaberrima* Steud.) varieties. Sixty-four hybrids and their pollen parents showed significant variation in field performance as well as heterosis for 8 studied agronomic traits. Field performance for all agronomic traits showed significant differences among the 64 crosses and their pollen parents at p≤ 0.05 and 0.01 probability level. The highest heterobeltiosis (heterosis over pollen parent) was scored for filled grain number per panicle (101.48%), followed by panicle yield (61.70%), spikelet number per panicle (73.33%), 1000-grain weight (46.00%), grain filling percentage (33.50%), panicle length (28.80%), plant height (27.74%) and days to flowering (16.00%). The parental lines of promising hybrids with higher yield potential showed relatively higher general combining ability. Two hybrids AF-zhong9A/RL1 and II-32A/RL1 with highest panicle yield were identified as partial interspecific hybrids. Their common pollen parent RL1 was derived from interspecific multiple crosses and contained about 12.5% genomic genes from *Oryza glaberrima* according to its pedigree. This study suggests the existence of interspecific heterosis between the two cultivated rice species in agronomic traits including grain yield.

Keywords: Interspecific heterosis, GCA, *Oryza sativa* L, *Oryza glaberrima* Steud.
Abbreviations: GCA_General Combining Ability; AF_The CMS line with African rice cytoplasm

Introduction

The exploitation of distant hybrid vigor is a promising way for raising yield potential in crops. Numerous studies have shown the yield advantages of being more heterozygous in hybrids. Genetic distance was reported to be positively correlated to the heterosis in maize (Lariepe et al., 2012), carrot (Jagoz et al., 2011) and rice (Luo et al., 1996; Xu et al., 2002; Luo et al., 1999; Phetmanyseng et al., 2010). While the genetic distance between the parents was emphasized to be positively correlated to heterosis of the hybrids, interspecific heterozygosity was demonstrated to be associated with a decrease in interspecific hybrid fertility (Moehring et al., 2011).

The crossability of the two cultivated rice species (*O. sativa* and *O. glaberrima*) and sterility of interspecific hybrids was first reported by Hiroko et al. (1961), when he performed investigations on crosses of two cultivated rice species, *Oryza sativa* and *Oryza glaberrima*. Grain yield advantage was reported in NERICA rice varieties, which are inbred lines derived from crosses between *O. sativa* and *O. glaberrima* (Jones et al., 1997; Dingkuhn et al., 1998). Various studies such as genetic mapping of agronomic traits (Aluko et al., 2003), genetic diversity (Barry et al., 2007), the molecular profiling analysis (Ndjiondjop et al., 2008) and drought resistance of inbred lines derived from crosses between *O. sativa* and *O. glaberrima* (Efisue et al., 2009) were also done in recent years. However, little is known about the grain yield and heterosis of the interspecific F_1 hybrids of *O. sativa* and *O. glaberrima* because of their sterility status. In the present study, a partial diallel cross plan was made among 8 male sterile lines and 8 pollen lines. The pollen lines included 4 lines derived from interspecific crosses containing about 12.5% to 25% of *O. glaberrima* genomic background. The partially fertile interspecific hybrids, produced in this study, provided the possibility to evaluate the interspecific grain yield heterosis between the two cultivated rice species *Oryza sativa* and *Oryza glaberrima*. The objectives of this study were to (1) evaluate the field performance and heterosis of the hybrids on eight agronomic traits including panicle yield and yield components, (2) identify male sterile and pollen parental lines with high general combining ability (GCA) and field performance, (3) select heterotic combinations of Cytoplasmic Male Sterility (CMS) or Photo-Thermosensitive Genetic Male Sterility (PTGMS)/ Restorer lines and (4) detect interspecific heterosis between *O. sativa* and *O. glaberrima* in grain yield potential of the partial interspecific hybrids.

Results

Pollen sterility of CMS lines

Pollens of the CMS lines used in this study were examined under light microscope for evaluating pollen sterility. All the three types of CMS (WA-CMS, ID-CMS and AF-CMS) lines were found completely sterile and showed typical abortive pollens (unstained withered and spherical sterile pollens) with similar morphology (Fig. 1).

Fertility restoration of F_1

Grain filling percentage (GFP) was used as the main criterion for estimating fertility restoration behavior among the hybrids. In this study, GFP of the hybrids varied from 0.0% (II-32A/RL4) to 86.83% (M1S/RL2). The difference of GFP between all the 64 crosses and the 8 pollen parents was significant at 0.01 of probability (Table 3). The occurrence of sterile F1s between CMS lines and pollen parents indicates that the pollen parents RL2, RL3, RL4 and Paddy do not possess effective restoring genes for the CMS lines, whereas the other 4 pollen parents RL1, R207, Minghui63 and Mianhui725 are effective restorer lines with strong restoration ability. For each F_1 hybrid, pollen fertility and spikelet fertility were calculated to determine their fertility restoration. Both fertile and sterile F_1 hybrids were observed in this research (Fig. 2).

