Molecular evolution of rice S_5^n and functional comparison among different sequences

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The wide compatibility gene, S_5^n , can overcome embryo sac sterility between *indica* and *japonica* subspecies of rice. Therefore, it is very important to characterize the features of the S_5^n sequence to reveal the origin and evolution of S_5^n . In this paper, 26 cultivated rice haplotypes and 22 wild rice accessions harboring S_5^n were used to sequence S_5^n . The results showed that 15 genotypes among the 48 materials were fully consistent with control cultivar 02428 (CK). The other 33 accessions had different degrees of variation in the S_5^n sequence. Variations in the coding region mainly occurred in the second exon and eight materials showed a 10-bp deletion at 1710–1719 bp, including wild (*O. nivara*) and cultivated rice, such as IRW501 and Yuetai B. S_5^n sequences were not biased and evolved neutrally. The 48 materials could be divided into 4 categories using a phylogenetic tree of the amino acid sequences. Most of the wild rice clustered together, and the cultivated rice clustered into another group. Eight cultivated rice and *O. nivara* (wild rice) clustered in another group, which were found to lack 10 consecutive bases in exon 2. Eight rice varieties with high numbers of differences in their S_5^n coding regions were crossed with testers (typically *indica* and *japonica*) to produced test cross F_1 populations. The F_1 s were examined for their ability to overcome *indica-japonica* hybrid sterility. The result showed that the embryo sac fertility of S_5^n -containing hybrids increased significantly compared with control hybrids, but there were no differences among the materials with divergent sequences, indirectly proving that S_5^n is a non-functional gene.

rice (*Oryza sativa* L.), hybrid sterility, S_5^n , wide compatibility gene, *O. ruvifipogon*, *O. nivara*

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Rice is one of the most important staple food crops of Asian populations, and high yield is a major aim for scientists. Asian cultivated rice (*Oryza sativa* L.) can be classified into two main subspecies, *indica* and *japonica*, which were produced by significant differentiation and reproductive isolation. Intersubspecific (*indica*×*japonica*) hybrids have great biological superiority, but their partial or complete sterility has limited the full exploration of strong heterosis in the hybrids. Therefore, the key to exploiting heterosis is to overcome the sterility of intersubspecific hybrids [1].

Many studies have shown that embryo sac and pollen

sterility mainly lead to sterility in *indica-japonica* hybrids, and both of them have strong influences on hybrid fertility [2,3]. Different allelic interactions at the S_5 locus on chromosome 6 are the main genetic reason for embryo sac sterility. There are three alleles at the S_5 locus i.e. $S_5^i S_5^i$ (*indica*), $S_5^j S_5^j$ (*japonica*) and $S_5^n S_5^n$ (wide compatibility allele) [4]. When wide compatible varieties ($S_5^n S_5^n$) are crossed with *indica* and *japonica* test-varieties, their hybrids are fertile. After nearly 20 years of research, rice geneticists cloned S_5^n and found that S_5^i and S_5^j differed by two nucleotides, which caused two amino acid replacements in the corresponding protein, and resulted in intersubspecific hybrid sterility [5–8]. S_5^n has a 136-bp DNA sequence deletion in the 5' terminus, causing loss of the signal peptide, which

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prevents the transportation of the protein across the membrane. That study provided an effective way to identify S_5^n and laid the foundation for the development of fertile interspecific hybrids [9].

We designed S_5^n functional molecular markers based on the sequence of S_5^n and identified 48 new germplasms with S_5^n from the Chinese rice core collection and wild rice [10,11]. In the present study, DNA of the 48 lines containing S_5^n was fully sequenced. Nucleotide sequence variation was analyzed among these materials and the expression products were predicted based on the open reading frame (ORF) of S_5^n . Finally, phylogenetic trees based on the nucleotide and amino acid sequence of S_5^n were built. In addition, whole-mount eosin B-staining confocal laser scanning

microscopy (WE-CLSM) was employed to observe the embryo sac fertility in test-cross hybrids of S_5^n -variant materials crossed with typical *indica* and *japonica* testers. The objectives of our research were to: (i) evaluate S_5^n differentiation and origin in cultivated and wild rice, and (ii) to compare sequences of S_5^n containing genotypes and their capability to overcome embryo sac sterility.

