Pak. J. Bot., 51(6): 2089-2095, 2019. DOI: http://dx.doi.org/10.30848/PJB2019-6(10)

MOLECULAR BREEDING OF FRAGRANT EARLY-SEASON HYBRID RICE USING THE *BADH2* GENE

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Abstract

Fragrance is an important index of rice quality. Consumers value high-quality fragrant rice. In this study, Badh2-1, a new functional molecular marker of *Badh2*, was designed for marker-assisted selection (MAS) of fragrant rice containing high levels of 2-acetyl-1-pyrroline (2AP). Badh2-1 MAS was then used to breed fragrant early-season two-line male sterile rice lines YuS-1 and YuS-5, restorer lines R66-1, R66-7, R66-15 and fragrant early-season hybrid rice varieties Yuliangyou 120, Yuliangyou 125 and Yuliangyou 130. Each of these contains 2AP in the range 414-734 ppb (ug/kg) and the hybrids exhibit increased yield and relatively good rice quality. The breeding of fragrant early-season rice will improve the crop's organoleptic qualities, which will increase the price and thus the economic motivation for planting early-season rice, helping to improve the food security of China and other nations.

Key words: Fragrant rice; Early-season hybrid rice; Badh2-1; Marker-assisted selection; 2-acetyl-1-pyrroline.

Introduction

Rice is the staple food for more than half the people of the world and demand is increasing because of the expanding rice-eating population, especially in many developing countries in Africa and Asia (Khush & Jena, 2009). Rice grains with fragrance appeal to consumers, and the retail price of fragrant rice is higher than that of conventional rice varieties. This is demonstrated by the retail price of jasmine fragrant rice produced in Thailand, which worldwide has a market value that is twofold higher than that of non-fragrant rice (Qiu & Zhang, 2003).

Over one hundred volatile compounds have been detected in fragrant rice, but the principal compound responsible for fragrance is thought to be 2AP (Widjaja *et al*., 1996, Jezussek *et al*., 2002). 2AP is present in a number of other higher plants, such as bread flowers (*Vallaris glabra* Ktze) (Wongpornchai *et al*., 2003), pandan leaves (*Pandanus amaryllifolius*) (Laksanalamai & Ilangantileke, 1993), pearl millet (Seitz *et al*., 1993), taro (Wong *et al*., 1996) and aromatic vegetable soybean (Wu *et al*., 2009). In addition, 2AP is considered an important aroma component of the crust of white bread and of roasted foods (Hofmann & Schieberle, 1998).

Map-based gene cloning has shown that the synthesis and accumulation of 2AP in rice grains are associated with the gene *BADH2*, which encodes a betainealdehyde dehydrogenase with 15 exons and 14 introns (Bradbury *et al*., 2005, Fitzgerald *et al*., 2008). Several studies suggested that the presence of 2AP is attributable to a recessive allele of *BADH2* gene with fragment deletions, including a 7-bp deletion on the 2nd exon, an 8-bp deletion on the 7th exon, and an 803-bp deletion between exons 4 and 5 (Bradbury *et al*., 2005, Shi *et al*., 2008, Kovach *et al*., 2009, Shao *et al*., 2011).

Increasing the economic value of rice by breeding of fragrant rice is one of the most important targets of modern rice-breeding project. However, fragrance is an unstable trait in selection because of the recessive nature of the gene and the difficulty of assessing the fragrance of individual plants. As the instability in action of fragrance gene Badh2 and the complexity of fragrance determination, MAS is considered to be one of the most useful methods for fragrance gene detection. Several primers have been designed on the location of the 8-bp deletion and three SNPs on the 7th exon of Badh2 to detection fragrant plants in an F2 population of Kyeema (fragrant rice) crossed with Gulfmont (non-fragrant) by using PCR to amplify fragrance-enhancing alleles (Bradbury *et al*., 2005a). These primers were further used to exam the fragrance alleles of nine fragrant and three non-fragrant rice varieties (Lu *et al*., 2008). Two functional markers, one located in the 7-bp deletion on the 2nd exon and the other in the 8-bp deletion on the 7th exon, have been developed to detection the fragrance gene among rice varieties with 24 have fragrance and 10 without fragrance (Shi *et al*., 2008). In short, these markers improve the efficiency of selection and accelerate the breeding process by using marker assistant selection of fragrant gene in rice.