Sources of variation for agronomic traits of the hybrids

In this investigation, analysis of variance for all 8 agronomic traits showed a significant difference ($P \leq 0.01$). The variations of these traits could be due to contributions of male parents (pollen parents), female parents and their interactions.

The field performance of the agronomic traits such as days to flowering, spikelet number per panicle, filled grain number per panicle, plant height, grain filling percentage and panicle yield were more affected by the male parents, whereas the female parents played relatively important roles in panicle length variation. In addition, the interactive effects of female and male parents were also considerably important source of variation for days to flowering, spikelet number per panicle and filled grain number per panicle (Table 3). The relative contribution of the variation sources for the field performance and heterobeltiosis was similar on most measured agronomic traits, except panicle yield, in which the variation of field performance was more affected by pollen parents. However, heterobeltiosis was almost equally affected by both parents.

Field performance and heterobeltiosis of the hybrids

Wide variations were observed on field performance of all 8 agronomic traits among 64 crosses (Table 4). The exceptionally wide variations were detected for filled grain per panicle, grain filling percentage and panicle yield, ranging from 0~230 grains, 0.00%~86.83% and 0.91~5.87g per panicle, respectively.

Low grain filling (0.00%~15.02%) was observed in 18 sterile hybrids derived from 6 CMS lines and 3 pollen parents (RL2, RL4 and Paddy). This indicates that their pollen parents did not possess effective restorer genes. The panicle yield was positively correlated with spikelet number per panicle (r= 0.64**), the 1000-GW (r=0.55**) and the grain filling percentage (r=0.70**). Both positive and negative heterobeltiosis were observed in all the traits measured in the 64 crosses (Table 4). The negative mean of heterobeltiosis for days to flowering and plant height (both were -0.90%) could be beneficial to crop production for enhancing early maturation and lodging resistance, respectively. The negative heterobeltiosis for grain filling percentage (-28.46%) could be attributed to the lack of fertility restoration ability of the pollen parents in some CMS/restorer combinations. Positive heterobeltiosis for spikelets per panicle (8.53%) and grain weight (10.47%) resulted in a positive mean of panicle yield heterobeltiosis (3.08%) despite the existence of sterile hybrids. The positive mean heterobeltiosis identified for panicle length, spikelet number per panicle, 1000-GW and panicle yield indicates that the genes controlling these traits from the parents interacted favorably and resulted in positive grain yield heterosis in most hybrids.

General combining ability of the parental lines

Significant negative and positive general combining ability (GCA) was observed among 16 parental lines for the 8 measured traits (Table 5). GCA for panicle yield of the pollen parents varied from 0.47 (RL3) to 2.41 (Mianhui725) while that of the female parents were from 0.76 (IR58025A) to 2.22 (AF-Zhong9A). A high GCA for panicle yield was observed in parental lines and female parents such as AF-zhong9A, II-32A, Zhenshan97A and M5S as well as male parents Mianhui725, RL1, Minghui63 and R207. Female parents Jin23A, AF-jin23A, IR58025A and male parent L3 showed relatively lower GCA.

Yield performance of promising hybrids

Nine hybrids with high panicle yield and good agronomical traits, designated as promising hybrids, were selected for further analysis (Table 6). The female parents of the 9 promising hybrids included 4 CMS lines and 1 PTGMS line. Their pollen parents included 3 commercial restorer lines and 2 lines (RL1, RL2) derived from interspecific crosses. The parental lines of the promising hybrids, except for Jin23A, which had the shortest growth duration, had relatively higher panicle yield GCA compared to the remaining parents (Table 5). The highest panicle yield was scored by AF-Zhong9A /RL1 (5.87g) followed by of II-32A/RL1 (5.77g). The two hybrids shared a common pollen parent (RL1), which was derived from an interspecific multiple cross RAM152/ Paddy//PaddyF_2/Mianhui725. About 12.5% of the genomic genes of RL1 came from an African rice (*Oryza glaberrima*) variety RAM152.

These hybrids could be regarded as partial interspecific hybrids according to their genomic composition. The high panicle yield of the two partial interspecific hybrids demonstrated their high grain yield potential. Though the difference in panicle yield of the top 7 promising hybrids were not significant, the panicle yield of AF-Zhong9A/RL1 was significantly higher than that of the rest 59 hybrids including Zhenshan97A/Minghui63 (Shanyou63) which was a commercial hybrid variety widely cultivated in China (Supplementary Table 1).

