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Full Length Research Paper

Application of subspecies-specific marker system identified from *O. sativa* to *O. glaberrima* accessions and *O. sativa* × *O. glaberrima* F_1 interspecific progenies

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Interspecific hybrids (F_1's) between Asian rice (*Oryza sativa* 2n=24 AA) and African rice (*Oryza glaberrima* 2n=24 AA) are almost completely sterile. This hybrid sterility barrier is mainly caused by an arrest of pollen development at the microspore stage. Intersubspecific F_1 hybrid sterility is mainly caused by cryptic chromosomal aberrations and allelic interaction between *indica* and *japonica*. To identify *O. glaberrima* specific loci, 67 subspecies-specific (SS) sequenced-tagged site (STS) marker were used to evaluate 30 *O. glaberrima* accessions, which could be classified into sub eleven groups. SPI (subspecies-prototype index) of *O. glaberrima* accessions ranged from 51.67 to 60.00, suggesting intermediate subspecific type based on whole-genome. Some informative markers for classifying O. *glaberrima* accessions, called reference markers, S01054, S01160, S02085, S02140, S03041, and S08107, showed *indica* alleles, which might have contributed to genomic diversification of *O. glaberrima*. Ten (14.9%) SS markers generated *glaberrima*-specific alleles, implying loci adjacent with these markers could be a key for interspecific hybrid sterility. Only 40 (59.7%) SS markers might be useful in *O. glaberrima* analysis, as other markers did not amplify heterozygous alleles in F_1 of *O.sativa* x *O. glaberrima*.

Key words: Subspecies-specific marker (SS), STS markers, indels, subspecies-prototype (SP), *Oryza sativa*, *Oryza glaberrima*.

INTRODUCTION

The genus *Oryza* is known to consist of two cultivated species, Asian rice (*Oryza sativa* 2n=24=AA) and African rice (*Oryza glaberrima* 2n=24=AA) and 22 wild species (2n=24, 48) representing 10 genomic types namely, AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK (Vaughan, 1994; Aggarwal et al., 1997).Unlike *O. sativa*, *O. glaberrima* has no known subspecies.

It might have arose from an African wild species, *O. barthii* independently of the origin of *O. sativa* from the Asian form of *O. perennis* (Semon et al., 2005).The two species are adapted to diverse environments and has its own ecologically adapted and useful traits (Glaszmann, 1987; Sarla et al., 2005).

A number of different markers such as isozyme (Glaszmann, 1987), protein (Bi et al., 1997), RFLP (Qian et al., 1995) RAPD (Chin et al. 2003;), simple sequence repeat SSR (McCouch et al., 2002; Chen et al., 2002; Ni et al., 2002), AFLP Cho et al., 1999), STS (Chin et al., 2007; Edwards et al., 2004), SNPs (McNally et al., 2009; Feltus et al., 2004), and chloroplast DNA (Sun et al., 2002) have been utilized to estimate the extent of genetic diversity in *O. sativa.* In *O. glaberrima,* estimates of genetic diversity based on molecular markers are comparatively few, markers such as isozyme, RFLP SSR and SNPs have been used to estimates genetic diversity in *O. glaberrima* and its close genetic relationship to *O. barthii* (Lorieux et al., 2000; Semon et al., 2005). Even though diversity in *O. glaberrima* are significantly lower than those

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than those in *O. sativa,* it has been shown to harbors genes that have allowed the species to survive and prosper in West Africa with minimal human intervention (Barry et al., 2006; Wang et al., 1992).

Even though O. *glaberrima* and *O. sativa* share the same genome, with minor sub-genomic differences which do not hinder normal chromosome pairing and gamete formation in the hybrids (Nayar, 1973); yet, F_1 hybrids between them shows complete sterility irrespective of the combinations of parental varieties (Pham and Bougero, 1993). Various causes such as meiotic irregularities (Heuer et al., 2003), low proportion of viable pollen, low pollen germination, cytoplasm and its interaction effects from male side and early elimination of female gametes and zygotes from female side (Porteres, 1956; Kitampura, 1962) have been ascribed for sterility. Other causes of sterility are due to segregation distortion (Causse et al., 1994; Lorieux et al., 2000), presence of sterility loci in O. glaberrima, (Koide et al., 2008; Sano 1986; Doi et al., 1999; Li et al., 2008), hybrid breakdown (Li et al., 1997; Kubo and Yoshimura, 2005) and suppressed recombination (Ikehashi, 1982; Neiman and Linksvayer, 2006); hindering easy transfer of useful genes between the two species. Some QTLs and epistatic interaction controlling hybrid-sterility have also been identified (Li et al., 2008).

It is of interest to understand the genetic structure of *O. glaberrima* as information on its diversity and structure is expected to assist plant breeders in the selection of parents for hybridization and also to identify materials that harbor alleles of value for plant improvement. Molecular markers have increasingly become useful tools for evaluating genetic diversity and determining cultivar identity. Compared to morphological markers, molecular markers can reveal differences among accessions at the DNA level and thus provide a more direct, reliable, and efficient tool for germplasm conservation and management.

Subspecies-specific (SS) or species-specific genomic regions could be inherited in a conserved manner to each of subspecies and species from which the SS regions were originated in the progenies of inter-subspecific or inter-specific crosses (Tanksley et al., 1992; Wang et al., 2001). Thus SS regions may provide a clue in understanding the mechanisms for reproductive barriers including inter-subspecific hybrid sterility and for the differentiation of rice subspecies.

OBJECTIVES

The purpose of this study was to evaluate the extent of genetic differentiation between diverse collections of *O. glaberrima* accessions using 67 subspecies-specific (SS) markers. We were interested in developing molecular markers in identifying O. sativa and/or *O.glaberrima* loci, and it usefulness in interspecific crosses.

MATERIALS AND METHODS

Plant material

Thirty accessions of *O. glaberrima* were obtained from the germplasm collection at International Rice Research Institute *(*IRRI) in the Philippines and Africa Rice Centre, Benin in West Africa, 12 accessions of *O. sativa*, representing both *indica* and *japonica* subspecies and 16 F_1 progenies from cross between *O. sativa × O. glaberrima* were used. The F_1s were produced by making crosses in the screenhouse of IRRI between 4 elite indica cultivars (IR64, PSBRc 18 -irrigated, IR 69502-6-SRN-3-UBN-1-B – rainfed and IR55423-01-upland cultivar) and several accessions of O. *glaberrima* referred to as RAM which were received from Mali in West Africa and have been field tested as drought tolerance. *O. sativa* was used as female parent in crosses with *O. glaberrima*. The F_1 plants were intermediate in morphological characteristics but were highly sterile. Variety names source/origin are given in Table 1.

Primer designing

A set of 67 STS markers used in this study were design by Chin et al. (2007) using an online-service software primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) to detect the insertion/deletion (InDel) polymorphism between the genome sequence of Nipponbare *(japonica*) and 9311 (*indica)*. The amplicon size for each primer set was determined so that the amplicon contained at least 5% InDel difference of its whole size, 100 to 400 bp. These markers covers the whole chromosomes at an 2 to 3 cm interval based on the sequence information available at RGP for *japonica* and NCBI for *indica* and are distributed throughout the 12 chromosomes

PCR amplification

The protocols for PCR amplification and detection for STS markers were similar as described in Temnykh et al. (2000) with some modifications. Each 25 µl reaction mixture contained 50 ng DNA, 5 pmol of each primer, 2 µl PCR buffer [100 mM Tris (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 2 µg gelatin], 250 µM of each dNTPs and 0.5 unit Taq polymerase. The thermocycler profile was: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 48°C or 55°C, 2 min at 72°C, and 5 min at 72°C for final extension using the MJ research PCR system. PCR amplicons were resolved by electrophoresis in 3% agarose gels and marker bands were revealed using the silver-staining protocols as described by Panaud et al. (1996).