1 Materials and methods

1.1 Materials

Twenty-six cultivated rice haplotypes and 22 accessions of wild rice (Table 1) were used for S_5^n sequencing. The plants

Table 1 Names and sources of 48 lines/cultivars with S_5^n gene^{a)}

Species	No.	Name	Type	Resource
Wild rice	SXW006, SXW008, SXW009, SXW010, SXW013, SXW014, SXW016, SXW017, SXW018, SXW021, SXW022, SXW024, SXW032	Suixi <i>O. rufipogon</i>		Guangdong
	YLW004, YLW006	Yulin <i>O. rufipogon</i>		Guangxi
	GZW032	Gaozhou <i>O. rufipogon</i>		Guangdong
	DXW051, DXW062	Dongxiang <i>O. rufipogon</i>		Jiangxi
	CHW001	Conghua <i>O. rufipogon</i>		Guangdong
	IRW496, IRW501, IRW644	<i>O. nivara</i>		IRRI
Cultivated rice	N74	Haobuka	<i>japonica</i>	Yunnan
	N75	Sabbangqishiluo	<i>japonica</i>	Yunnan
	N77	Mubanggu	<i>japonica</i>	Yunnan
	N81	Xiaohonggu	<i>japonica</i>	Yunnan
	N84	Laozaogu	<i>indica</i>	Yunnan
	N94	Mowanguneiza	<i>japonica</i>	Yunnan
	N101	Fanhaopi	<i>indica</i>	Yunnan
	N103	Piwusheng	<i>indica</i>	Yunnan
	N115	Feienuo2	<i>indica</i>	Guizhou
	N150	Baoxie-7B	<i>indica</i>	Hunan
	N161	Teqingxuanhui	<i>indica</i>	Hunan
	INL3	L202	<i>japonica</i>	America
	INL5	Jackson	<i>japonica</i>	America
	INL49	M18	<i>japonica</i>	Guangdong
	INL43	Cypress PB-6	<i>japonica</i>	America
	INL44	Gulfmont	<i>japonica</i>	America
	INL46	APIV	<i>indica</i>	Guangdong
	INL56	M12	<i>japonica</i>	Guangdong
	INL69	LKJ001	<i>indica</i>	Fujian
	CC31	Huibeizi	<i>japonica</i>	Yunnan
	CC42	Zhongqijiaqing	<i>indica</i>	Yangtze River
	ZB23	Madhukar	<i>indica</i>	India
	ZB31	CW kezhen 2A	<i>indica</i>	Guangdong
	ZB54	Cisanggarung	<i>indica</i>	Unknown
	ZB71	Doddi	<i>indica</i>	Taiwan
	ZB89	Yuetai B	<i>indica</i>	Guangdong

a) Three self-cross progeny plants were used for sequencing in wild rice SXW008, SXW017 and SXW021; Two in wild rice, SXW013, SXW014 and SXW018. Other materials were sequenced using 3 plants.

from self-cross progeny of some wild rice, including SXW008, SXW010, SXW013, SXW014, SXW016, SXW017, SXW018, SXW021, SXW022, SXW032, YLW004, YLW006, DXW051, DXW062 and CHW001, were selected for S_5^n sequencing because these wild rice accessions are heterozygous at the S_5 locus and it was difficult to sequence them. Eight rice varieties with significant differences in their S_5^n sequences were crossed with *indica* (Guanglu'ai 4 and Nantehao) and *japonica* testers (Taichung 65) to prepare test-cross hybrids (F_1). Typical hybrids (Guanglu'ai 4×Taichung 65) and (Nantehao×Taichung 65) were used as controls (CK). All materials were planted at the experimental farm of the South China Agricultural University (SCAU).