Heterobeltiosis and heterotic hybrids

Twenty out of 64 hybrids expressed positive heterobeltiosis for panicle yield. Among them 9 hybrids with more than 25% heterobeltiosis were regarded as heterotic hybrids (Table 7). These heterotic hybrids were crosses between 5 female parents (4 CMS lines and 1 PTGMS line) and 4 pollen parents including 3 commercial restorer lines and one line (RL1) derived from interspecific crosses. The 9 heterotic hybrids were not exactly matched to the 9 promising hybrids as described before. Four of the 9 heterotic hybrids shared a common pollen parent R207, which had a relatively low panicle yield of 2.63g. Among them only Zhenshan97A/ R207 was also listed as a promising hybrids. On the other hand, 4 out 9 promising hybrids shared another common

Table1. Male sterile lines as female parents.

Female Lines	Pedigree	Origin	MS Type
Jin23A	Jin23B: Huangjin3//FeigaiB/M	Hunan	WA-CMS
Zhenshan97A	Zhenshan97B:Zhen-zhuai11/Shan'aixuan4	Jiangxi	WA-CMS
II-32A	II-32B: Zhenshan97/IR665	Hunan	ID-CMS
IR58025A	IR58025B: IR48483A /Pusa167-120-3-2	IRRI	WA-CMS
AF-Zhong9A	WAB375/IRAT268//Zhong9///Zhong9B_6F_1	HZAU	AF-CMS
AF-Jin23A	RAM3/Jin23//Jin23B_7F_1	HZAU	AF-CMS
M1S	Peiai64s/ Peiai64s//IR7109	HZAU	PTGMS
M5S	Peiai64s/ Peiai64s//CNA6187	HZAU	PTGMS

WA-CMS = Wild Abortive Cytoplasmic Male Sterile line, ID-CMS = Indonesia Paddy rice Cytoplasmic Male Sterile line, AF-CMS = African Cytoplasmic Male Sterile line, Photo-Thermosensitive Male Sterile line, HZAU: Huazhong Agricultural University.

Table2. Restorer lines as pollen parents.

Male Lines	Pedigree*	Origin
RL1	RAM152/ Paddy //PaddyF2/Mianhui725 F6	HZAU
RL2	RAM3/Jin23B//Wanxian98 F7	HZAU
RL3	RAM152/ Paddy //PaddyF2/Jiachunnian F6	HZAU
RL4	RAM152/ Paddy //PaddyF2/Jiachunnian F6	HZAU
Paddy	Local variety	Indonesia
R207	R432/N422	Hunan
Minghui63	IR30/Gui630	Fujian
Mianhui 725	Peiai64/Mianhui501	Sichuan

*RAM3 and RAM152 are African rice (*Oryza glaberrima* Steud) varieties.

pollen parent Mianhui725, which had a relatively higher panicle yield of 4.14g. Among them 2 crosses AF-Zhong9A/ Mianhui725 and II-32A/ Mianhui725 appeared again in the list of 9 heterotic hybrids. Nevertheless, the 2 partial interspecific hybrids AF-zhong9A/RL1 and II-32A/RL1 ranked as the top 2 promising hybrids. They reappeared as most heterotic hybrids with highest heterobeltiosis for panicle yield of 61.7% and 58.5%, respectively. The difference of the panicle yield heterobeltiosis of the top 4 hybrids (AF-Zhong9A/RL1, II-32A/RL1, Zhenshan97A/ R207 and Jin23A/R207) was not significant at $P \leq 0.05$ while they were significantly higher than the other 5 hybrids (Table 7).

However, all of the partial interspecific hybrids did not show outstanding performance in grain yield. Twelve out of 32 hybrids were sterile with grain filling percentage ranging from 0.00% (II-32A/RL4) to 14.30% (AF-jin23A/RL4). Ten partial interspecific hybrids were incompletely restored with grain filling percentage ranging from 19.70% (Jin23A/RL3) to 69.47% (M5S/RL2). Only 10 partial interspecific hybrids were fertile with grain filling percentage above 70%. Three fertile partial interspecific hybrids were selected as promising hybrids. In addition to the top two hybrids AF-Zhong9A/RL1 and II-32A/RL1, M1S/RL2 was also listed as a promising hybrid. The panicle yield (4.93g) of M1S/RL2 was slightly lower than that of the top 2 but statistically insignificant (Table 6).