Scoring of the SS –STS markers

The SS STS markers were scored as ‘a’ (*japonica* allele) or ‘b’ (*indica* allele) for each marker locus. The total number of ‘a’ from *japonica* varieties and ‘b’ from *indica* were counted. Since some markers showed variation in generating SS alleles among varieties within and inter-subspecies, the concept of subspecies-specificity (SS) was employed as follows:

Subspecies-specificity (SS) score of each marker = (Total number of expected alleles in each subspecies) / (Total number of varieties/accessions tested) × 100 (%)

For example, if a marker has a SS score of 100%, it means that the SS marker generated SS alleles for all the accessions without

Table 1. Plant materials in this study.

Species/ subspecies	Entry No.	Name	Source	Entry No.	Cross combination*	Source
Indica	1	IR55423-01	IRRI	43	IR64 × RAM54	IRRI
	2	IR60080-46-A		44	IR64 × RAM86	
	3	IR64		45	IR64 × RAM90	
	4	IR68703-AC-24-1		46	IR64 × RAM120	
	5	IR69502-6-SRN		47	IR64 × RAM134	
	6	PSBRC18		48	IR64 × RAM131	
	7	PSBRC82		49	PSBRC18 × RAM111	
Japonica	8	Hwacheongbyeo	Korea	50	IR55423-01 × RAM3	
	9	Ilpumbyeo		51	IR55423-01 × RAM24	
	10	Jinmibyeo		52	IR55423-01 × RAM163	
	11	Junambyeo		53	IR69502 × RAM118	
	12	TR22183	China	54	IR69502 × RAM121	
O. glaberrima	13	RAM3	Mali	55	IR69502 × RAM163	
	14	RAM24		56	IR60080-46-A × IG10	
	15	RAM54		57	IR68703-AC-24-1 × CG14	
	16	RAM86		58	IR60080-46-A × CG14	
	17	RAM90				
	18	RAM111				
	19	RAM118				
	20	RAM120				
	21	RAM121				
	22	RAM131				
	23	RAM134				
	24	RAM152				
	25	RAM163				
	26	IG10	Ivory coast			
	27	CG14				
	28	CG17				
	29	CG20				
	30	Acc.103477				
	31	TOG5674				
	32	TOG5681				
	33	TOG5860	Africa			
	34	TOG6472				
	35	TOG6508				
	36	TOG6589				
	37	TOG6597	Ivory coast			
	38	TOG6629				
	39	TOG6631	Africa			
	40	TOG7235				
	41	TOG7291				
	42	TOG7442				

Entry no. 43- 58 are F_1 progenies between *O. sativa* × *O. glaberrima.*

exception. A marker with SS scores equal to or higher than 93.3 (up to 2 exceptions out of the total number of accessions) was regarded as SS marker. In addition, the Subspecies-Prototype (SP) degree for each accession was calculated in order to describe the relative genomic inclination of each accession toward either subspecies as follows:

Subspecies-prototype (SP) degree = Total number of *japonica* SS alleles in each accession - Total number of *Indica* SS alleles in each accession of each accession / Total number of SS markers tested

If a variety has a SP degree close to 1 or -1, the variety is estimated to have the genomic constitution close to the prototype of *japonica* or *indica*, respectively.

RESULTS

Genotyping by subspecies-specific (SS) markers

The information of 67 SS STS markers used in this study is summarized in supplementary Table 1. For a marker to be confirmed as SS, a threshold of 93.3% was set. There was only one SS markers detected on chromosome 6, while 11 SS markers were identified on chromosome 3. The average number of SS markers was 5.6 per chromosome The BAC/PAC clones from which STS markers were originated and the marker location within BAC/PAC clones were denoted in a sequence order of base pairs. The SS markers which showed perfect SS scores were S01022, S02026, S02140, S03020, S03041, S04128, S06001, S07011, S09026B, S10003A, S11004A, S11006A and S12011B.

Classification of *O. glaberrima* accessions by SS markers

Figure 1 show the gel profile of an SS- STS marker applied to amplify 12 *O.sativa* varieties, 30 *O. glaberrima* accessions, and 16 F_1 progenies between *O.sativa* × *O. glaberrima.* As expected, most of the SS markers detected *O. sativa* alleles with only 7% (10 markers) detecting *O. glaberrima*-specific allele (Table 2). Estimated size of alleles present in only *O. glaberrima* ranged from 160 bp in SS marker S10003A to 610 bp in marker S11004A. Two SS markers S02085 and S02140 did not detect any *indica-japonica* alleles among the *O. glaberrima* accessions (Table 2).

The average value of *Indica*-prototype index which is similar to *indica* varieties of *O .sativa* (IPI) was about 50% for each *O. glaberrima* accessions and between 80 to 90% among *indica* varieties from IRRI (Figure 2). No IPI was observed in the *japonica* varieties of Korean origin while the IRRI type had about 15%. The 2 *japonicas* and the 5 *indica* varieties were similar in allelic composition to the IRRI varieties, even though they might have different plant types (Figure 2, Supplementary Tables 2 and 3); The subspecies-prototype index (SPI) of *O. glaberrima* accessions ranged between 51.67- 60.00, while the japonica species had very low SPI (0 to 13.24) (Supplementary table 2).

A total of 10 subgroups were identified based on 6 informative markers, called reference markers, S01054, S01160, S02085, S02140, S03041, and S08107 (Table 3). Each of the 10 subgroups revealed different markers showing the kind of mutation occurring at that specific locus (either as an *indica/japonica* allele mutating to *japonica, indica* or *O. glaberrima* alleles). For example in group IV, the *O. glaberrima* allele mutated from *japonica* allele as revealed by marker S01160. Also *O. glaberrima* accession TOG5674 could be distinguished from CG14 by the presence of additional *indica* allele revealed by marker S02085 and specific allele by S02140. Twenty-nine of the 30 *O. glaberrima* accessions (except TOG 6629) were observed to be segregating for different alleles (Table 3).

Forty-two percent SS markers detected heterozygous alleles between *japonica/glaberrima* in the F_1 progenies; whilst 34% markers also detected heterozygous alleles between *indica/glaberrima* in the F_1 progenies (Table 4 and Supplementary Tables 3). Some makers (13%) did not detect heterozygous alleles in the F_1 between *O.sativa* and *O. glaberrima* species; whilst others such as S09093A could not distinguish the heterozygous alleles between *indica and glaberrima*.

Comparative view of genome of *O. glaberrima* based on *O. sativa* spp *japonica* genome

A total of 38 loci among the *O. glaberrima* accessions had only *indica* alleles and 26 loci had only *Japonica* alleles, whilst only 1 loci showed both *indica* and *japonica* alleles (Figure 3). Some non-*sativa* alleles were detected on chromosome 1, 2, 9, 10, 11, and 12. Heterozygous alleles of *indica* and *glaberrima* (G+I) were identified on 3 loci on chromosomes 1, 2 and 3. Markers on inter-sub specific hybrid sterility QTLs, S05014B and RM413 on chromosome 5 and S08066 on chromosome 8, showed *indica* alleles in 29 O. *glaberrima* accessions.

DISCUSSION

Allele frequency of 30 *O. glaberrima* accessions

A small proportion of the SS-STS markers tested (10 in all=14.9% 10 in 67) did not amplify *O. sativa* allele but rather *O. glaberrima* specific alleles, and consist of 6 to 7 glaberrima specific alleles. Polymorphism between *O. glaberrima* at the DNA level has been reported to be low; few polymorphisms (37 to 41%) could be detected by Enriquez et al. (2001) using SSR markers. Further, Bimpong et al. (2004) observed 38% polymorphism between *O. sativa* × *O. glaberrima* parents using SSR markers. Those SS markers that detected *O. glaberrima* alleles might be related to the evolution of O. *glaberrima.* Very few STS markers have been used to detect polymorphism among *O. glaberrima* species. SSR have been used widely in genetic diversity studies (Semon et al., 2005; Garris et al., 2004; Senior, 1998); however, much work has not been done on the use of STS to detect genetic diversity in *O. glaberrima*. Bimpong et al. (2009) detected 9.9% (16 out of 162 STS markers) polymorphism between 10 accessions of *O. glaberrima* using STS markers. The other primers (54.5%) amplified *indica, japonica* or unspecific alleles in some genotypes. The lack of amplification of products in some SS markers

Supplementary Table 1. List of subspecies-specific primers.