1.2 Primer design

A total of five new primer pairs (Table 2) were used to amplify the DNA of S_5^n and full length sequences were obtained by these primers. All primers were synthesized by Shanghai Biological Engineering Technology Services Limited Company.

1.3 PCR amplification and sequencing

DNA was extracted according to the method of Yang et al. [10]. The PCR reaction reagent (20 μ L) included 20–30 ng template DNA, 0.15 μ mol/L each primer, 0.2 mmol/L dNTP, 1×PCR buffer, and 1 U *Taq* polymerase. The PCR reaction comprised pre-denaturation for 5 min at 94°C; followed by amplification for 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 5 min. PCR reactions were performed on the Mycycler thermal cycler (Bio-Rad Laboratories, USA). PCR products were separated on 0.8% agarose gels. Purified PCR products were sequenced by the Beijing Genomics Institute.

1.4 Data analysis

The full-length sequences were assembled by ContigExpress

software. Multiple sequence alignments were carried out using ClustalX software [12]. Polymorphic sites, transversions, and conversions of different S_5^n sequences were analyzed using MEGA 4.0 software [13]. Phylogenetic trees were constructed using the neighbor-joining (NJ) distance algorithm, maximum-parsimony (MP) and minimum-evolution (ME), respectively. Nucleotide diversity was calculated by DNAsp 5.0 software [14].

1.5 Embryo sac fertility observation

Whole-mount eosin B-staining confocal laser scanning microscopy (WE-CLSM) was used to observe embryo sac structure [15]. After blooming, 300 spikelets containing mature embryo sacs were collected and fixed in FAA (formaldehyde : acetic acid : 50% ethanol = 5:6:89) for at least 24 h. The florets were then washed with 50% ethanol and stored in 70% ethanol at 4°C. The ovaries were dissected in a petri dish under a binocular dissecting microscope, and hydrated sequentially in 50% ethanol, 30% ethanol and distilled water. The samples were pretreated in 2% aluminum potassium sulfate for 20 min and stained with 10 mg/L eosin B solution (dissolved in 4% sucrose) for 12 h at room temperature. The samples were then post-treated in 2% aluminum potassium sulfate for 20 min. After rinsing with distilled water three times, the samples were dehydrated with a series of ethanol solutions (30%, 50%, 70%, 90% and 100%). Subsequently, the dehydrated samples were transferred into a mixture of absolute ethanol and methyl salicylate (1:1) for 1 h, and then cleared in pure methyl salicylate solution for at least 1 h. The samples were scanned under a Leica SPE laser scanning confocal microscope. Pollen fertility and seed setting rate were counted according to the method of Shi et al. [16].

2 Results

2.1 PCR and sequence features of S_5^n

PCR amplification of 02428 (a compatible variety that carried S_5^n) was carried out using 5 pairs of primers, and 5 DNA fragments were obtained and sequenced. The full-length DNA sequence of S_5^n was obtained by assembling the 5 fragments, and was the same as that reported by Chen et al. [8], which showed that these 5 pairs of primers could be used to amplify the whole sequence of S_5^n . PCR amplification from 48 different rice materials was done using the same 5 primer pairs. The S_5^n sequence has three exons and two introns, which was consistent with 02428 (CK).

2.2 S_5^n sequence variation

Among the 48 S_5^n sequences, 15 haplotypes (Figure 1, Group 1) were similar to 02428, while sequences of the other 33 haplotypes were different from 02428. Twenty-

Table 2 Names and sequences of five primer pairs

Name	Sequence (5'→3')
S5-q1	CGTCTTGCTTCTTCATTCCC GTAGGTAAACACAGGCAGAG
S5-q2	CGTCTTGCTTCTTCATTCCC GTAGGTAAACACAGGCAGAG
S5-q3	GCGTGTAGTAGTATCATAAA GTCCCCAATCCTCAGCGTGT
S5-q4	TCGCTGCTCGTCGGTCAAGT GAGATAGGTTTAGCACAAAGC
S5-q5	TGGGGAACAGGGTTACTC GGAAGATGAAAAGGTCGC