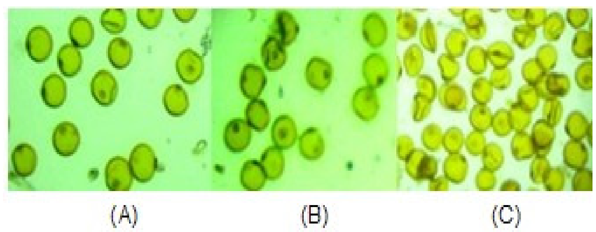

Fig 1. Sterile pollens of the 3 different types of CMS plants under microscope: (A) pollens produced by a WA-CMS plant; (B) pollens of an ID-CMS plant and (C) pollens of an AF-CMS plants. All three types of CMS produced similar unstained spherical or withered sterile pollens which resulted from arrested development at early microspore stage.

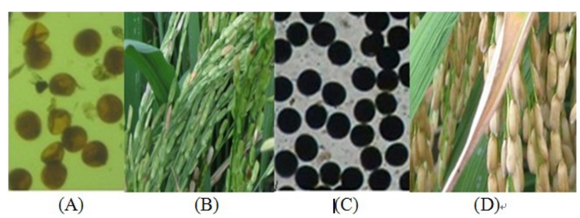

Fig 2. Pollen and spikelet fertility of F_1 hybrids: (A) light stained spherical and unstained withered sterile pollens from a sterile hybrid plant; (B) panicles of sterile F1 plants at maturity showing sterile spikelets; (C) dark stained round fertile pollens from a fertile F1 plant and (D) panicles of fertile F_1 plants at maturity showing high grain filling percentage.

Discussion

Reproductive barrier in the interspecific F_1 and progenies derived from *O. sativa* and *O. glaberrima* (Heuer et al., 2003) constituted a bottleneck for interspecific grain yield heterosis. Genetic strategies and methods have been suggested to increase the crossability (Abebrese et al., 2011) and the seed fertility of partial hybrid progenies of crosses between *O. glaberrima* and *O. sativa* (Ikeda et al., 2009). Genetic populations such as Recombinant Inbreed Lines (RILs), Chromosome Segment Substitution Lines (CSSLs) and Introgression Lines (ILs) with useful foreign allele traits in *O.sativa* genetic background have been developed via interspecific crosses between *O.sativa* and *O.glaberrima* (Gutierrez et al., 2010; Bimpong et al., 2010 and 2011) or wild rice species (Tian et al, 2006; Rangel et al., 2007). RL1, RL2, RL3 and RL4 used in this study were essentially introgression lines carrying about 12.5% to

Table 3. Mean Square of 8 agronomic traits of the 64 hybrids.

Source of variation	DL	DF		PH		SN		GFP	
		FP	Hb	FP	Hb	FP	Hb	FP	Hb
Rep	2	0.010	1.386E.-06	68.220	0.004	228.500	0.005	79.400	0.015
Male	7	2128.590**	0.040**	1995.880**	0.200**	17135.800**	0.595**	13600.300**	2.777**
Female	7	50.920*	0.004**	695.806*	0.066**	12.520**	0.077**	13.400*	0.562**
M x F	49	204.600**	0.016**	96.144**	0.008**	4338.600**	0.072**	888.200*	0.177**
Error	126	0.010	0.000	15.430	0.001	0.016	0.017	0.048	0.009

Source of variation	DL	PL		FG		1000- GW		PY	
		FP	Hb	FP	Hb	FP	Hb	P	Hb
Rep	2	1.770	0.002	631.700	0.025	0.003	25.100	0.758	5.734
Male	7	51.430**	0.014**	65958.000**	3.509	0.576**	1144.000	12.210**	26.800**
Female	7	40.140*	0.045**	19.290**	0.847**	0.028**	280.200*	4.593**	26.770*
M x F	49	9.650**	0.010**	7512.100**	0.025	0.058**	576.400**	2.136**	12.160**
Error	126	0.818	0.001	0.060	0.021	0.003	34.100	0.524	0.030

*and **= difference significant at P≤ 0.05 and P≤ 0.01 of probability, DL= degree of liberties, SE= standard error, FP = Field performance, Hb% = heterosis over pollen parent, Rep = replication, FxM = Female x Male, DF = Days to flowering, PH= plant height, PL= panicle length, SN = spikelet number per panicle, FG= filled grain number per panicle, GFP= grain filling percentage, 1000-GW = thousand grain weight, PY= Panicle Yield.

Table 4. Range and mean value of field performance agronomic traits of the 64 hybrids.