Chromosome	Marker	Physical location in Nipponbare pseudomolecule(bp)		Expected size of amplicon (bp)		Primer sequence		BAC clone 1)	SS score2 (%)
		start	stop	*Japonica*	*Indica*	Forward	Reverse		
1	S01022	4384676	4384975	300	312	catggatgatgcttccctct	ttgacagtggctccacaaag	AP002484	100
	S01054	10309451	10309687	237	266	gcgaagcctgctttttgat	cggagatttttccctaaaacaa	AP002070	96.7
	S01140	35147646	35147820	175	187	gctaggcagactctagctcatca	tggaacaagtagaagcagaagtca	AP003411	93.3
	S01157B	39802962	39803196	235	223	ccctcaatcatcgcaactgt	cagatgcagaaaagcgcata	AP006531	93.3
	S01160	40802478	40802660	183	179	ttgcgatttatttgccagtg	ccaggcatccaatgcttatt	AP004672	96.7
2	S02026	5345560	5345726	167	180	tggtccatcatattgccaac	tcctctcagatccgattttca	AP004184	100
	S02052	12020182	12020373	192	201	gcagtcggttctcaattggt	gattttccagcccattctca	AP005743	93.3
	S02054	14117145	14117316	172	157	tttgaagcacgagggatctt	ataaaggaccgatgcaaacg	AP004856	93.3
	S02057B	17440500	17440729	230	241	agcctcttctccctcctcac	tgcaaacaccataacaaccaa	AP004999	93.3
	S02081B	20964659	20964883	225	201	agcggcatattttgcatagc	tgttttgcaggacgcagtag	AP004876	96.7
	S02085	21636396	21636559	164	153	gcgagagtgtacccctttga	tgtgtaccttgcaccctgaa	AP006068	96.7
	S02140	32850429	32850633	205	220	tgggaggaggatattgtgga	tgacaggttgatgtgatggaa	AP005538	100
3	S03010B	2098371	2098585	215	203	gtgcggatttggttctgttt	gagggagaggccagattctt	AC118132	93.3
	S03020	4302802	4302984	183	168	tttctaggtacattttaagcaagca	catgaatttgaagctgcgagta	AC126223	100
	S03027	5713283	5713531	249	232	tgaacattttggtcgtctgg	ttgacgaagtcaccatagacg	AC105928	96.7
	S03041	8900833	8901024	192	201	gctgacattgtccgaggttt	ccgacgtccaacctaagc	AC139168	100
	S03046	10137125	10137381	257	248	tcacagtttacaggcggaatc	gcaccatgtatagaccattcca	AC137634	93.3
	S03048	10754658	10754836	179	159	gggatgggagaagggaataa	gccagctaggatgttgaagg	AC137267	96.7
	S03096	24299548	24299716	169	183	cacttgcaagctaagcacca	ccttcctgcttgacgagaaa	AC120505	96.7
	S03099	24995662	24995883	222	233	ctcccaggatgctcactcag	ataatccaagggcacagcac	AL731878	96.7
	S03120	27366848	27367089	242	254	tgtgcgctcgtgattatttt	aaggggagcagataatgcag	AC092779	96.7
	S03136	30109023	30109222	200	219	gcattaaggcacacaaagca	tgtttgtaatccgcatggaa	AC118133	96.7
	S03145	32174684	32174922	239	251	tcacctacaggaagcagcag	gccgtcgttgaagaagtagc	AC091494	93.3
4	S04058	20162588	20162832	245	226	gatccatgcagttgattgtga	tcgtcttatctaaaaagaaaatttga	AL662947	96.7
	S04060	20474915	20475135	221	203	tatggttttatccgccaacc	gctacaactaaaacaagaaacgtga	AL606598	96.7
	S04077B	24949310	24949483	174	201	atgtggatggtgggtcctat	agggttcatcgctaatgctg	AL606604	93.3
	S04077A	24958459	24958724	266	247	tcccaggtgaactacggact	cagcattttcagtggaagca	AL606604	93.3
	S04087A	27761378	27761633	256	248	atgtttggcaatccgctaag	aaagatggttgagcggaaga	AL606682	96.7
	S04097B	29346635	29346813	179	189	tccacagtctgtccgtgaaa	ctccttgtgctgcagaattg	AL662957	93.3
	S04128	34569925	34570087	163	181	tcacgggaaagctttggtat	aacttatgcagccaccatcc	AL606456	100
	S04129B	35102075	35102256	182	203	aatcgattcattcgcacaaa	ctttcatgctctgccattga	AL606637	93.3

Supplementary Table 1. Contd.

5	S05064	16966554	16966786	245	257	aaagcaagtcacaaacaaaataaa	tgcctcgattttcataagca	AC104272	93.3
	S05080A	20663155	20663383	229	254	tggccaactttgggaattta	aagagtcgtgcaaatgaaaaga	AC109595	96.7
6	S06001	546207	546437	231	244	agctcaatatcaggcaagcag	aaatgacacagttgaccttttgaa	AP000616	100
7	S07011	2543283	2543511	229	205	ctggatccaaggcatcattc	cttcgctctcaccatcaaca	AP004263	100
	S07048	8487589	8487745	157	172	catggcaccttgagagttga	acacatggagctggcttctc	AP005824	96.7
	S07050C	13437934	13438142	209	225	ctccacttatggcagcgaat	caagtgaagtgggagcaggt	AP003745	93.3
	S07050A	14531440	14531638	199	211	tacacgaacgaacgacaagg	cgctgatttgggtaggtctc	AP005200	96.7
	S07101	26848149	26848350	202	216	gcatgccaggatatggtctc	tcggtacacacctcctgtga	AP003832	93.3
	S07103	27558599	27558809	211	223	agcatggatccttcatccaa	actccgattttgcacttcg	AP005182	93.3
8	S08066	18904657	18904874	218	238	ttgttccgttgtgtgcaact	gatgcagcgacgtgaaatc	AP003947	96.7
	S08090	23079842	23080054	213	231	gcgtgtggaagaggagaaag	cagtgagaatctcgcagtcg	AP004693	96.7
	S08106	25773305	25773524	220	194	ttacggattgtcacggtttt	ggaatttgtcactggtttcca	AP005509	96.7
	S08107	25956924	25957152	229	240	ttggtaatgcccatgctaga	cacgattcggtcatttcaga	AP003888	93.3
9	S09000A	244321	244528	208	221	ccaattcacggtttaacaagg	gccatgaagcttcgttagga	AP006058	96.7
	S09026B	9142928	9143141	214	189	gggaggcagagggaactact	ttatcaggccaggtcctttg	AP005780	100
	S09040B	12641376	12641601	226	214	taatatcgcatggcaagacg	actttgcagaggcgacaaac	AP005637	96.7
	S09058	15942709	15942930	222	233	cgtgagaagtccagtccaca	attgatcgattgggggattt	AP005551	93.3
	S09062B	16864607	16864856	250	236	acgcataccgaatgtgacag	gttggcactcccgattaaaa	AP005559	93.3
	S09065	17914403	17914639	237	246	tgtgttcgacgtttgaccat	gggccagggtacattgaata	AP005574	96.7
	S09073	19077948	19078180	233	250	accaccctgaaccacaacat	tcactgggttctgtgtccaa	AC099403	96.7
	S09075B	19519638	19519866	229	211	gactaacgaacggggcctat	ggcagcccacactatttagg	AC108753	96.7
	S09075A	19575874	19576047	174	154	cctcactcacctggagaagg	cgtccacactaacggacaca	AC108753	93.3
	S09093A	22803693	22803950	258	232	caccgctctcactgtcattc	tccctcagccataaaaccag	AP006162	96.7
10	S10001	992379	992586	208	229	atcgtggtcgggattatgag	gcatcatggcttttgtgttg	AC078891	96.7
	S10003A	1715981	1716214	234	246	ataagacggacggtcaaacg	atctcttgtgggctttgtgg	AC025098	100
	S10013A	5180767	5180949	183	170	agtcgggtcatttcttagcc	ctacgtcctccgtttcacaa	AC083944	93.3
	S10019	10299169	10299319	151	163	atgcatctacatggcatttg	gatgctgagatgcgattgaa	AC123594	93.3
	S10026C	13594825	13595071	247	227	tacgtgtccttgtgcctgaa	tttcacccccactgtaaagg	AC021893	93.3
	S10071	20926684	20926850	167	158	tatggctcaaccctggaaac	cgtgctagtttgttcactgga	AC051633	96.7
	S10072	21129266	21129444	179	203	tgagtgttgcgttgtcttcc	tggtaaggccttgaagatgg	AC020666	96.7
11	S11004A	1081615	1081787	173	157	tctctggccttctactcatgg	ttgtgtttctacttggactcttttt	AC136970	100
	S11006A	1270331	1270591	261	248	atgcgccgtccaacttatac	tggtgcaaaggaatgaacaa	AC123525	100
	S11028	5463772	5463946	175	186	attcccctggggtagctaga	atgggtgaattgcagagaat	AC123523	96.7

Supplementary Table 1. Contd.