Figure 1 Mutations in bases of 48 materials carrying the S_5^n gene in wild and cultivated rice. ■, exon; —, intron; *, base position (the first transcription starting point of S_5^n 5' is + 1). 1) The base changed from T to C, the amino acid changed from Leucine to Proline; 2) the base changed from C to A, the amino acid changed from Phenylalanine to Leucine; 3) the base changed from T to C, the amino acid changed from Methionine to Threonine; 4) the base changed from C to T, the amino acid changed from Proline to Serine; 5) the base changed from C to T, the amino acid changed from Alanine to Valine. Group 1, Sabbangqishiluo, Piwusheng, M12, Feienuo2, M18, Cisanggarung, Cypress PB-6, Baoxie-7B, LKJ001, Mubanggu, Gulfmont, L202, Zhongqijiaqing, Jackson, and Huibeizi. Group2, SXW006, SXW008, SXW009, SXW010, SXW013, SXW014, SXW016, SXW017, SXW018, SXW021, SXW022, SXW024, SXW032 and DXW051. Group 3, DXW062. Group 4, CHW001. Group 5, YLW004. Group 6, YLW006. Group 7, IRW496. Group 8, RW644. Group 9, Teqingxuanhui and CW kezhen 2A. Group 10, Haobuka. Group 11, APIV. Group 12, GZW032. Group 13, IRW501. Group 14, Yuetai B, Madhukar, and Doddi. Group 15, Mowanggunzeiza, Laozaogu, Xiaohonggu. Group 16, Fanhaopi.

four variable sites, including eight conversions, two transversions, and 14 deletions were found among these 33 haplotypes sequences. Transitions were more frequent than transversions (4:1), suggesting that gene sequence variations in rice S_5^n show conversion bias. The open reading frames (ORF) of four rice haplotypes (IRW496, Haobuka, CW kezhen 2A and Teqingxuanhui) among the 33 haplotypes were similar to 02428. Sequence variations in the ORF mainly occurred in exon 2, which has 6 variable sites. Ten consecutive bases were lost in exon 2 of 8 materials, including 7 cultivated rice and 1 wild rice (Yuetai B, Madhukar, Doddi, Xiaohonggu, Laozaogu, Mowanggunzeiza, Fanhaopi, and IRW501 (*O. nivara*). Four materials out of these 8 materials belong to Yunnan province of China.

Nucleotide polymorphism analysis showed that the nucleotide diversity (π) was not very high (0.00134) for either cultivated or wild rice and there were no significant differences between wild and cultivated rice (Table 3). Six different populations of wild rice and cultivated rice produced 0.00095 and 0.00081 nucleotide polymorphism for S_5^n , respectively. There was also no significant difference in the coding region of wild rice (0.00111) and cultivated rice (0.00119).

2.3 Construction of phylogenetic trees based on S_5^n sequence

Phylogenetic trees of the S_5^n nucleotide sequence were constructed using NJ, MP, and ME, and the 3 trees were very similar. Figure 2 shows the phylogenetic tree built by

NJ with 1000 interior branch tests. According to this model, 17 cultivated rice were clustered with 02428 (CK) as a separate group (class I); 8 cultivated rice and *O. nivara* (wild rice) clustered in another group (class II); some cultivated rice from Yunnan and other Southeast Asian cultivated rice clustered together in another group (class III), and wild rice (*O. rufipogon*) from Guangdong, Jiangxi and Guangxi were clustered together in the last group (class IV).