F1 hybrids	DF (d)	PH(cm)	SN(N)	GFP
FP				
Range	77.00~145.00	89.00~136.00	154.00~315.00	0.00~86.83
Mean	105.97	115.65	252.00	51.57
SE	0.83	0.84	0.18	0.21
Hb%				
Range	-28.70~16.00	-26.33~27.70	-55.05~73.30	-100.00~33.50
Mean	-0.90	-0.90	8.53	-28.46
SE	0.01	0.01	0.02	0.03

F1 hybrids	PL (cm)	FG(N)	1000GW (g)	PY(g/p)
FP				
Range	25.00~35.00	0.00~230.00	19.30~27.50	0.91~5.87
Mean	30.24	127.04	24.00	3.59
SE	0.55	0.36	0.43	1.58
Hb%				
Range	-23.89~28.00	-100~101.48	55.00~46.00	-80.56~61.70
Mean	2.41	-22.48	10.47	3.08
SE	0.01	0.04	0.05	3.33

PH= plant height, DF = Days to flowering, PL= panicle length, SN= spikelet number per panicle, FG= filled grain number per panicle SE= standard error, GFP= grain filling percentage, 1000GW = Thousand Grain Weight PY= panicle yield, Hb% = Heterobeltiosis, FP = field performance

25% of *O. glaberrima* genes in the genetic background of *O. sativa*. Therefore, the hybrids produced from crosses between sterile lines and these introgression lines are considered as partial interspecific hybrids. Ten out of the 32 partial interspecific hybrids, produced by pollen parents RL1, RL2, RL3 and RL4, showed more than 70% of the grain filling percentage. Our study on these fertile partial interspecific hybrids revealed the possibility of exploiting interspecific heterosis of grain yield and yield components in rice. Yield increase in rice was considered to depend on panicle yield increase (Janoria et al., 1991). In this study, panicle yield referred to the grain yield potential and considered to be more reliable and accurate than grain yield per plant. This is because of the fact that among the grain yield components, panicles number per plant was more susceptible to the uncontrolled environmental factors such as field fertility irregularity, pests attack, uneven distribution and small F_1 populations. Two partial interspecific hybrids AF-Zhong9A/RL1 and II-32A/RL1 showed good field performance as well as heterobeltiosis of panicle yield. Their outstanding performance demonstrated the existence of the interspecific heterosis on grain yield potential. Their common pollen parent (RL1) was derived from multiple crosses of *Ozyza glaberrima* Steud. variety RAM152 and *Oryza sativa* L. varieties Paddy and Mianhui725. The RL2, RL3 and RL4 were also derived from interspecific crosses. However, none of them showed strong restoration ability when crossed with the 6 CMS lines. The lack of effective restorer gene was found in RL2 and RL4, which produced hybrids with average grain filling percentage of 9.83% and 4.29%, respectively. The RL3 that produced incomplete fertile hybrids, with grain filling percentage around 49.41%, could be regarded as a weak restorer line. RL1, RL2, RL3 and RL4 are derived from 3 different interspecific crosses (Table 2). The genomic compositions of these lines are different due to their pedigree and artificial selection. The selection of introgression lines with genomic composition containing benefit genes from *Ozyza glaberrima* and effective restorer gene is the key for the success of interspecific hybrid rice breeding. General combining ability (GCA) is an important parameter for evaluating the parental line in commercial rice production (Wang et al., 2009). The parental lines (AF-Zhong9A, II-32A and RL1) of 2 top promising

Table 5. GCA of the 16 parental lines used in this study.

Parental lines	General Combining Ability							
	DF	PH	PL	SN	FG	GFP	1000-GW	PY
Female lines								
AF-Zhong9A	0.66	11.14	2.98	13.56	0.59	-2.02	- 0.18	2.22
II-32A	2.41	5.10	-0.20	1.27	-7.96	-5.17	0.24	2.06
Zhenshan97A	0.53	-2.44	-1.21	-13.65	-14.42	-2.80	0.67	1.97
M5S	0.41	-2.23	-0.78	3.31	45.80	16.80	0.15	1.63
M1S	0.20	-0.73	-0.03	14.81	54.59	17.00	0.04	1.87
Jin23A	-11.67	8.02	-27.92	0.07	-4.44	-1.10	- 0.02	1.04
AF-jin23A	-0.47	-3.90	-0.84	-15.02	-33.58	-10.32	-0.60	1.01
IR58025A	-1.79	-12.31	-17.08	-0.03	-2.48	-2.59	-0.04	0.76
$LSD_{(0.05)}$	0.04	2.24	0.52	18.04	14.00	3.95	0.43	0.53
Male lines								
Mianhui 725	-4.60	-1.36	1.38	20.67	61.79	17.12	0.14	2.41
RL1	-5.72	-11.48	-2.08	-29.23	40.17	23.18	0.11	1.86
Minghui63	1.78	2.39	0.28	-9.27	41.04	17.70	0.02	1.68
R207	0.03	-9.69	-2.14	-34.52	33.46	22.45	-0.03	1.43
RL3	2.61	-11.19	4.46	-0.23	1.56	-2.93	- 0.25	0.47
RL2	-4.97	6.22	0.62	40.06	-53.74	-24.66	a	a
RL4	-29.42	-2.98	-61.62	0.23	16.93	22.28	a	a
Paddy	-28.95	26.44	-65.54	1.90	-4.57	-5.85	a	a
$LSD_{(0.05)}$	0.04	2.24	0.52	18.04	14.00	3.95	0.03	0.42

a: Data of 1000-GW and PY were not scored because pollen lines of RL2, RL4 and Paddy produced sterile hybrid.