In our study, a new functional molecular marker of *Badh2* located in the 8-bp deletion on the 7th exon was developed, and this new functional molecular marker was used for MAS and breeding of fragrant early-season two-line male sterile line rice, restorer lines and new early-season hybrid rice varieties with relatively good rice quality.

Experimental materials and methods: We used the following rice lines: Zhongxiang-1, a fragrant rice variety with relative high yield and good rice quality; Zhong 2B, an early-season fragrant three-line maintainer line rice; Xiangling 628S, an early-season two-line male sterile line, which is used extensively for early-season rice production in China; and Thailand-2935, a conventional rice variety introduced from Thailand. The segregation populations of Xiangling 628S × Zhong2B and Zhongxiang-1 × Thailand-2935 were used to select early-season two-line male sterile lines and restorer lines.

Badh2 **functional molecular marker development, DNA extraction and genotype identification:** A new functional molecular marker was designed for the 8 bp deletion on the 7th exon of *Badh2*. During seedling stage, DNA of each plant was extracted, the method was according to the protocol as following. The volume of the PCR reaction was contained 10 μL, including 1 μL of 10×PCR buffer (25 mM/L $MgCl_2$), 0.1 mM dNTPs, 1 μM primer pairs, 0.1 U Taq DNA polymerase, 25 ng template DNA and 4.95 μL double-distilled H_2O. The PCR conditions included an beginning denaturation at 95ºC for 3 min, then 35 cycles of 30 s at 95ºC, 40 s at 55ºC and 40 s at 72ºC, and a final extension step at 72ºC with 8 min. PCR products of no more than 300 bp were separated using 8% non-denaturing polyacrylamide gels. Finally, the genotype of each plant was identified.

Fragrance phenotype identification of parents and segregation population individuals: The 2AP content of the parents' grain and segregation population individuals were determined using GC-MS (gas chromatograph-mass spectrometry) as described by Ying *et al*., 2011. Grain of the fragrant rice Zhong2B was used as a positive control. One gram of whole meal flour was extracted for 3 h at 80°C in a 1:1 (v/v) mixture of anhydrous ethanol and methylene chloride, using 0.5 mg L^{-1} of 2,4,6-trimethylpyridine (TMP) as internal standard. After standing at room temperature for 30 min, an aliquot of the supernatant was introduced into the GC-MS, Agilent, Santa Clara, CA, USA). The carrier was helium gas (99.999% purity) at a pressure of 34.5 kPa, and the injector and GC-MS interface temperatures were set to 170℃. The initial temperature of the HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm; J&W, Folsom, CA, USA) was set to 50ºC. After 2 min at 50ºC, the temperature was increased to 280ºC at a rate of 10ºC /min. The extract was introduced directly into the mass spectrometer, which was operated in the electron impact (EI) mode with an ionization voltage of 70 eV and an ion source temperature of 230ºC. The standard deviations for measurements of 2AP were typically ≤1%.

MAS of fragrant early-season two-line male sterile rice: Non fragrance early-season two-line male sterile line Xiangling628S was crossed with fragrant early-season three-line maintainer line rice Zhong 2B to produce F_1, then after F_1 plants selfing the F_2 segregation population was constructed. Further in the progeny of segregation population, according to the selection target of fragrant early-season two-line male sterile line, the new developed functional molecular marker was uesd to identify the genotyping of the fragrant plants.

MAS of fragrant early-season restorer line rice: Thailand-2935, a non-fragrant conventional rice variety with good quality, was crossed with fragrant conventional rice variety Zhongxiang-1 to produce the F_1, then, F_1 plants selfing the F_2 segregation population was obtained. The newly-developed molecular marker was then used to identify the genotypes of the fragrant plants in this population, and fragrant early-season two-line conventional rice with relatively good grain quality was bred.

The screening of new excellent fragrant early-season hybrid rice varieties: The selected fragrant early-season two-line male sterile lines were crossed with the newly-bred fragrant early-season restorer lines to produce novel hybrid rice varieties. These new hybrid rice combinations were planted in the natural paddy field of the China National Rice Research Institute in a 10-row plot with 20 plants per row by 15×21 cm spacing under a randomized complete block design with three replications. Finally, the agronomic traits including plant height, effective panicle number, number of grain, number of grain per panicle, 1000-grain weight and yield per plant *et al*., of each combination were examined to identify high quality new early-season hybrid rice varieties with fragrance and an improved yield.