2.4 Amino acid sequence variation and construction of phylogenetic tree

The full length S_5^n coding region was 1074 bp and encoded

Table 3 DNA sequence polymorphism analysis and detection of neutral evolution in 48 materials carrying S_5^n in cultivated and wild rice^{a)}

Tape of material	Location	S	π
<i>O. rufipogon</i> and <i>O. sativa</i>	Coding region	5	0.00154
	Intron	3	0.00173
	Whole region	10	0.00134
<i>O. rufipogon</i>	Coding region	5	0.00111
	Intron	3	0.00123
	Whole region	10	0.00095
<i>O. sativa</i>	Coding region	3	0.00119
	Intron	1	0.00076
	Whole region	5	0.00081

a) S= number of segregating sites; π =nucleotide diversity.

2.5 Effects of different sequences of S_5^n on embryo sac fertility of intersubspecific hybrids

3 Discussion

3.1 S_5^n evolution

More than 300 different rice varieties (lines) carrying S_5^n have been identified, including *indica* and *japonica*, since Ikehashi and Araki proposed the “wide compatibility gene (S_5^n) theory” in 1986 [10,11,17]. S_5^n distribution rates in *indica* and *japonica* subspecies are almost identical [10]. S_5^n was also found in common wild rice (*O. rufipogon*) indigenous to China, including those in Guangdong, Guangxi, Jiangxi, Hainan and Hunan provinces or Autonomous region [11,18–20]. Moreover, S_5^n was also found in *O. nivara*, which is closely related to *O. rufipogon*. These results indicated that S_5^n might have originated from all rice, including *O. sativa* (*indica* and *japonica*), *O. rufipogon*, and *O. nivara*, and suggested that it is of widespread significance. The structural features of the S_5^n sequence were similar among cultivars and wild rice. DNA base sequence variation in S_5^n of *O. sativa* (*indica* and *japonica*), *O. rufipogon*, and *O. nivara* were also nearly the same. Variations in *indica* and *japonica* rice occurred at almost the same frequency, suggesting that variation of the S_5^n sequence was not biased. The coefficient of S_5^n nucleotide polymorphism (0.00134) analysis showed that the polymorphism rate was not very high. The above results indicated that S_5^n in wild rice and cultivated rice perhaps evolved simultaneously by

Figure 3 Mutations in S_5^m amino acid sequences. L1 included 20 materials, namely Sabbangqishiluo, Piwusheng, M12, Feienuo2, M18, Cisanggarung, Cypress PB-6, Baoxie-7B, LKJ001, Mubanggu, Gulfont, L202, Zhongqijiaqing, Jackson, Huibeizi, Teqingxuanhui, CW kezhen 2A, Haobuka, IWR496 and GZW032. L2 included 16 materials, namely SXW006, SXW008, SXW009, SXW010, SXW013, SXW014, SXW016, SXW017, SXW018, SXW021, SXW022, SXW024, SXW032, DXW051, YLW006, and CHW001. L3 included three materials, namely IWR644, APIV, and DXW062. L4: YLW004. L5 included four materials, namely IRW501, Yuetai B, Madhukar, and Doddi. L6 included 4 materials, namely Mowanguneiza, Fanhaopi, Laozaogu, and Xiaohonggu.

Figure 4 Protein phylogenetic tree constructed by neighbor-joining with 1000 interior branch tests.

parallel evolution, as a neutral gene.

It was speculated that S_5^m in *O. sativa* may have originated in Yunnan. Whole sequence analysis of 48 cultivated and wild rice haplotypes showed that 15 materials yielded similar sequence to that of 02428, and these rice varieties were mainly from Yunnan Province of China and the U.S. The S_5^m -containing cultivar, 02428, was also native to Pangxiegou, Yunnan Province [17,21]. Our laboratory detected some materials carrying S_5^m and most of them are from Yunnan [10]. In addition, China's cultivated rice varieties carrying S_5^m are mainly from Yunnan. Therefore, Yunnan may be one origin of S_5^m . Apart from Yunnan, the origin area of *Javanese* rice maybe also one of the sources of S_5^m , because many rice varieties that developed by crossing with *Javanese* rice from the US were found to harbor S_5^m . In this study, phylogenetic analysis of the nucleotide and protein sequences of S_5^m also showed that Yunnan Province is the origin of S_5^m .