Table 6. The 9 promising hybrids with high yield potential and their pollen parents.

Genotypes	DF	PH	PL	SN	FG	GFP	1000-GW	PY
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Hybrids								
AF-zhong9A/RL1	101.00**	127.67	31.26**	294.00	244.00	83.16	24.0*	5.87
II-32A/RL1	101.00**	115.67**	28.88**	265.00	210.00	79.16	27.5	5.77
II-32A/Mianhui725	98.00**	123.00*	34.17	313.67	246.00	78.42	23.5*	5.73
AF-zhong9A/Mianhui725	101.00**	129.33	28.66*	267.00	206.33	75.29	26.0*	5.40
Zhenshan97A/Mianhui725	98.00**	113.67**	30.10**	310.33	230.33	74.22	22.0**	5.06
Jin23A/Mianhui725	106.00**	105.33**	29.49**	293.67	209.33	71.76	23.6**	4.95
MIS/RL2	98.00**	125.00**	27.81**	232.67*	202.00	86.83	24.3**	4.93
Zhenshan97A/Minghui63 (Shanyou63)	102.00**	113.00**	30.51**	234.67*	187.67	80.31	25.1**	4.71*
Zhenshan97A/R207	101.00**	111.00**	27.65**	206.33**	167.00*	80.81	26.0*	4.01**
Pollen Parents								
RL2	101.00	125.67**	28.64**	242.33**	185.67	76.76	22.8**	4.24**
Mianhui725	106.00	111.00**	32.38	245.33	200.33	71.15	26.4	4.14**
Minghui63	106.00	106.33*	29.24**	218.33**	166.00**	75.92	22.6**	3.75**
RL1	103.00	103.33**	25.87**	198.00*	135.00**	68.42*	22.9*	3.63**

*and ** = significant at $P \leq 0.05$ and $P \leq 0.01$ of the probability, PH = Plant Height, DF = Day to Flowering, PL = Panicle Length, GFP = grain filling percentage, 1000-GW = Thousand Grain Weight, PY = Panicle Yield, SN = Spikelet Number per panicle, FG = Filled Grain per panicle.

hybrids have shown the highest GCA for panicle yield, when compared to other 13 parental lines used in this study, except Mianhui725. RL1 which carried about 12.5% genomic background of *O. glaberrima* was found to be a strong restorer with relatively higher GCA. Therefore, RL1 could be regarded as a good introgression restorer line for producing heterotic interspecific hybrids. Heterotic loci responsible for heterosis were reported in maize (Tang et al., 2007) and in rice (Hua et al., 2002; Luo et al., 2011). The existence of heterotic loci is fundamental to produce heterotic hybrids. The high yield scored by the 2 partial interspecific hybrids AF-Zhong9A/RL1 and II-32A/RL1 suggests that the interspecific heterotic loci in these hybrids may play an important role for their strong heterosis on grain yield. Therefore, further molecular analysis and screening of interspecific heterotic loci will enhance the understanding of the mechanisms of interspecific heterosis and facilitate its utilization in rice hybrid breeding.

Materials and methods

Parents and cross design

Eight male sterile lines comprised of 3 Cytoplasmic Male Sterility (CMS) lines with wild abortive cytoplasm (WA), 1 CMS line with Indonesian paddy rice cytoplasm (ID), 2 CMS lines with African rice cytoplasm (AF), and 2 PTGMS lines were used as female parents (Table 1). There were 8 male sterile lines, among them 2 PTGMS lines and 2 African rice cytoplasmic male sterile lines, AF-Jin23A and AF-Zhong9A, were new lines developed in our lab of Huazhong Agricultural University (Table 2). A total of 64 crosses were made at

Table7. The 9 heterotic hybrids with high heterobeltiosis on panicle yield and yield components.

Hybrids	Heterobeltiosis %			
	GFP	SN	1000-GW	PY
AF-zhong9A/RL1	21.54	48.48	4.08*	61.70
II-32A/RL1	15.70	33.83	20.08*	58.50
Zhenshan97A/R207	19.20	26.50*	46.00	52.47
Jin23A/ R207	25.51	34.64	35.00*	47.65
II-32A/R207	16.18	36.67	38.00	41.87*
II-32A/Mianhui725	20.14	34.64	33.00*	38.40*
M5S/ R207	17.00	32.44	33.00*	38.38*
AF-zhong9A/Mianhui725	5.81*	9.87*	-1.51*	30.43*
Zhenshan97A/Minghui63(Shanyou63)	4.37*	26.49*	-16.67*	25.60*

SN = spikelet number per panicle, FG = Filled grain per panicle, GFP= grain filling percentage, PY= Panicle Yield, Hb%= heterobeltiosis

the experimental station of Huazhong Agricultural University in Hainan, China following the Factorial Mating Scheme (NC Design II) in March 2010.