Results

Badh2-1, the new functional molecular marker of *Badh2:* Sequencing of *Badh2* among fragrant and non-fragrant rice varieties showed there was an 8-bp deletion located in the 7th exon of *Badh2* from the fragrant rice varieties (Suppl. Fig. 2). Based on the sequencing results, we designed a new indel marker Badh2-1 (Forward primer: GTTGCATTTACTGGGAGT, Reverse primer: GAAAAGGACAACATTGAGAA). Analysis using Badh2-1, together with fragrance phenotyping, showed that all the rice varieties with *Badh2* genotypes identical to Zhong2B were fragrant (Table 1, Fig. 3A). Therefore the functional marker Badh2-1 reliably distinguishes between fragrant and non-fragrant rice varieties (Fig. 3A).

The fragrance phenotype and genotyping identification of parent varieties: Zhong2B: An early season fragrant three-line maintainer line rice variety. GC-MS analysis indicated that the 2AP content was 550 ppb (ug/kg) (Table 4). Badh2-1 detection showed that the genotype of Zhong2B was consistent with the original sequencing results of Zhong2B, with an 8-bp deletion in the 7th exon of *Badh2* (Fig. 3A, Suppl. Fig. 2).

Zhongxiang-1: A late-season fragrant conventional rice variety with good rice quality. GC-MS analysis indicated that the 2AP content was 682 ppb (ug/kg) (Table 4). Badh2-1 detection showed that the genotypes of Zhongxiang-1 and Zhong2B were identical (Fig. 3A). Sequencing of *Badh2* from Zhongxiang-1 showed that there was a deletion of 8 bp from exon 7 (Suppl. Fig. 2).

Xiangling 628S: An early season two-line male sterile line. Agronomic traits analysis indicated that the plant height of Xiangling628S was 67.4 cm, and tiller number was 12.2, with 132.4 spikelets per panicle. There was almost no 2AP in Xiangling 628S. Badh2-1 detection showed that the *Badh2* genotype of Xiangling628S was identical with those of non-fragrance rice varieties, having no deletion on the 7th exon (Table 1, Table 4, Fig. 3A).

Thailand-2935: A conventional non-fragrant variety with good rice quality, introduced from Thailand. Agronomic traits analysis indicated that the plant height of Thailand-2935 was 91.5 cm, panicle number was 5.4, spikelets number per panicle was 142.8, seed setting rate was 87.9%, and 1000-grain weight was 21.5 g. GC-MS analysis indicated that there was almost no 2AP content in *Thailand-2935*. Badh2-1 detection showed that the *Badh2* genotype of Thailand-2935 was identical to those of non-fragrance rice varieties (Table 2, Table 4, Fig. 3A).

Breeding of early-season two-line male sterile line rice using Badh2-1 MAS: Xiangling628S was crossed with Zhong2B, and then the Xiangling628S×Zhong2B F_2 segregation population containing 1500 plants was constructed. Badh2-1 was used to scan the genotype of each plant in this F_2 population, and 287 plants with *Badh2* genotypes identical with that of fragrant rice were identified (Fig. 3B). After selection according to male sterility phenotype, together with other agronomic traits, such as tiller number per plant, plant height, spikelet number per panicle, *etc*., 35 two-line male sterile individuals with fragrance were selected. In the next series of segregation populations, Badh2-1 genotyping, male sterility phenotype and other agronomic traits were considered as the basis of pedigree selection. Finally, in the year 2014, we obtained two excellent early-season two-line male sterile rice selections, YuS-1 and YuS-5, with respective 2AP contents of 650 ppb (ug/kg) and 511 ppb (ug/kg). The agronomic characteristics of YuS-1 and YuS-5 are described in Suppl. Fig. 1; Fig. 1A, D, H; Table 1; Table 4.

