



# Development of a new fragrant and good eating quality rice variety with stripe virus disease resistance by molecular marker-assisted gene pyramiding

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## Abstract

Rice (*Oryza sativa* L.) is one of the most important staple crops providing near half of the global population. With the advance of living standards, people demand for novel rice varieties with better quality of eating and fragrance. Unfortunately, the practical application of pesticides to control rice stripe disease results a significant deterioration of rice quality and nutrition. Therefore, it is an urgent need to develop disease resistant rice varieties with good eating quality and fragrance. Here, we present the strategy and practical development of a new rice variety, Shangshida No. 15, by molecular marker-assisted gene pyramiding. 'Shangshida No. 15' displayed the aroma trait and the same good eating quality phenotypes as that of Jinfeng rice, a famous rice variety for its good eating quality and high yield in the Yangtze River region of China. Shangshida No. 15 exhibited significantly higher average plant height and 1000-grain weight, while its growth duration was approximately 2 weeks shorter than those of Jinfeng. In conclusion, our study demonstrated a breeding strategy for obtaining high quality rice through molecular marker-assisted gene pyramiding, and the resulting new variety could facilitate further attempts to develop more high eating quality rice varieties together with stripe disease-resistant traits without affecting yields.

**Key words:** Eating quality, agronomic traits, molecular marker, gene pyramiding, stripe disease resistance gene

## Introduction

Rice (*Oryza sativa* L.) is a pivotal cereal crop that acts as a staple food for half of the world's population. To meet the demands of the food market, many new rice varieties are developed every year. They may

have high yields and high resistances to various diseases but the eating quality of most of them needs to be further improved. Therefore, one of the major goals of a rice-breeding program is to develop varieties that not only have high yields with resistance to various diseases but also have good eating quality and fragrance.

Rice stripe disease, which is caused by rice stripe virus (RSV), seriously restricts rice production in the subtropical regions of East Asia. Since 2000, this disease has spread to the Middle and Lower Yangtze River areas of China, resulting in a severe loss of production (Wang 2006; Wang et al. 2008). Currently, conventional methods controlling rice stripe disease depend mainly on the use of pesticides to control the insect vector *Laodelphax striatellus* spreading the disease, which are expensive and harmful to the environment. Therefore, resistant rice varieties developed by introducing resistance genes into elite varieties is likely an efficient, economic and safe way to prevent this disease. Presently, molecular markers for stripe disease resistance were based on the *Stv-b<sup>1</sup>* gene (Yao et al. 2009; Chen et al. 2009; Li et al. 2009; Pan et al. 2009; Zhang et al. 2009). However, this gene has not been accurately mapped and cloned. Zhang et al. (2011) cloned a new rice stripe resistance gene, *qSTV11<sup>KAS</sup>*, from Kasalath rice variety (Zhang et al. 2011) that is highly resistant to RSV (Sun et al. 2006). *qSTV11<sup>KAS</sup>* is the first gene that has been cloned for rice stripe disease resistance. Later, Xu et al. (2013) developed molecular markers for this gene.

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Of equal importance is the improvement of the fragrance of rice grains. Presently, at least 13 fragrance alleles have been reported, but only do *badh2-E2*, which possesses a 7-bp deletion in its exon 2, and *badh2-E7*, which has an 8-bp deletion in its exon 7, exist in most of the Chinese fragrant rice varieties (Shi et al. 2008; Xu et al. 2011). The corresponding molecular markers of those alleles have been successfully developed (Shi et al. 2013).

Rice eating quality is mainly affected by three physicochemical properties i.e., amylose content (AC) (Juliano et al. 1981), gel consistency (GC) (Cagampang et al. 1973), and gelatinisation temperature (GT) (Little et al. 1958). Rice grains possess approximately 90% of starch; therefore, genes affecting starch biosynthesis are associated with AC, GC and GT mediated eating quality of rice. Tian et al. (2009) reported that there are 10 genes, such as *Wx*, *SSII-3*, *SBE3*, *AGPiso*, *SSIV-2*, *ISA*, *AGPlar*, *SSI*, *SSSIII-2* and *PUL*, that play important roles in modulating the contents of AC, GC and GT in rice. Molecular markers for rice eating quality have been widely reported (Tian et al. 2010; Han et al. 2004; Bao et al. 2006). Xie et al. (2013) studied the molecular marker genotypes of 10 genes that are related to rice eating quality in 22 important Shanghai rice varieties and found that Jinfeng, Baonong34 and Yinxiang18 are optimal genotypes for eating quality among tested 16 non-soft *japonica* rice varieties (Xie et al. 2013). Jinfeng rice is famous for its high and stable yield and good eating quality, which is better than that of Ilpumbyeo rice, a South Korea's optimal eating quality rice variety (Lu et al. 2004). In 2000, Jinfeng rice was listed as a high-quality rice variety in Shanghai and was well received by consumers. However, this variety does not have stripe disease resistance (Xu et al. 2013).