12	S12011B	1884649	1884804	156	177	tgggggagttctgaaatctg	ttaagttcggtgccccataa	AL935154	100
	S12030	3843516	3843732	217	235	tccacatgtaaaccgctgaa	tgagtgatataacaacacacaacca	AL732376	96.7
	S12109B	27415607	27415770	164	173	ggactcggataaccgcatta	ggaacgcagcgaaagaat	AL732378	93.3

1BAC clone information is available at International Rice Genome Sequencing Project (IRGSP). g Threshold of SS score for identification of SS marker was 93.3 (%) (See Materials and Methods).

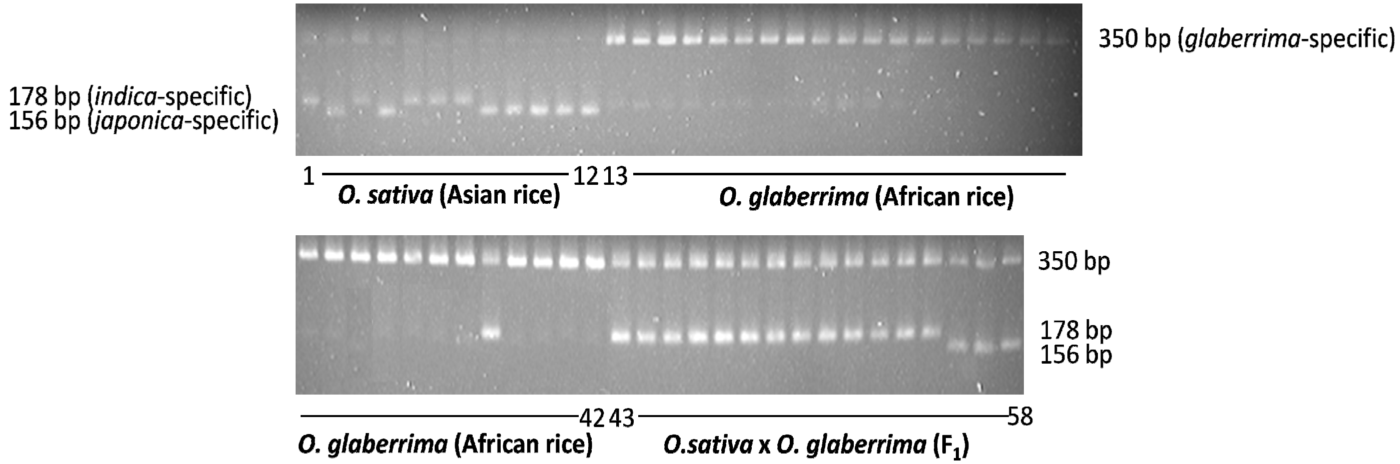

Figure 1. Genotyping of *O. glaberrima* accessions by subspecies-specific (SS) STS markers. (a) 1 to 12 : *O. sativa* (*indica* allele : 178bp and *japonica* allele : 156 bp). 13 to 42: *O. glaberrima* (350 bp non-*O.sativa* allele). (b) 43 to 58 : showed both the alleles of *sativa* and *glaberrima*, but the band size of F1's were different, implying the amplified regions of *O. sativa* and *O. glaberrima* were neighbored.

markers has been a common obser-vation in the STS markers (Wang et al., 2009; Perry et al., 1999) and it may reflect divergences in the sequences flanking the SS STS loci, leading to the production of possible null alleles, or totally restricting amplification.

The informative markers (called reference markers) used to classify O. *glaberrima* accessions into 10 subgroups (S01054, S01160, S02085, S02140, S03041, and S08107), might have contributed to genomic diversification of *O. glaberrima,* as they were able to show the kind of mutation occurring at that specific locus (either as an *indica/japonica* allele mutated to *japonica, indica* or *O. glaberrima* alleles); suggesting a continuous mutation of some SS markers were associated in terms of inheritance; and might have contributed to genomic diversification of *O. glaberrima*.. For example, 'TOG5674' can be distinguished from 'CG14' by additional *indica* allele of S02085 and specific allele of S02140.

Whole genome analysis has revealed the structural similarities between *O sativa* and *O. glaberrima* species, (Park et al., 2003,***)*** but due to the high frequency of polymorphism in subspecies-specific (SS) loci, it is assumed that there is a relationship between *sativa* × *glaberrima* F1 hybrid sterility and SS loci. *O. glaberrima* had *indica* alleles at two loci associated with inter-subspecific F_1 hybrid sterility on chromosome 5 and chromosome 8 (Figure 3).

Application of SS-STS markers to study the relations within *O. glaberrima* species

The SS STSs markers used in the study were able to revealed different alleles both from *japonica* and *indica* sources among the *O. glaberrima* accessions. The efficiency of STS

Table 2. Subspecies-specific STS markers generating *glaberrima* (G)-specific alleles.

Name of varieties/lines	S01160	S02085	S02140	S03145	S09093A	S10003A	S10013A	S11004A	S12011B
IR55423-01	J1)	I	I	I	I	I	I	J	I
IR60080-46-A	I	J	J	J	J	J	I	J	J
IR64	I	I	I	I	I	I	I	I	I
IR68703-AC-24-1	I	J	J	J	J	J	I	J	J
IR69502-6-SRN	I	I	I	I	I	I	I	I	I
PSBRC18	J	I	I	I	I	I	I	I	I
PSBRC82	I	I	I	I	I	I	I	I	I
Hwacheongbyeo	J	J	J	J	J	J	J	J	J
Ilpumbyeo	J	J	J	J	J	J	J	J	J
Jinmibyeo	J	J	J	J	J	J	J	J	J
Junambyeo	J	J	J	J	J	J	J	J	J
TR22183	J	J	J	G+J	J	J	J	J	J
RAM3	G	G	I	G+I	G	G	G	G	G
RAM24	G	G	I	G+I	G	G	G	G	G
RAM54	G	G	I	G+I	G	G	G	G	G
RAM86	G	G	I	G+I	G	G	G	G	G
RAM90	G	G	I	G+I	G	G	G	G	G
RAM111	G	G	I	G+I	G	G	G	G	G
RAM118	G	G	I	G+I	G	G	G	G	G
RAM120	G	G	I	G+I	G	G	G	G	G
RAM121	G	G	I	G+I	G	G	G	G	G
RAM131	G	G	I	G+I	G	G	G	G	G
RAM134	G	G	I	G+I	G	G	G	G	G
RAM152	G	G	I	G+I	G	G	G	G	G
RAM163	G	G	I	G+I	G	G	G	G	G
IG10	G	G	I	G+I	G	G	G	G	G
CG14	G	G	I	G+I	G	G	G	G	G
CG17	G	G	I	G+I	G	G	G	G	G
CG20	J	G	I	G+I	G	G	G	G	G
Acc.103477	G	G	I	G+I	G	G	G	G	G
TOG5674	I	G+I	G	G+I	G	G	G	G	G
TOG5681	I	G+I	I	G+I	G	G	G	G	G
TOG5860	I	G	I	G+I	G	G	G	G	G
TOG6472	I	G	I	G+I	G	G	G	G	G
TOG6508	G+I	G	I	G+I	G	G	G	G	G
TOG6589	G+I	G	I	G+I	G	G	G	G	G
TOG6597	G+I	G	I	-	G	G	G	G	G
TOG6629	G+I	G+I	I	-	I	G+I	G+I	J	G+I
TOG6631	I	G	I	G+I	G	G	G	G	G
TOG7235	I	G	I	G+I	G	G	G	G	G
TOG7291	I	G	G	G+I	G	G	G	G	G
TOG7442	I	G	I	G+I	G	G	G	G	G
IR64 × RAM54	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR64 × RAM86	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR64 × RAM90	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR64 × RAM120	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR64 × RAM134	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR64 × RAM131	G+I	G+I	I	-	I	G+I	G+I	G+I	G+I
PSBRC18 × RAM111	G+J	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR55423-01 × RAM3	G+J	G+I	I	G+I	I	G+I	G+I	G+J	G+I
IR55423-01 × RAM24	G+J	G+I	I	G+I	I	G+I	G+I	G+J	G+I

Table 2. Contd.