The breeding of early-season restorer line rice using Badh2-1 MAS: Zhongxiang-1 was crossed with Thailand-2539. The functional molecular marker Badh2-1 was used to scan the genotype of each of the 2539 plants in the F_2 population (Fig. 3C). After taking into consideration other agronomic traits, 97 plants with excellent performance were selected. Then in the next series of segregation populations, Badh2-1 genotyping and pedigree selection were used to identify fragrant and non-fragrant rice plant phenotypes. Finally, excellent early-season restorer line rice selections R66-1, R66-7, R66-15, with 2AP contents of 734, 653, and 695 ppb (ug/kg) respectively, were selected in 2014 (Suppl. Fig. 1; Fig. 1B, E, I; Table 2).

The identifications of new early-season hybrid rice with fragrance: The hybrid rice combinations YuS-1×R66-1, YuS-1×R66-5, YuS-1×R66-17, YuS-5×R66-1, YuS-5×R66-5 and YuS-5×R66-17 tested in a comparative trail. YuS-1×R66-7 gave the highest yield, of 7784±5 kg/ha, and was named Yuliangyou120 (Table 3). Badh2-1 detection showed that the *Badh2* genotype of Yuliangyou120 was identical with that of fragrant rice variety Zhong2B (Fig. 3A). Yuliangyou120 contained 596 ppb (ug/kg) of 2AP (Fig. 2C, Table 4), while sequencing of its *Badh2* showed that there was an 8-bp deletion on the 7th exon (Suppl. Fig. 2).

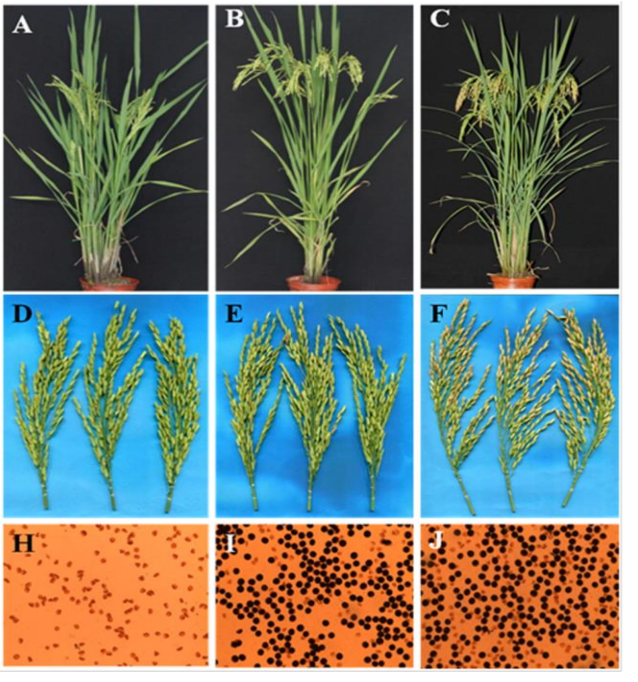

Fig. 1. The agronomic trait performances of selected rice lines. A, B, C, the plant morphologies of YuS-1, R66-1, and Yuliangyou120; D, E, F, the panicles of YuS-1, R66-1, and Yuliangyou120; H, I, J, the pollen fertility of YuS-1, R66-1, and Yuliangyou120, shown by staining with I_2-KI solution.