Hybridization

Before crossing, pollen sterility of CMS lines was checked by staining pollen grains with 1% potassium iodide-iodine I_2-KI) solution at heading stage. About 10 spikelets from each plant were sampled in the morning before flowering. Photo-Thermosensitive Genetic Male Sterile (PTGMS) lines were fertile when cultivated in Hainan in winter-spring season due to the short photoperiod and low temperature. So, artificial emasculation was used before hand pollination. The pollinated panicles were bagged and labeled.

Field experiments

Pre-germinated seeds of the F_1 hybrids and their corresponding pollen parents were sown in nursery beds at the Huazhong Agricultural University Experimental Station in Wuhan on 15th May, 2010. 30-day-old seedlings were then transplanted with 20 plants per plot at 15 x 20 cm^2 spacing in three replications following a Randomized Completed Block Design (RCBD). At harvest, a random sample of 5 plants per plot was taken to measure the following agronomic traits: Days to Flowering (DF), Plant Height (PH), Pollen Fertility (PF), Spikelet Number per panicle (SN), Filled Grain number per panicle (FG), Grain Filling Percentage (GFP), Panicle Length (PL), 1000-Grain Weight (GW), Panicle Yield (PY). Those parameters collected from the field constituted the field performance of the entries.

Data analysis

Statistic analysis was made using Statistix 8.1 software. Analysis of variance was done by F-test with significance of mean squares. The LSD (least significant difference $p \leq 0.05$, 0.01) was used to compare the means. Heterobeltiosis (Hb%) $=\{(F_1 - MP)/MP\} \times 100$, where F_1 is the average performance of the F_1 and MP is the average performance of the male parent. General Combining Ability (GCA) of the parental lines was evaluated in this study following the formula of Gorz et al in (1987). GCA (female) = $X_i - Y$ and GCA (male) = X_j- Y, where X_i is the mean of hybrids with a given female averaged over all replications, X_j is the mean of hybrids with a given male averaged over replications and Y is the experimental mean.

Conclusion

Partial interspecific hybrids were obtained by crossing the indica male sterile lines and introgression lines carrying about 12.5% to 25% genomic background of *O. glaberrima*. Two fertile partial interspecific hybrids AF-Zhong9A/RL1 and II-32A/RL1 showed higher heterobeltiosis of yield traits and higher panicle yield over commercial high yielding indica hybrids. This suggested the existence of the interspecific heterosis on grain yield potential. Further molecular analysis on the genomic composition and interspecific loci of those partial interspecific hybrids is essential for understanding the interspecific heterosis between the two cultivated rice species.

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References

Abebrese SO, Akromah R and Dartey PKA (2011) Crossability of selected progeny from interspecific crosses between *Oryza sativa* and *Oryza glaberrima* (NERICAs). Afr J Agri Res. 6: 79 – 83.

Aluko GK (2003) Genetic mapping of agronomic traits from the interspecific cross of *Oryza sativa* (L.) and *Oryza glaberrima* (Steud). PhD Dissertation. 127 P

Barry MB, Pham JL, Noyer JL, Billot C, Courtois B and Ahmadi N (2007) Genetic diversity of the two cultivated rice species (*O. sativa & O. glaberrima*) in Maritime Guinea. Euphytica. 154 : 127 – 137

Bimpong IK, Carpena AL, Mendioro MS, Fernandez JR, Ramos J, Reversat G and Brar DS (2010) Evaluation of *Oryza sativa* x *Oryza glaberrima* derived progenies for resistance to rootknot nematode and identification of introgressed alien chromosome segments using SSR markers. Afr J Biotechnol. 9: 3988 - 3997

Bimpong IK, Chin JH, Ramos J and Koh HJ (2011) Application of subspecies- specific marker system identified from *Oryza sativa* to *Oryza glaberrima* accessions and *Oryza sativa* x *Oryza glaberrima* F_1 interspecific progenies. Genet Mol Biol. 3:7– 24

Dingkuhn M, Jones MP, Johnson DE and Sow A (1998) Growth and yield potential of *Oryza sativa* and *O-glaberrima* upland rice cultivars and their interspecific progenies. Field Crops Res. 57: 57-69

Efisue AA, Tongoona P, Derera J, Ubi EB and Oselebe OH (2009) Genetics of morpho-physiological traits in segregating populations of interspecific hybrid rice under stress and non-stress conditions. J Crop Improv. 23: 383-401.