IR55423-01 × RAM163	G+J	G+I	I	G+I	I	G+I	G+I	G+J	G+I
IR69502 × RAM118	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR69502 × RAM121	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR69502 × RAM163	G+I	G+I	I	G+I	I	G+I	G+I	G+I	G+I
IR60080-46-A × IG10	G+I	G+J	H	G+J	G+J	G+J	G+I	G+J	G+J
IR68703-AC-24-1 × CG14	I	G+J	H	G+J	G+J	G+I	G+I	G+J	G+J
IR60080-46-A × CG14	I	G+J	H	G+J	G+J	G+J	G+J	G+J	G+J
Alleles in *glaberrima*[2] (bp)	176	NULL	NULL	270 and 290	240	300	160	610	350

[1] J: *japonica*-specific allele, I: *indica*-specific allele, G: alleles-present in *O. glaberrima* accessions, G+I: alleles in glaberrima and indica allele, G+J: alleles in glaberrima and japonica allele. [2] Estimated size of alleles only found in *O. glaberrima* not in *O. sativa* in basepair (bp). 'NULL' represents no amplification of *indica-japonica* alleles in *O. glaberrima* accessions.

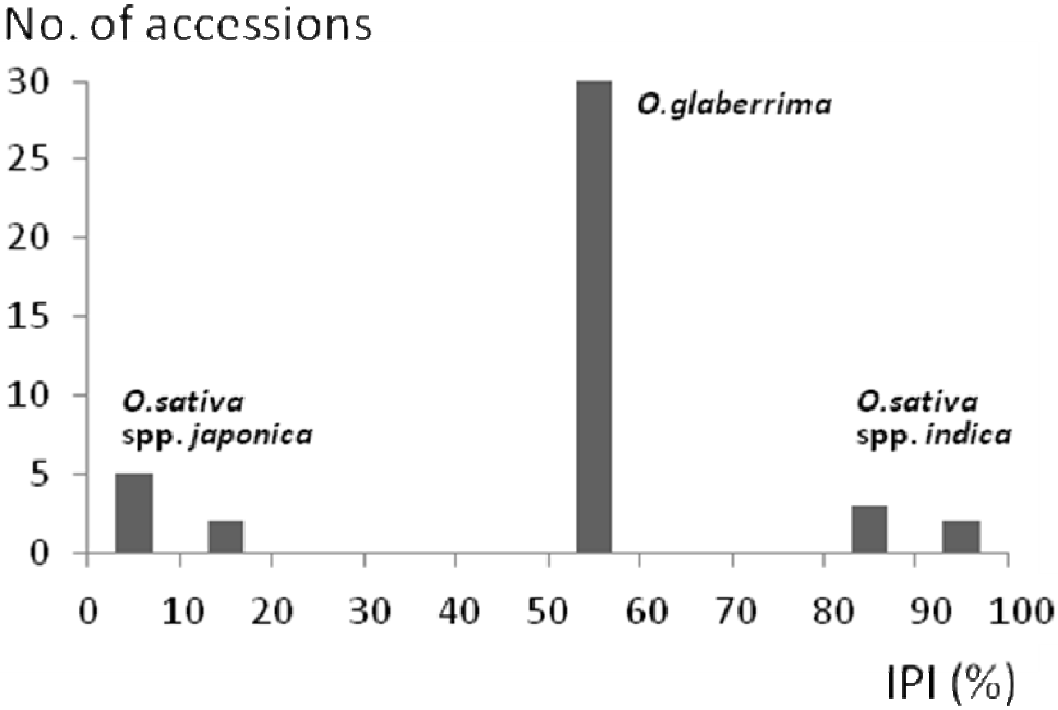

Figure 2. IPI (*Indica*-prototype index: similarity to *indica* varieties of *O.sativa*) of 30 *O. glaberrima* accessions.

Table 3. Successful polymorphic markers between *O. glaberrima* and *O. sativa.*

Subspecies of *O.sativa*[1]	**Chromosome**													**Marker frequency[2]**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**	
indica	3	2	4	1	0	0	3	1	4	3	1	1	23	34.33%
japonica	1	3	5	6	2	1	3	1	5	0	0	1	28	41.79%
indica/japonica	1	1	1	0	0	0	0	0	0	2	1	1	7	10.45%
-	0	1	1	1	0	0	0	2	1	2	1	0	9	13.43%

[1] Two bands from parents represent hetero alleles were successfully amplified when *O.glaberrima* accessions were crossed with corresponding subspecies of *O.sativa*. [2] Total number of markers out of total of 67 subspecies-specific STS markers.

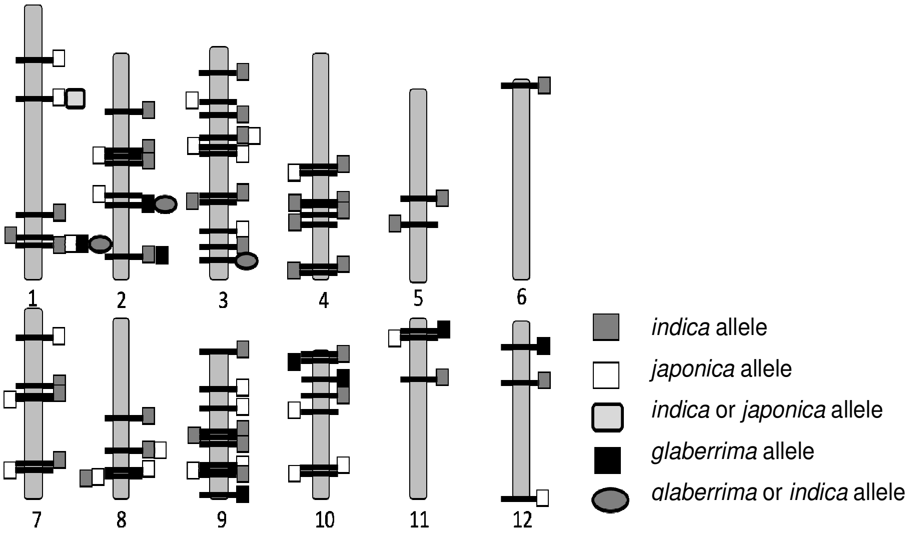

Figure 3. Comparative view of alleles of *O.glaberrima* based on Nipponbare genome.

Table 4. Reference markers classifying 30 *O.glaberrima* accessions.