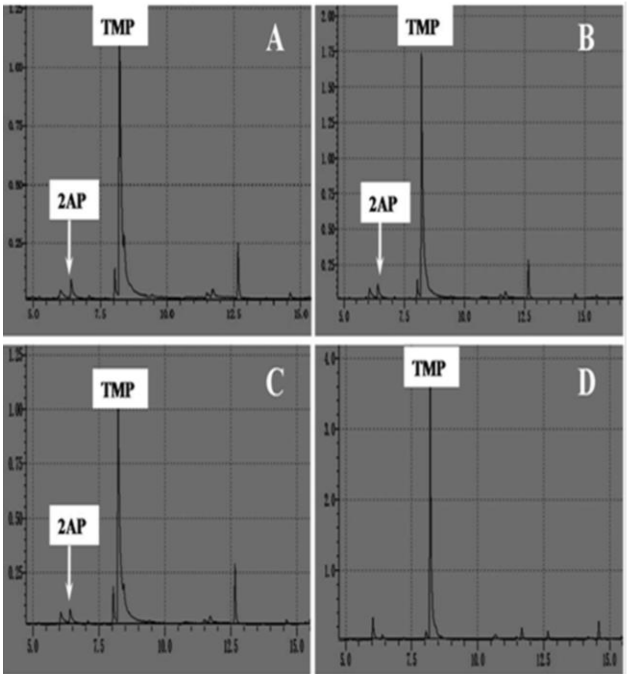

Fig. 2. Chromatograms of 2AP in selected rice lines. A, YuS-1. B, R66-1. C, Yuliangyou120. D, Luliangyou17 control. TMP: 2,4,6-trimethylpyridine internal standard.

Discussion

MAS saves time and simplifies rice breeding, facilitating introduction of an important allele to an elite line in a relatively short time. Until recently, MAS applied in rice breeding has mostly been related to resistance to diseases such as rice blast and bacterial leaf blight, with few applications to other agronomic traits such as fragrance. Wang *et al*., (2009) used one CAPS marker to identify the Wx-mq homozygous and heterozygous genotypes in the offspring in segregation populations of the high-yielding rice cultivar "Wu-xiang-jing 14" crossed with the high-quality Japonica rice cultivar "Kantou 194" which has a low amylose content and translucent endosperm, developing the new variety "Nan-jing 46" within 9 years (Wang *et al*., 2009, Yao *et al*., 2010). Although molecular markers have been developed for various indels and SNPs on different exons of *Badh2*, fragrant rice varieties (especially fragrant early-season hybrid rice) bred using MAS have not been reported until now.

Generally, these functional molecular markers of *Badh2* were developed using the old conventional fragrant rice varieties, with poor agronomic traits, including inferior plant type, low yield and weak disease resistance *etc*., so it is difficult to introduce just the fragrance trait from old conventional fragrant rice varieties into modern cultivated rice varieties while excluding other inferior agronomic traits. Currently, because of the recessive nature of *Badh2*, some functional markers targeted to rare allelic variation in old conventional fragrant varieties with unstable expression of *Badh2* cannot be applied widely for breeding fragrant rice. Shao *et al*., (2013) have shown the diversity of *Badh2* in different fragrant rice varieties including *O. indica*, *japonica* and *rufipogon*. Their results indicate that the 8-bp deletion on the 7th exon of *Badh2* occurs in a large number of fragrant rice varieties, including some fragrant three-line restorer lines and maintainer rice lines. Zhong 2B, a three-line maintainer line rice, was bred by our research group with *Badh2* introduced from Ru9502008, a famous conventional fragrant rice, and showing an 8-bp deletion on the 7th exon of *Badh2*. So, new fragrant rice varieties may easily be bred by using Zhong 2B as the donor parent of *Badh2*, together with MAS using the functional marker Badh2-1.

The area of early-season hybrid rice is continuously decreasing because of its high cost and minimal yield advantages (Chen *et al*., 2007). In China, planting early-season rice, including early-season hybrid rice, can increase the cropping index, and would increase the total yield of rice. In order to improve food security, the government of China encourages farmers to produce early-season rice. However, the poor quality of early-season rice, with associated low prices, provides little incentive for farmers to produce it. This applies especially to early-season hybrid rice as the seed is relatively costly compared to conventional early-season rice varieties. Yuliangyou120, Yuliangyou125 and Yuliangyou130 are fragrant early-season hybrid rice varieties, the yields of which are higher by 1.83%, 0.87% and 0.55% respectively compared to the control Luliangyou17. In addition, the 2AP contents of these varieties are 596 ppb, 478 ppb and 414 ppb, respectively, indicating improved organoleptic properties compared to the control. Thus planting fragrant rice, especially fragrant early-season hybrid rice, not only increases the total yield but especially improves rice quality. The fragrance of early-season rice makes it more desirable to consumers, increasing the price accordingly. This will boost the motivation of farmers to produce fragrant early-season hybrid rice which will result in a greater planting area of early-season rice and enhanced national food security.