3.2 S_5^m sequence diversity and effect on embryo sac sterility of intersubspecific hybrids

Chen et al. [8] revealed that a 136-bp deletion of S_5^m resulted in a lack of the N-terminal signal peptide, so the expression products could not be properly transported and were deposited in the cell and produced fertile embryo sacs. This loss of function did not occur because of expression the products of S_5^j or S_5^i or their interaction. According to this mechanism, S5 at both ends of TAAT motif in the first exon lack 136 bp sequence than other location, so no matter

Figure 5 Abnormal embryo sac structures of *indica-japonica* hybrids F_1 . (a) Normal embryo. E, egg; P, polar nuclei; A, antipodal cells. (b) Normal embryo sac after fertilization. (c) Embryo sac degradation (Arrows). (d) and (e) Abnormal small embryo sacs. (f) Embryo sac without female germ unit. (g) and (i) Embryo sac with abnormal position of polar nuclei (Arrow). Bar: 40 μm .

what variation S_5 sequences have, it could not affect its power to overcome embryo sac sterility of intersubspecific hybrids. The present study confirmed this hypothesis. Our laboratory identified 48 materials carrying S_5^n and their genomic sequences had a deletion of 136-bp at both ends of the TAAT motif. Thirty-three materials showed different degrees of variation in the S_5^n sequence. In particular, 8 varieties were found to lack 10 consecutive bases in exon 2, which resulted in reduction of 43 amino acids in the encoded protein compared with control S_5^n amino acid sequence (02428). We prepared different crosses with different S_5^n sequences and found that their embryo sac fertility

was significantly higher than control hybrids (typical *indica* and *japonica*). S_5^n sequence variation does not affect their ability to overcome embryo sac sterility in hybrids, and further verified that the S_5^n gene is a non-functional gene. These results are in agreement with Chen et al. [8], who reported that S_5^n is a non-functional gene. It is worth noting that some test cross hybrids, such as Doddi $\times$ Taichung65 and L202 $\times$ Guangluai 4, produced significantly lower embryo sac fertility than the average embryo sac fertility. In addition, the main types of embryo sac abortion were also different. Further research is needed to determine whether non S_5 loci interact and cause low embryo sac fertility.

Table 4 Pollen fertility, embryo sac fertility, and seed set of testcross F₁^{a)}

Name of parents and hybrids	Pollen fertility (%)	Embryo sac fertility (%)	Seed set (%)
Guanglu'ai4×Taichung65 (CK)	37.00	26.17	20.16
Nantehao×Taichung65 (CK)	14.42	54.81	29.80
Madhukar	84.65	97.92	91.30
Madhukar×Guanglu'ai4	54.60	92.08	87.60
Madhukar×Nantehao	76.50	92.14	86.90
Madhukar×Taichung65	55.80	78.13	70.30
Doddi	82.80	91.60	88.10
Doddi×Guanglu'ai4	90.96	98.21	92.25
Doddi×Nantehao	70.48	95.72	92.22
Doddi×Taichung65	80.98	68.02	59.99
Yuetai B	92.90	75.51	66.40
Yuetai B×Guanglu'ai4	80.25	98.76	52.90
Yuetai B×Nantehao	81.20	96.30	73.60
Yuetai B×Taichung65	66.08	85.96	77.20
Huibeizi	86.10	97.48	82.60
Huibeizi×Guanglu'ai4	88.60	98.54	74.60
Huibeizi×Nantehao	81.80	98.24	76.80
Huibeizi×Taichung65	85.88	90.71	81.40
L202	92.95	—	88.30
L202×Guanglu'ai4	87.50	76.60	66.60
L202×Nantehao	77.78	82.61	63.90
L202×Taichung65	60.85	84.03	64.00
Jackson	95.90	96.60	68.13
Jackson×Guanglu'ai4	87.37	83.45	68.96
Jackson×Nantehao	80.70	96.27	68.90
Jackson×Taichung65	91.92	92.97	80.71
Piwusheng	—	—	75.10
Piwusheng×Guanglu'ai4	88.70	97.29	85.70
Piwusheng×Nantehao	90.57	77.58	74.90
Piwusheng×Taichung65	86.00	97.87	91.23
Cisanggarung	89.11	79.01	72.05
Cisanggarung×Guanglu'ai4	66.50	92.09	59.13
Cisanggarung×Nantehao	74.40	81.88	62.10
Cisanggarung×Taichung65	93.00	92.13	69.70