Marker-assisted selection (MAS) is a highly efficient approach to select for new genotypes in early stages of rice breeding and is widely used in rice breeding programs. It is a promising method that allows pyramiding of several genes in a new rice variety. In this study, our aim was to develop a new rice variety with fragrance, good eating quality, and stripe disease resistance, by molecular marker-assisted gene pyramiding.

## Materials and methods

### Rice cultivars and breeding of new variety

Three rice (*Oryza sativa* L.) cultivars with high yields were used as donor parents to develop a new rice

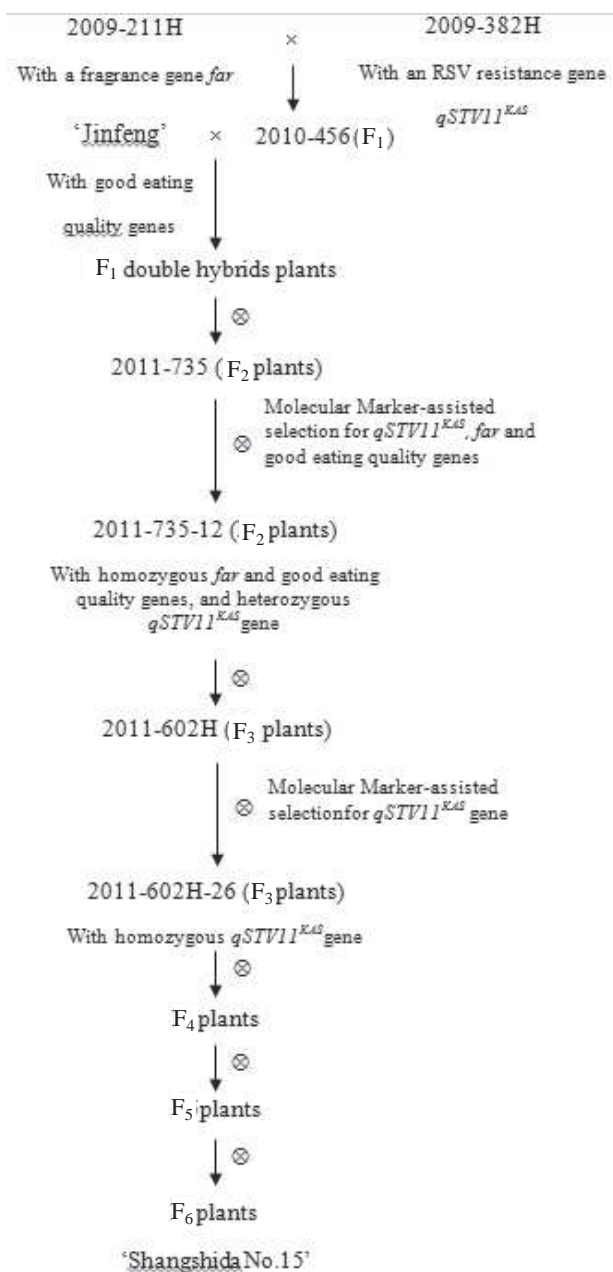
variety, Shangshida No. 15, with fragrance, good eating quality, and stripe disease resistance in the genetic background of Jinfeng rice variety. The three parents included an intermediate '2009-382H' rice variety for breeding the red pericarp rice variety Shangshida No. 7, which also contains a RSV resistance gene, *qSTV11<sup>KAS</sup>* derived from Kasalath. Kasalath is an intermediate 2009-211H variety for breeding the fragrant giant-embryo rice Shangshida No. 8 (Zhu et al. 2013) that harbours a *fgf* gene (*badh2-E7*) with a 8-bp deletion in exon 7 of the betaine aldehyde dehydrogenase (BADH2) gene (Xu et al. 2011), and Jinfeng rice, a high eating quality rice variety with better genotypes for the genes controlling eating quality as the recipient parent. Detailed breeding program was shown in Fig. 1.