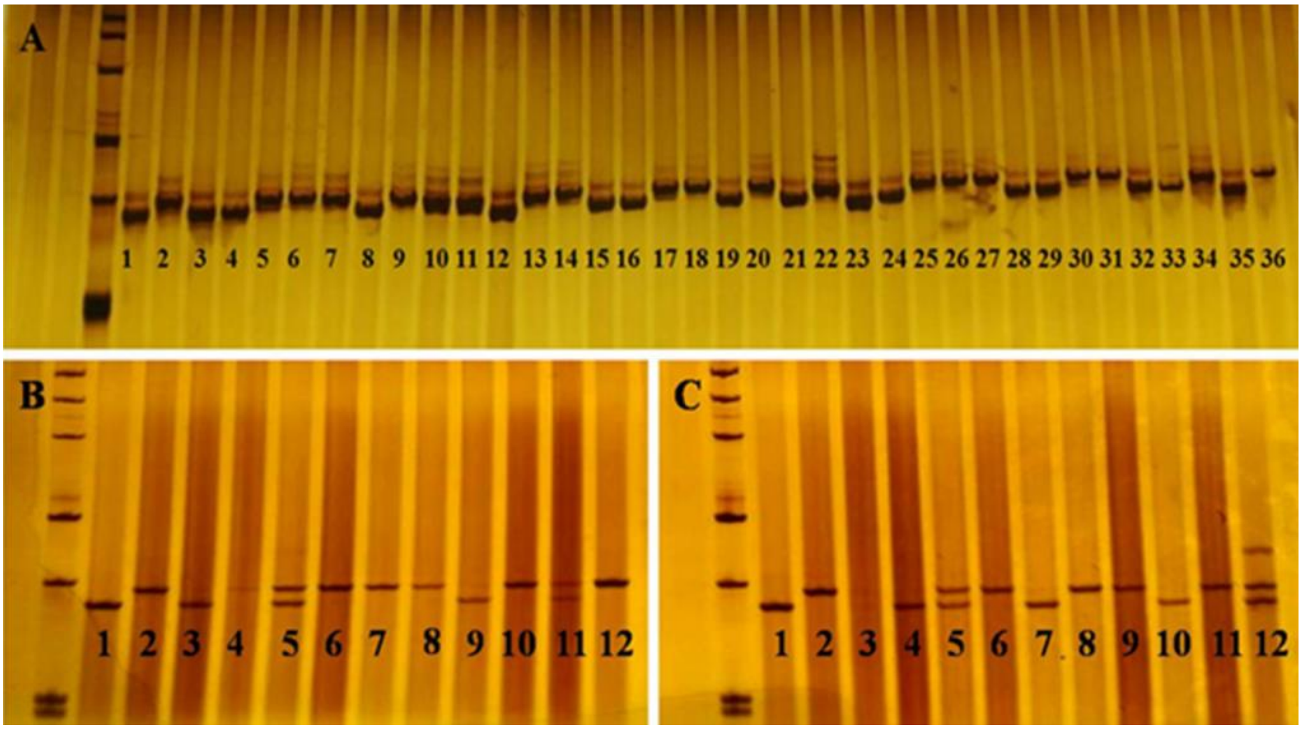

Fig. 3. Gels showing genotyping by Badh2-1 of different rice varieties and segregation population individuals. A: Rice varieties with or without fragrance; 1, Zhong 2B; 2, Xiangling628S; 3, Zhongxiang-1; 4, Basmati370; 5, Thailand-2935; Full information on the rice varieties detected is listed in Suppl. Table 1. B: The genotypes of Badh2-1 in the Xiangling628S×Zhong 2B F_2 population; 1, Zhong2B; 2, Xiangling628S; 3-12, the individuals in the F_2 population. C: the genotypes of Badh2-1 in Zhongxiang-1×Thailand-2935 F_2 population, 1, Zhongxiang-1; 2, Thailand-2935; 3-12, the individuals in the F_2 population.

Table 1. The agronomic trait performances of parents and bred early-season two-line male sterile line rice.