a) "—" denotes missing value.

Table 5 Types of abnormal mature embryo sacs in different rice combinations (%)

Combinations	Total abortion rate of embryo sac	Embryo sac degeneration	Abnormal small embryo sac	Embryo sac with abnormal polar nuclei	Embryo sac without egg apparatus	Embryo sac without female germ unit	Other types of abnormal embryo sac
Guanglu'ai4×Taichung65 (CK)	73.83	45.80	23.40	4.67	0.00	0.00	0.00
Nantehao×Taichung65 (CK)	45.19	27.90	9.62	7.69	0.00	0.00	0.00
Piwusheng×Nantehao	22.42	0.00	0.00	21.80	0.00	0.00	0.60
Piwusheng×Taichung65	2.13	0.00	0.00	1.42	0.00	0.70	0.00
Madhukar×Guanglu'ai4	7.92	1.25	2.50	3.75	0.00	0.00	0.40
Madhukar×Nantehao	8.76	1.38	3.69	3.69	0.00	0.00	0.00
Madhukar×Taichung65	21.88	0.00	15.63	6.25	0.00	0.00	0.00
Cisanggarung×Guanglu'ai4	7.91	0.56	0.56	5.09	0.00	0.00	1.70
Cisanggarung×Nantehao	18.13	5.00	0.63	12.50	0.00	0.00	0.00

(to be continued)

(continued)

Combinations	Total abortion rate of embryo sac	Embryo sac degeneration	Abnormal small embryo sac	Embryo sac with abnormal polar nuclei	Embryo sac without egg apparatus	Embryo sac without female germ unit	Other types of abnormal embryo sac
Cisanggarung×Taichung65	7.87	0.00	3.15	4.72	0.00	0.00	0.00
DoddixGuanglu'ai4	1.79	0.00	0.60	1.19	0.00	0.00	0.00
DoddixNantehao	4.28	1.07	0.00	3.20	0.00	0.00	0.00
DoddixTaichung65	31.98	0.00	1.16	29.00	0.00	0.00	1.74
Yuetai B×Guanglu'ai4	1.24	0.00	0.00	1.24	0.00	0.00	0.00
Yuetai B×Nantehao	3.70	0.46	0.00	3.24	0.00	0.00	0.00
Yuetai B×Taichung65	14.04	1.69	2.24	9.55	0.56	0.00	0.00
Huibeizi×Guanglu'ai4	1.46	0.49	0.00	0.98	0.00	0.00	0.00
Huibeizi×Nantehao	1.76	0.00	0.00	1.76	0.00	0.00	0.00
Huibeizi×Taichung65	9.29	0.88	3.54	3.98	0.44	0.44	0.00
L202×Guanglu'ai4	23.40	14.90	3.54	4.96	0.00	0.00	0.00
L202×Nantehao	17.39	4.35	6.09	6.69	0.00	0.00	0.00
L202×Taichung65	15.97	0.69	11.10	4.17	0.00	0.00	0.00
Jackson×Guanglu'ai4	16.55	5.04	7.19	4.31	0.00	0.00	0.00
Jackson×Nantehao	3.73	2.23	1.49	0.00	0.00	0.00	0.00
Jackson×Taichung65	7.03	0.00	3.78	2.16	0.00	1.08	0.00

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