Group	Name	I	J	-	G	H	G+I	G+J	SPI=I/(I+J+H)	Reference markers		Allele change from G1[1]
G1	RAM86	36	24	0	7	0	1	0	60.00			
	RAM90	36	24	0	7	0	1	0	60.00			
	RAM111	36	24	0	7	0	1	0	60.00			
	RAM118	36	24	0	7	0	1	0	60.00			
	RAM120	36	24	0	7	0	1	0	60.00			
	RAM121	36	24	0	7	0	1	0	60.00			
	RAM131	36	24	0	7	0	1	0	60.00			
	RAM134	36	24	0	7	0	1	0	60.00			
	RAM152	36	24	0	7	0	1	0	60.00			
	RAM163	36	24	0	7	0	1	0	60.00			
	IG10	34	24	2	7	0	1	0	58.62			
	CG14	36	24	0	7	0	1	0	60.00			
	CG17	36	24	0	7	0	1	0	60.00			
	TOG6472	35	25	1	6	0	1	0	58.33			
	TOG7235	36	24	1	6	0	1	0	60.00			
G2	RAM3	35	25	0	7	0	1	0	58.33	S08107		I->J
	RAM24	35	25	0	7	0	1	0	58.33			
	RAM54	35	25	0	7	0	1	0	58.33			
G3	Acc.103477	35	25	0	7	0	1	0	58.33	S03041		I->J
G4	CG20	36	25	0	6	0	1	0	59.02	S01160		G->J
G5	TOG5681	35	25	1	5	0	2	0	58.33	S02085		G->G+I
G6	TOG5674	34	25	1	6	0	2	0	57.63	S02085	S02140	G->G+I, I->G
G7	TOG6508	34	25	1	6	0	2	0	57.63	S01160		I->G+I
	TOG6589	34	24	2	6	0	2	0	58.62			
G8	TOG5860	35	23	2	6	1	1	0	59.32	S01054		J->H
	TOG6631	36	24	0	6	1	1	0	59.02			
	TOG7442	36	24	0	6	1	1	0	59.02			
G9	TOG6597	33	22	5	6	1	1	0	58.93	S01054	S01160	J->H, G->G+I
G10	TOG7291	35	24	0	7	1	1	0	58.33	S01054	S02140	J->H, I->G

[1] *O. glaberrima* accessions in different groups can be distinguished using corresponding reference markers by observation of allele change. For example, 'TOG5674' can be distinguished from 'CG14' by additional *indica* allele of S02085 and specific allele of S02140.

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study.

Accession name	**Entry No.**	**S01022**	**S01054**	**S01140**	**S01157B**	**S01160**	**S02026**	**S02052**	**S02054**	**S02057B**	**S02081B**	**S02085**	**S02140**	**S03010B**	**S03020**	**S03027**	**S03041**	**S03046**	**S03048**	**S03096**	**S03099**	**S03115**	**S03120**	**S03136**
IR55423-01	1	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
IR60080-46-A	2	J	J	J	I	I	J	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J
IR64	3	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
IR68703-AC-24-1	4	J	J	J	I	I	I	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J
IR69502-6-SRN	5	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	J	I	I
PSBRC18	6	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
PSBRC82	7	I	I	I	I	I	I	I	I	P	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Hwacheongbyeo	8	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Ilpumbyeo	9	J	J	-	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Jinmibyeo	10	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Junambyeo	11	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
TR22183	12	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
RAM3	13	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM24	14	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM54	15	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM86	16	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM90	17	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM111	18	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM118	19	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM120	20	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM121	21	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM131	22	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM134	23	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM152	24	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
RAM163	25	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
IG10	26	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
CG14	27	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
CG17	28	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
CG20	29	J	J	I	J	J	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
Acc.103477	30	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	J	J	J	I	I	J	J	I
TOG5674	31	J	J	I	J	I	I	I	J	I	J	G+I	G	I	J	I	I	J	J	I	I	J	J	I
TOG5681	32	J	J	I	J	I	I	I	J	I	J	G+I	I	I	J	I	I	J	J	I	I	J	J	I
TOG5860	33	J	H	I	J	I	I	I	J	I	-	G	I	I	J	I	I	J	J	I	I	J	J	I
TOG6472	34	J	J	I	J	I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (contd).

TOG6508	35	J	J	I	J	G+I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
TOG6589	36	J	J	I	J	G+I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
TOG6597	37	J	H	I	J	G+I	I	I	J	I	J	G	I	I	J	I	-	J	J	I	I	J	J	I
TOG6629	38	H	H	I	J	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
TOG6631	39	J	H	I	J	I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
TOG7235	40	J	J	I	-	I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
TOG7291	41	J	H	I	J	I	I	I	J	I	J	G	G	I	J	I	I	J	J	I	I	J	J	I
TOG7442	42	J	H	I	J	I	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	J	I
IR64×RAM54	43	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR64×RAM86	44	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR64×RAM90	45	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR64×RAM120	46	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR64×RAM134	47	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR64×RAM131	48	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	H	I
PSBRC18×RAM111	49	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	H	I
IR55423-01×RAM3	50	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	H	I
IR55423-01×RAM24	51	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR55423-01×RAM163	52	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	H	I
IR69502×RAM118	53	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	J	H	I
IR69502×RAM121	54	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	J	H	I
IR69502×RAM163	55	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	J	H	I
IR60080-46-A×IG10	56	J	J	H	H	G+I	-	H	J	H	J	G+J	H	H	J	H	H	H	G+J	H	H	J	J	H
IR68703-AC-24-1×CG14	57	J	J	H	H	I	-	H	J	H	J	G+J	H	H	J	H	H	J	G+J	I	H	J	J	H
IR60080-46-A×CG14	58	J	J	H	H	I	-	I	H	H	H	G+J	H	H	J	H	H	J	G+J	I	I	J	J	H
Specific alleles (Neither I or J)						G=176			I=H	P=260	N	N		I=H					Hetero G					

* Alleles: I:*indica*; J: *japonica*; H: hetero (I and J); G: *glaberrima*-specific; N: non-amplified allele of *O.glaberrima*; P: TR22183 specific; S: smear bands

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (continued).

Accession Name	**Entry No.**	**S03145**	**S04058**	**S04060**	**S04077A**	**S04077B**	**S04087A**	**S04097B**	**S04128**	**S04129B**	**S05064**	**S05080A**	**S06001**	**S07011**	**S07048**	**S07050C**	**S07050A**	**S07101**	**S07103**	**S08066**	**S08090**	**S08106**	**S08107**
IR55423-01	1	I	I	I	I	I	-	I	I	I	I	I	J	I	I	I	-	I	I	I	I	I	I
IR60080-46-A	2	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J	I	J	J	J

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (continued).

IR64	3	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
IR68703-AC-24-1	4	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J	I	J	J	J
IR69502-6-SRN	5	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
PSBRC18	6	I	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I
PSBRC82	7	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Hwacheongbyeo	8	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Ilpumbyeo	9	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Jinmibyeo	10	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Junambyeo	11	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J
TR22183	12	G+J	J	J	J	J	J	J	J	J	J	J	J	J	I	I	I	J	J	J	J	-	J
RAM3	13	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J
RAM24	14	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J
RAM54	15	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J
RAM86	16	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM90	17	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM111	18	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM118	19	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM120	20	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM121	21	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM131	22	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM134	23	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM152	24	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
RAM163	25	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
IG10	26	G+I	I	J	I	I	I	I	I	-	-	I	I	J	I	I	J	I	J	I	I	J	I
CG14	27	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
CG17	28	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
CG20	29	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
Acc.103477	30	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I
TOG5674	31	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	-
TOG5681	32	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	-
TOG5860	33	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	-
TOG6472	34	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	-
TOG6508	35	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	-
TOG6589	36	G+I	I	J	I	I	I	I	I	I	I	I	I	-	I	I	J	I	J	I	J	J	-
TOG6597	37	-	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	-	I	J	J	I
TOG6629	38	-	I	H	I	I	I	I	I	-	I	I	H	H	I	H	H	I	H	I	I	H	I
TOG6631	39	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	I

Supplementary Table 2. Contd.