Traits	Plant height (cm)	Length of flag leaf (cm)	Width of flag leaf (cm)	Valid panicles per plant	Length of panicle (cm)	Seeds number per panicle	Seed length (mm)	Seed width (mm)	Seed setting rate (%)	1000- grain weight (g)	Grain weight per plant (g)
Xiangling628S	67.36±0.12	29.62±0.12	2.32±0.03	12.22±0.23	19.82±0.35	132.42±1.36	8.24±0.19	1.99±0.06	/	/	/
Zhong 2B	93.67 ±0.32	40.90 ±0.22	1.97±0.01	8.23±0.15	22.70± 0.26	165.67± 2.45	9.87± 0.31	2.99± 0.04	86.2±1.32	24.90±0.21	28.01±0.12
YuS-1	71.61± 0.21	32.43 ±0.13	1.92±0.06	19.63±0.31	22.33± 0.29	165.37±2.38	8.79± 0.26	2.55± 0.03	/	/	/
YuS-5	75.42± 0.11	34.28 ±0.25	1.97±0.04	17.54±0.32	24.23± 0.36	171.43± 3.46	8.89± 0.23	2.37± 0.04	/	/	/

Table 2. The agronomic trait performances of parents and bred early-season restorer line rice.

Traits	Plant height (cm)	Length of flag leaf (cm)	Width of flag leaf (cm)	Vaild panicles per plant	Length of panicle (cm)	Seeds number per panicle	Seed length (mm)	Seed width (mm)	Seed setting rate (%)	1000- grain weight (g)	Grain weight per plant (g)
Tailand rice 2935	91.15±0.44	43.15±0.20	2.22±0.04	5.36±0.16	23.73±0.31	142.81±1.31	9.53±0.31	2.50±0.10	87.9±1.56	21.51±0.23	17.02±0.21
Zhongxiang-1	108.62±0.23	47.71±0.36	2.13±0.02	10.41±0.18	27.51±0.42	147.15±1.41	9.17±0.32	2.57±0.09	71.3±2.11	17.6±0.35	17.35±0.24
R66-1	90.58 ±0.13	33.57 ±0.39	1.93 ±0.01	6.33 ±0.16	21.53 ±0.36	157.23 ±2.56	9.60±0.28	2.62 ±0.05	91.1 ±3.48	26.23 ±0.45	21.79 ±0.31
R66-7	98.17±0.12	35.19±0.15	1.95±0.03	8.21±0.19	25.61±0.29	158.75±3.23	9.61±0.27	2.64±0.07	89.3±4.52	24.71±0.37	22.87±0.36
R66-15	102.23±0.24	35.76±0.43	1.98±0.02	8.97±0.17	26.13±0.34	167.13±3.51	9.68±0.34	2.71±0.08	88.2±2.65	25.59±0.21	23.12±0.19

Table 3. The agronomic trait performances of bred new early-season hybrid rice.

Traits	Plant height (cm)	Length of flag leaf (cm)	Width of flag leaf (cm)	Valid panicles per plant	Length of panicle (cm)	Seeds number per panicle	Seed length (mm)	Seed width (mm)	Seed setting rate (%)	1000- grain weight (g)	Grain weight per plant (g)	Yield/ha (kg/ha)	Increased %
Yuliangyou120 (YuS-1/R66-7)	96.34±0.35	39.12±0.42	1.78±0.01	13.24±0.21	27.43±0.41	214.13±1.37	9.79±0.13	2.61±0.08	89.8±1.12	25.35±0.45	27.58±0.35	783.5±5.1	+1.83
Yuliangyou125 (YuS-1/R66-1)	80.33 ±0.31	43.67 ±0.25	2.00 ±0.03	9.67 ±0.15	22.63 ±0.25	211.00 ±2.35	9.71 ±0.21	2.68 ±0.12	91.2 ±2.23	24.75 ±0.32	25.16 ±0.19	710.8±10.2	+0.87
Yuliangyou128 (YuS-1/R66-15)	90.12 ±0.21	38.75 ±0.36	1.97 ±0.02	11.12±0.18	24.23 ±0.37	215.04 ±2.36	9.61 ±0.31	2.64 ±0.06	81.6 ±3.21	25.45 ±0.24	27.06 ±0.21	7541.6±7.6	-1.33
Yuliangyou130 (YuS-5/R66-1)	101.25±0.26	39.15±0.28	1.82±0.04	14.31±0.26	26.12±0.34	201.67±2.21	9.12±0.16	2.13±0.05	88.1±1.11	24.34±0.21	26.73±0.38	7685.2±6.3	+0.55
Yuliangyou141 (YuS-5/R66-7)	98.76±0.33	37.27±0.24	1.67±0.02	13.58±0.31	27.34±0.36	178.65±1.19	8.76±0.21	2.34±0.04	87.5±1.56	23.12±0.36	27.41±0.41	7315.4±5.1	-4.29
Yuliangyou145 (YuS-5/R66-15)	106.24±0.27	39.14±0.35	2.01±0.01	15.12±0.36	29.89±0.43	198.44±2.23	8.89±0.16	2.54±0.08	88.9±2.35	24.77±0.15	28.12±0.32	7612.3±11.5	-0.41
Luliangyou 17 (CK)	90.47±0.26	26.93±0.12	1.73±0.01	9.33±0.21	20.60±0.15	156.00±3.38	9.83±0.25	2.67±0.06	79.7±2.59	26.25±0.27	23.88±0.12	7643.8±7.6	/