Efisue AA, Tongoona P and Derera J (2009) Screening early-generation progenies of interspecific rice genotypes for drought-stress tolerance during vegetative phase. J Crop Improv. 23: 174 - 193.

Gorz HJ, Hakins FA, Pedersen JF and Ross WM (1987) Combining ability effects for mineral elements in forage sorghum hybrids. Agronomy. Faculty Publications. Pp 283.

Gutierrez AG, Silvio JC, Olga XG, Cesar PM, Fernado C, Gustavo P, Joe T and Mathias L (2010) Identification of a rice stripe necrosis virus resistance locus and yield component QTLs using *Oryza sativa* X *O.glaberrima* introgression lines. Plant Biol. 10: 6 – 15

Heuer S and Kouame MM (2003) Assessing hybrid sterility in *oryza glaberrima* x *O. sativa* hybrid progenies by PCR marker analysis and crossing with wide compatibility varieties. Theor Appl Genet. 107: 902-909.

Hiroko M, Hinata K and Oka HI (1962) Comparison between two cultivated rice species *Oryza sativa* L. and *Oryza glaberrima* Steud. Jap J Breeding 12 : 153 – 165

Hua J and Yong Z X (2002) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proceed Natl Acad Sci USA. 100: 2574–2579.

Ikeda R, Yoshimi S and Inoussa A (2009) Seed fertility of F_1 hybrids between upland rice NERICA cultivars and *Oryza sativa* L. or *O.glaberrima* Steud. Breeding Sci. 59: 27 - 35

Jagoz B (2011) The relationship between heterosis and genetics distance based on RAPD and AFLP markers in carrot. Plant Breed. 30: 574 – 579.

Janoria MP, Rhodes AM and Shrivastava MN (1991) Determination of characters for panicle yield in early maturing, semi-dwarf varieties of rice under two fertility environments. Indian J Genet Plant Breed. 5: 102 – 106

Jones MP, Dingkuhn M, Johnson DE and Sow A (1998) Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies. Field Crops Res. 57: 57–69.

Lariepe A (2012) The genetic Basis of the heterosis: Multiparental quantitative traits loci mapping reveals contrasted levels of apparent overdominance among traits of agronomical interest in maize (*Zea mays* L.). Genetics l : 795-811.

Luo LJ, Mei H, Yu X, Wang Y, Zhong D, Ying C, Li Z, Paterson AH (1999) Yield heterosis performance and their parental genetic diversity in rice. Chinese J Rice Sci.13: 6 – 10

Luo XJ, Wu S, Tian F, Xin XY, Zha XJ, Dong XX, Fu YC, Wang XK, Yang JS, Sun CQ (2011) Identification of heterotic loci associated with yield-related traits in Chinese common wild rice (*Oryza rufipogon* Griff.). Plant Sci. 181: 14-22.

Luo XJ, Li Yuan J and McCouch SR (1996) Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theor Appl Genet. 92: 637 – 643

Moehring AJ (2011) Heterozygosity and its unexpected correlations with hybrid sterility. Evolution. 9: 2621-2630.

Ndjiondjop MN (2008) Molecular profiling of interspecific lowland rice populations derived from IR64 (*Oryza sativa*) and Tog5681 (Oryza *glaberrima*). Afr J Biotechnol. 7: 4219 - 4229.

Phetmanyseng X, Xie F, Hernandez J E, Boirromeo T H (2010) Hybrid rice heterosis and genetic diversity of IRRI and Lao rice. Field Crops Res.117: 18 – 23

Rangel NP, Brondani RPV, Coelho ASG, Rangel PHN and Brondani C (2007) Comparative linkage mapping of *Oryza glumaepatula* and *Oryza sativa* interspecific crosses based on microsatellite and expressed sequence tag markers. Genet Mol Biol. 30: 614 - 622

Tang JH (2007) Detection of quantitative trait loci and heterotic loci for plant height using an immortalized F2 population in maize. Chinese Sci Bull 52: 477-483.

Tian F, Li DJ, Fu Q, Zhu ZF, Fu YC, Wang XK and Sun CQ (2006) Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. Theor Appl Genet. 112: 570 – 580

Xu W, Virmani SS, Hernandez JE, Sebastian LS (2002) Genetic diversity in parental line and heterosis of the tropical rice hybrids. Euphytica. 127: 139 – 148

Wang YW, Gao Shan and Zhang Chunyan (2009) Combining Abilities of Quantitative Characters in Long-staple Cotton and Their Inheritance Analysis. J Anhui Agric Univ. 37: 7972 - 7974