### Markers for MAS and detection

The primer sequences of functional markers that were tagged for *qSTV11<sup>KAS</sup>*, *fgf*, *Wx*, *SSII-3*, *SBE3*, *AGPiso*, *SSIV-2*, *ISA*, *AGPlar*, *SSI*, *SSSIII-2* and *PUL* genes were based on published reports (Supplementary Table 1 available at <http://www.isgpb.co.in>). PCR analyses of *qSTV11<sup>KAS</sup>*, *fgf*, *SSII-3*, *SBE3*, *AGPiso* and *SSIV-2* were performed in a total reaction volume of 20  $\mu$ l (Supplementary Table 2 available at <http://www.isgpb.co.in>) and gene-specific programs (Supplementary Table 3 available at <http://www.isgpb.co.in>). PCR products of *fgf*, *SBE3* and *AGPiso* were separated by electrophoresis for 40 mins on a 2% agarose gel after digestion with *AluI*, *SpeI* and *EcoRI*, respectively, at 37°C for 8 hours, while those of *qSTV11<sup>KAS</sup>*, *SSII-3* and *SSIV-2* were separated by electrophoresis directly on a 4% agarose gel for 60 mins. PCR amplifications of *Wx*, *ISA*, *AGPlar*, *SSI*, *SSSIII-2* and *PUL* were carried out in a total volume of 50  $\mu$ l (Supplementary Table 4 available at <http://www.isgpb.co.in>) and gene-specific programs (Supplementary Table 5 available at <http://www.isgpb.co.in>). Products of those PCRs were sequenced in Beijing Genomics Institute.

### Evaluation of agronomic traits and rice eating quality

For agronomic trait evaluation, Shangshida No. 15 rice and Jinfeng rice were planted in three separate field trials in Shanghai (field area: 13.3 m<sup>2</sup> each). The agronomic traits including plant height, effective panicles, spikelet length, filled grains per panicle, total grains per panicle, rate of setting seeds, 1000-grain weight, grain yield, sowing date, heading date, maturing



**Fig. 1. The breeding program to develop a new variety in this study**

date and whole growth duration were measured in these two varieties.

For eating quality evaluation, mature grains of Shangshida No. 15 and Jinfeng were collected. AC and GC content evaluation were determined based on the method of Juliano et al. (1981) and Cagampang et al. (1973), respectively. GT was evaluated according to the National Standards NY147-88 (Anonymous 1989) which was expressed as the alkali spreading value (ASV). All evaluations were performed in five

replications. The sensory assessments of the rice grains were performed using a rice taste meter (SAT1A, Japan) in accordance with the operation protocol. In addition, to compare the flavor of 'Shangshida No. 15' and Jinfeng, we performed aroma determination using the 1.7% KOH method on rice seeds as previous description with the little modification (Berner et al. 1986).

Rice eating quality was measured by three physiological parameters, viz., AC contents in per cent as waxy =  $\geq 2$ , very low = 5 to  $\geq 12$ , low = 13 to  $\geq 20$ , intermediate = 21 to  $\geq 25$  and high =  $> 25\%$  (Juliano et al. 1981); GC contents in mm as soft =  $\geq 50$ , medium = 36 to 49 and hard = 27 to  $\geq 35$  mm (Cagampang et al. 1973) and GT as the alkali spreading value (ASV) in degree centigrade, ASV Score: 1-3 high (774°C); 4-5 low to intermediate (70°C to 74°C) and 6-7, high (55°C to 69°C) (National Standards NY147-88)

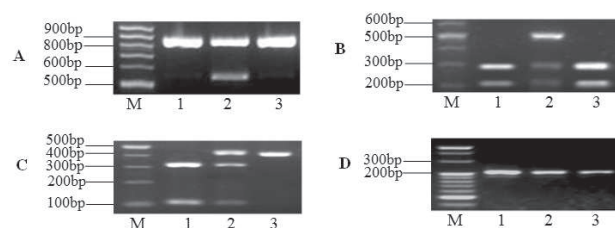
## Results

### Genotyping of the three parental varieties

Before the breeding program, we genotyped the 10 genes that are related to rice eating quality (*Wx*, *SSII-3*, *SBE3*, *AGPiso*, *SSIV-2*, *ISA*, *AGPlar*, *SSI*, *SSSIII-2* and *PUL*) in '2009-382H', '2009-211H' and Jinfeng rice, which indicated that Jinfeng rice carried the microsatellite allele (CT)<sub>18</sub> of *Wx*, while both '2009-382H' and '2009-211H' genotypes had the (CT)<sub>17</sub> allele. Using markers reported by Bao et al. (2006), we found that '2009-211H' and Jinfeng also carry *SSIIa-TT*, while '2009-382H' was heterozygous for *SSIIa-TT/GC* (Fig. 2A). By using the C/G SNP in the 3' untranslated region (3'-UTR) of *SBE3* (Han et al. 2004), we showed that '2009-211H' and Jinfeng both had homozygous *SBE3* genotype, while '2009-382H' was heterozygous for *SBE3* (Fig. 2B). The PCR fragment for *AGPiso* (Tian et al. 2010) in Jinfeng rice could be fully digested by *EcoRI*, and that in 2009-382H could be partially digested by *EcoRI*, while that in '2009-211H' could not be digested by *EcoRI* (Fig. 2C). The genotypes of *SSIV-2* among three parent varieties were found to be identical (Fig. 2D), so were *ISA*, *AGPlar*, *SSI*, *SSSIII-2*, *SSIV-2* and *PUL*, and therefore, genotypes with *qSTV11<sup>KAS</sup>*, *fgr*, *Wx*, *SSII-3*, *SBE3* and *AGPiso* were selected from the selfed progenies.