TOG7235	40	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	I
TOG7291	41	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	I
TOG7442	42	G+I	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	J	J	I
IR64×RAM54	43	G+I	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	H
IR64×RAM86	44	G+I	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I
IR64×RAM90	45	G+I	I	H	I	I	I	I	I	I	I	I	I	H	-	I	H	I	H	I	I	H	I
IR64×RAM120	46	G+I	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I
IR64×RAM134	47	G+I	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I
IR64×RAM131	48	-	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I
PSBRC18×RAM111	49	G+I	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	-	I	H	I
IR55423-01×RAM3	50	G+I	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I
IR55423-01×RAM24	51	G+I	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I
IR55423-01×RAM163	52	G+I	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I
IR69502×RAM118	53	G+I	G	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I
IR69502×RAM121	54	G+I	G	H	I	I	I	I	-	I	I	I	H	H	I	I	H	I	H	I	I	H	I
IR69502×RAM163	55	G+I	G	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I
IR60080-46-A×IG10	56	S	S	H	H	H	H	H	H	H	H	H	I	J	I	I	J	H	J	H	J	G+H	J
IR68703-AC-24-1×CG14	57	S	S	H	H	H	H	H	I	I	H	H	H	J	H	H	J	H	J	I	J	G+H	J
IR60080-46-A×CG14	58	S	S	H	H	H	H	H	H	H	H	H	H	J	H	H	J	H	J	H	J	G+H	J
Specific alleles (Neither I nor J)		G=270,290	Hetero G																				

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (continued).

Accession Name	**Entry No.**	**S09000A**	**S09026B**	**S09040B**	**S09058**	**S09062B**	**S09065**	**S09073**	**S09075A**	**S09075B**	**S09093A**	**S10001**	**S10003A**	**S10013A**	**S10019**	**S10026C**	**S10071**	**S10072**	**S11004A**	**S11006A**	**S11028**	**S12011B**	**S12030**	**S12109B**
IR55423-01	1	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I
IR60080-46-A	2	J	J	J	J	J	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J
IR64	3	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
IR68703-AC-24-1	4	J	J	J	J	J	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J
IR69502-6-SRN	5	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
PSBRC18	6	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (continued).

BRC82	7	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Hwacheongbyeo	8	J	J	I	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Ilpumbyeo	9	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
Jinmibyeo	10	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	-	J
Junambyeo	11	I	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J
TR22183	12	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J	J	J
RAM3	13	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM24	14	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM54	15	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM86	16	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM90	17	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM111	18	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM118	19	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM120	20	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM121	21	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM131	22	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM134	23	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM152	24	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
RAM163	25	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
IG10	26	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
CG14	27	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
CG17	28	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
CG20	29	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
Acc.103477	30	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG5674	31	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG5681	32	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG5860	33	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG6472	34	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG6508	35	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG6589	36	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG6597	37	I	J	-	I	-	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG6629	38	I	H	-	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	J	H	I	G+I	I	H
TOG6631	39	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG7235	40	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG7291	41	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
TOG7442	42	I	J	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
IR64×RAM54	43	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H

Supplementary Table 2. Genotyping results with 67 SS-STS markers in this study (continued).

IR64×RAM86	44	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR64×RAM90	45	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR64×RAM120	46	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR64×RAM134	47	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR64×RAM131	48	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
PSBRC18×RAM111	49	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR55423-01×RAM3	50	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
IR55423-01×RAM24	51	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
IR55423-01×RAM163	52	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
IR69502×RAM118	53	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR69502×RAM121	54	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR69502×RAM163	55	I	H	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
IR60080-46-A×IG10	56	J	J	J	I	H	H	J	J	H	G+J	I	G+J	G+I	H	J	J	J	G+J	J	I	G+J	I	J
IR68703-AC-24-1×CG14	57	J	J	J	H	H	H	J	J	H	G+J	H	G+I	G+I	H	J	J	J	G+J	J	H	G+J	H	J
IR60080-46-A×CG14	58	J	J	J	H	H	H	J	J	H	G+J	I	G+J	G+J	I	J	J	J	G+J	J	I	G+J	H	J
Specific alleles (Neither I nor J)											G=240		G=300	G=160					G=610			G=350		

* Alleles: I:*indica*; J: *japonica*; H: hetero (I and J); G: *glaberrima*-specific; N: non-amplified allele of *O.glaberrima*; P: TR22183 specific; S: smear bands

Supplementary Table 3. Allele constitution of parents and F$_1$ progenies to elucidate useful polymorphic markers in *O. sativa × O. glaberrima* breeding programs.

Crosses	**Entry No.**	**Description**	**S01022**	**S01054**	**S01140**	**S01157B**	**S01160**	**S02026**	**S02052**	**S02054**	**S02057B**	**S02081B**	**S02085**	**S02140**	**S03010B**	**S03020**	**S03027**	**S03041**	**S03046**	**S03048**	**S03096**	**S03099**	**S03120**	**S03136**	**S03145**
Indica × Glaberrima	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	15	RAM54	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	43	IR64×RAM54	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	16	RAM86	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	44	IR64×RAM86	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	17	RAM90	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	45	IR64×RAM90	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	20	RAM120	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I

Supplementary Table 3. Contd.

Indica × *Glaberrima*	46	IR64×RAM120	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	23	RAM134	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	47	IR64×RAM134	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	22	RAM131	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	48	IR64×RAM131	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	I	-
	6	PSBRC18	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	18	RAM111	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	49	PSBRC18×RAM111	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	I	G+I
	1	IR55423-01	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	13	RAM3	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	50	IR55423-01×RAM3	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	-	H	H	I	I	H	I	G+I
	1	IR55423-01	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	14	RAM24	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	51	IR55423-01×RAM24	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	1	IR55423-01	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	25	RAM163	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	52	IR55423-01×RAM163	H	H	I	H	G+J	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	19	RAM118	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	53	IR69502×RAM118	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	21	RAM121	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	54	IR69502×RAM121	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	25	RAM163	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	55	IR69502×RAM163	H	H	I	H	G+I	I	I	H	I	H	G+I	I	I	H	I	I	H	H	I	I	H	I	G+I
Japonica × *Glaberrima*	2	IR60080-46-A	J	J	J	I	I	J	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J
	26	IG10	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	56	IR60080-46-A×IG10	J	J	H	H	G+I	-	H	J	H	J	G+J	H	H	H	H	H	H	G+J	H	H	J	H	S
	4	IR68703-AC-24-1	J	J	J	I	I	I	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J
	27	CG14	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	57	IR68703-AC-24-1×CG14	J	J	H	H	I	-	H	J	H	J	G+J	H	H	J	H	H	J	G+J	I	H	J	H	S
	2	IR60080-46-A	J	J	J	I	I	J	J	J	J	J	J	J	J	J	J	J	J	J	I	J	J	J	J
	27	CG14	J	J	I	J	G	I	I	J	I	J	G	I	I	J	I	I	J	J	I	I	J	I	G+I
	58	IR60080-46-A×CG14	J	J	H	H	I	-	I	H	H	H	G+J	H	H	J	H	H	J	G+J	I	I	J	H	S

Supplementary Table 3. Allele constitution of parents and F_1 progenies to elucidate useful polymorphic markers in *O.sativa* × *O. glaberrima* breeding programs (continued).

Crosses	Entry No.	Description	S04058	S04060	S04077A	S04077B	S04087A	S04097B	S04128	S04129B	S05064	S05080A	S06001	S07011	S07048	S07050C	S07050A	S07101	S07103	S08066	S08090	S08106	S08107	S09000A	S09026B
Indica × Glaberrima	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	15	RAM54	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J	I	J
	43	IR64×RAM54	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	16	RAM86	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	44	IR64×RAM86	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	17	RAM90	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	45	IR64×RAM90	I	H	I	I	I	I	I	I	I	I	I	H	-	I	H	I	H	I	I	H	I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	20	RAM120	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	46	IR64×RAM120	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	23	RAM134	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	47	IR64×RAM134	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	22	RAM131	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	48	IR64×RAM131	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	6	PSBRC18	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I	I	I	I	I	I	I	I
	18	RAM111	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	49	PSBRC18×RAM111	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	-	I	H	I	I	H
	1	IR55423-01	I	I	I	I	-	I	I	I	I	I	J	I	I	I	-	I	I	I	I	I	I	I	I
	13	RAM3	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J	I	J
	50	IR55423-01×RAM3	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I	I	H
	1	IR55423-01	I	I	I	I	-	I	I	I	I	I	J	I	I	I	-	I	I	I	I	I	I	I	I
	14	RAM24	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	J	I	J
	51	IR55423-01×RAM24	I	H	I	I	I	I	I	I	I	I	I	H	I	I	H	I	H	I	I	H	I	I	H
	1	IR55423-01	I	I	I	I	-	I	I	I	I	I	J	I	I	I	-	I	I	I	I	I	I	I	I
	25	RAM163	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	52	IR55423-01×RAM163	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	19	RAM118	I	J	I	I	I	I	I	I	I	I	H	J	I	I	J	I	J	I	I	J	I	I	J

Supplementary Table 3. Contd.