Table 4. The rice quality performances and 2AP content detections of parents and selected early-season two-line male sterile lines, restorer lines and new early-season hybrid rice varieties.

Variety name	Milled rice length/mm	Milled rice length/ milled rice width	Amylose content/%	Alkali spreading value	Gel consistency/ mm	Protein content/ %	2AP content/ppb
Xiangling628S	7.9 ± 0.02	2.6 ± 0.1	13.5 ± 1.1	5.9 ± 0.2	62 ± 3.2	7.6 ± 0.1	/
Zhong 2B	8.3 ± 0.05	2.4 ± 0.3	17.8 ± 1.2	4.8 ± 0.3	55 ± 2.1	8.2 ± 0.2	550.1 ± 3.3
Tailand rice 2935	8.0 ± 0.03	2.4 ± 0.2	13.2 ± 1.5	6.1 ± 0.1	78 ± 3.1	5.6 ± 0.1	/
Zhongxiang-1	8.2 ± 0.03	2.3 ± 0.4	15.6 ± 1.3	5.7 ± 0.5	72 ± 2.5	6.3 ± 0.4	681.5 ± 7.6
YuS-1	7.6 ± 0.01	2.1 ± 0.1	18.3 ± 1.4	3.9 ± 0.3	53 ± 3.4	8.7 ± 0.3	650.3 ± 4.6
YuS-5	7.9 ± 0.05	2.0 ± 0.3	16.5 ± 1.6	6.1 ± 0.2	71 ± 2.1	9.2 ± 0.5	510.8 ± 3.9
R66-1	8.1 ± 0.02	1.9 ± 0.1	17.4 ± 3.2	5.1 ± 0.1	56 ± 1.9	5.8 ± 0.1	733.5 ± 5.3
R66-7	7.7 ± 0.01	1.8 ± 0.2	18.1 ± 2.2	5.3 ± 0.3	55 ± 2.8	7.4 ± 0.2	652.8 ± 6.7
R66-15	7.4 ± 0.06	2.0 ± 0.4	15.2 ± 1.5	5.2 ± 0.2	76 ± 3.4	7.1 ± 0.3	694.7 ± 2.1
Yuliangyou120 (YuS-1/R66-7)	7.8 ± 0.05	2.0 ± 0.2	19.2 ± 1.3	3.8 ± 0.1	64 ± 2.1	8.1 ± 0.1	596.4 ± 3.8
Yuliangyou125 (YuS-1/R66-1)	8.3 ± 0.08	1.8 ± 0.1	17.9 ± 1.4	5.1 ± 0.2	67 ± 3.6	9.3 ± 0.4	478.3 ± 1.6
Yuliangyou130 (YuS-5/R66-1)	7.5 ± 0.07	1.7 ± 0.1	19.7 ± 4.1	3.9 ± 0.3	54 ± 4.5	7.7 ± 0.3	413.9 ± 5.1
Luliangyou 17(CK)	6.4 ± 0.02	1.6 ± 0.2	24.9 ± 2.4	3.2 ± 0.2	60 ± 2.7	6.8 ± 0.1	/

Acknowledgement

This research was financially supported by China Natural Science Foundation (No. 31501285), Zhejiang Science and technology projects (Grant No: 2015C32045), China National Key Research and Development Program (2016YFD0101801), and the National S&T Major Project (2016ZX08001006).

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(Received for publication 14 April 2018)