	53	IR69502×RAM118	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	21	RAM121	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	54	IR69502×RAM121	I	-	I	I	I	I	-	I	I	I	H	H	I	I	H	I	H	I	I	H	I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	25	RAM163	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	55	IR69502×RAM163	I	H	I	I	I	I	I	I	I	I	H	H	I	I	H	I	H	I	I	H	I	I	H
Japonica × *Glaberrima*	2	IR60080-46-A	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J	I	J	J	J	J	J
	26	IG10	I	J	I	I	I	I	I	-	-	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	56	IR60080-46-A×IG10	S	H	H	H	H	H	H	H	H	H	I	J	I	I	J	H	J	H	J	G+H	J	J	J
	4	IR68703-AC-24-1	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J	I	J	J	J	J	J
	27	CG14	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	57	IR68703-AC-24-1×CG14	S	H	H	H	H	H	I	I	H	H	H	J	H	H	J	H	J	I	J	G+H	J	J	J
	2	IR60080-46-A	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J	I	J	J	J	J	J
	27	CG14	I	J	I	I	I	I	I	I	I	I	I	J	I	I	J	I	J	I	I	J	I	I	J
	58	IR60080-46-A×CG14	S	H	H	H	H	H	H	H	H	H	H	J	H	H	J	H	J	H	J	G+H	J	J	J

Supplementary Table 3. Allele constitution of parents and F_1 progenies to elucidate useful polymorphic markers in *O.sativa x O.glaberrima* breeding programs (continued).

Crosses	Entry No.	Description	S09040B	S09058	S09062B	S09065	S09073	S09075A	S09075B	S09093A	S10001	S10003A	S10013A	S10019	S10026C	S10071	S10072	S11004A	S11006A	S11028	S12011B	S12030	S12109B
indica×*glaberrima*	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	15	RAM54	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	43	IR64×RAM54	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	16	RAM86	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	44	IR64×RAM86	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	17	RAM90	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	45	IR64×RAM90	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	20	RAM120	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J

Supplementary Table 3. Contd.

indica×*glaberrima*	46	IR64×RAM120	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	23	RAM134	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	47	IR64×RAM134	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	3	IR64	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	22	RAM131	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	48	IR64×RAM131	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	6	PSBRC18	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	18	RAM111	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	49	PSBRC18×RAM111	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	1	IR55423-01	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I
	13	RAM3	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	50	IR55423-01×RAM3	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
	1	IR55423-01	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I
	14	RAM24	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	51	IR55423-01×RAM24	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
	1	IR55423-01	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	J	I	I	I	I	I
	25	RAM163	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	52	IR55423-01×RAM163	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+J	H	I	G+I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	19	RAM118	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	53	IR69502×RAM118	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	21	RAM121	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	54	IR69502×RAM121	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
	5	IR69502-6-SRN	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	25	RAM163	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	55	IR69502×RAM163	H	I	H	I	H	H	H	I	I	G+I	G+I	H	H	H	H	G+I	H	I	G+I	I	H
Japonica × *Glaberrima*	2	IR60080-46-A	J	J	J	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J
	26	IG10	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	56	IR60080-46-A×IG10	J	I	H	H	J	J	H	G+J	I	G+J	G+I	H	J	J	J	G+J	J	I	G+J	I	J
	4	IR68703-AC-24-1	J	J	J	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J
	27	CG14	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	57	IR68703-AC-24-1×CG14	J	H	H	H	J	J	H	G+J	H	G+I	G+I	H	J	J	J	G+J	J	H	G+J	H	J
	2	IR60080-46-A	J	J	J	J	J	J	J	J	J	J	I	I	J	J	J	J	J	J	J	J	J
	27	CG14	J	I	I	I	J	J	I	G	I	G	G	I	J	J	J	G	J	I	G	I	J
	58	IR60080-46-A×CG14	J	H	H	H	J	J	H	G+J	I	G+J	G+J	I	J	J	J	G+J	J	I	G+J	H	J

markers to determine relations within the AA genome species is well documented. Robeniol et al. (1996) using only 14 STS markers accurately determined the genome composition of O. *meridionalis as* an AA genome species and most distantly related specie to *O. sativa*, and *O. longistaminata* the second most distantly related.

The SS markers which detected *glaberrima*-specific alleles suggest that loci adjacent to these markers could be a key for interspecific hybrid sterility. It may be interesting to compare these SS markers with other alleles of other wild rice species. The detection of heterozygous alleles between *japonica/glaberrima* and between *indica /glaberrima* in the F1s, suggest caution when applying some SS markers to other rice species and implying their distinguished association to *O. glaberrima* genome. Only 40 (59.7%) of the SS markers might be useful in the *O. glaberrima* analysis, as other markers did not detect any amplification of heterozygous alleles in F_1 progenies between *O.sativa* × *O. glaberrima*. This might be due to minute genomic differences between *O.sativa* and *O. glaberrima (*Ohmido and Fukui, 1995; Park et al., 2003*)*. Also, some markers did not generate heterozygous allele in the F_1's, suggesting that those loci are unique in *O.sativa* or *O. glaberrima* (S01160, S02052, S03099, S07048, S07050C, S08090, S08107, S09000A and S09058). Minor difference in the sequence of some markers might have affected the recombination in PCR amplicon region of some markers such as S02054, S02081, S04060, and S08106), and are allelic-specific (S03020 and S03046). Some small cryptic changes and mutations in PCR amplification region of some markers might have caused some markers not align well during PCR amplification, that is, S03048.

O. glaberrima had *indica* alleles at two loci associated with intersubspecific F_1 hybrid sterility on chromosome 5 and chromosome 8. Interspecific hybrids (F_1's) between *O.sativa* and *O. glaberrima* are almost completely sterile. This hybrid sterility barrier is mainly caused by an arrest of pollen development at the microspore stage **(**Heuer et al., 2003; Peltier, 1953). Intersubspecific F_1 hybrid sterility is mainly caused by cryptic chromosomal aberrations and allelic interaction between *indica* and *japonica* (Chin et al., 2007). The SS (subspecies-specific) STS marker were able to classify the *O. glaberrima accessions* into 10 sub-groups. Subspecies-prototype index (SPI) of *O. glaberrima* accessions ranged from 51.67 to 60.00, suggesting intermediate subspecific type based on whole-genome.

Comparative view of genome of *O. glaberrima* based on *O. sativa* spp. *japonica* genome

A total of 23 and 22 loci showed only *indica* and *japonicas* alleles respectively whilst 4 loci showed both *indica* and *japonica* alleles. Some non-sativa alleles which were detected on chromosomes 1, 2, 3, 9, 10, 11, and 12 might be *O. glaberrima* specific alleles (Figure 3), The heterozygous alleles of indica and *O. glaberrima* (G+I) identified on 3 loci on chromosomes 1, 2 and 3, suggests that non-sativa regions might be located on aligned BAC clones of *O. glaberrima*. This information can be useful in further studies involving the F_1 hybrid sterility between *O.sativa* and *O. glaberrima*.

Conclusion

The informative markers identified in this study might be very useful in studying the diversification of *O. glaberrima*; Loci adjacent to the SS markers which detected *glaberrima*-specific alleles could be a key for interspecific hybrid sterility between *O. sativa* × *O. glaberrima.* The detection of heterozygous alleles between *japonica* and *glaberrima* and between *indica* and *glaberrima* by some SS markers suggest that caution must be taken when applying some SS markers to other rice species and implying their distinguished association to *O. glaberrima* genome.

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