### New variety breeding program and molecular marker-assisted selection

The detailed breeding program to develop the ideal new variety is depicted in Fig. 1. The F<sub>1</sub> (2010-456)

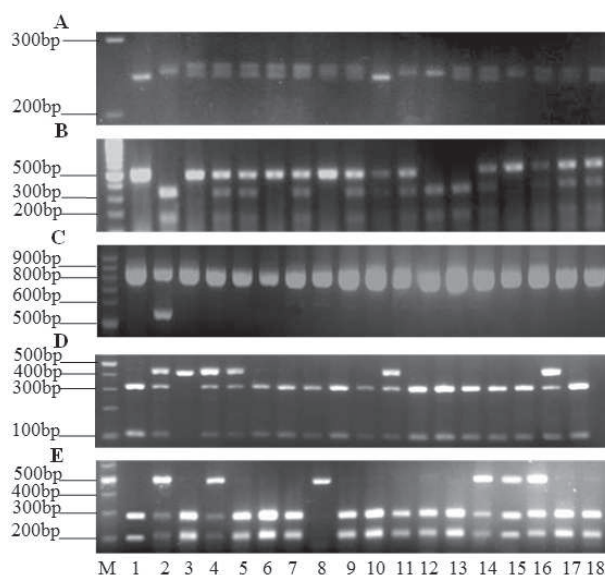


**Fig. 2. Genotype identification of the three parental varieties. A, B, C and D represent the identification of the *SSIIa-TT*, *SBE3*, *AGPiso* and *SSIV-2* genes respectively. Lanes 1, 2 and 3 represent Jinfeng, '2009-382H' and '2009-211H' respectively; M represents the DNA ladder**

hybrids between '2009-211H' and '2009-382H' were generated in March 2010 and planted in May 2010 in Shanghai, which were heterozygous for *qSTV11*<sup>KAS</sup> and *fgr*. The '2010-456' F<sub>1</sub> plants were used as the donor to generate F<sub>1</sub> double hybrids with Jinfeng as the recipient parent. The resulting double hybrid F<sub>1</sub> plants were grown in Hainan Province in April 2011 to generate F<sub>2</sub> seeds, named as '2011-735', which were planted in Shanghai in May 2011. The F<sub>2</sub> populations were genotyped for the *qSTV11*<sup>KAS</sup>, *fgr*, *SSII-3*, *SBE3*, *AGPiso* and *Wx* genes (Fig. 3). The selected '2011-735-12' plant was heterozygous for *qSTV11*<sup>KAS</sup>, but homozygous for other five genes, notably, its genotypes for four eating-quality genes (*SSII-3*, *SBE3*, *AGPiso* and *Wx*) were identical to those in 'Jinfeng'. F<sub>3</sub> seeds of the '2011-735-12' plant were harvested and planted in Hainan Province in December 2012 and resulting F<sub>3</sub> plants, named as '2011-602H-26', were homozygous for *qSTV11*<sup>KAS</sup> and other five eating quality genes. The individual selection of expected plants was repeated from F<sub>4</sub> to the F<sub>5</sub> generations by keeping planting F<sub>4</sub> and F<sub>5</sub> seeds in Shanghai and the Hainan Province. In April 2013, F<sub>6</sub> seeds were harvested in Hainan Province, which showed stable genotypes and displayed relatively good agronomic traits. Plants homozygous for all six genes were further identified with the help of available markers, and the final product, a new elite fragrant rice variety, was named as Shangshida No. 15.

#### **Agronomic traits and eating quality of Shangshida No. 15 and Jinfeng rice**

The average plant height (108.22±0.37 cm) and 1000-grain weight (26.20±0.06 g) of Shangshida No. 15 were significantly higher than those of Jinfeng rice (plant height, 101.56±0.77, 1000-grain weight, 24.10±0.07, P<0.01) (Table 1). Other agronomic traits such as effective panicles, spikelet length, filled grains per panicle and total grains per panicle in Shangshida No.



**Fig. 3. Genotype identification of F<sub>2</sub> populations. A, B, C, D and E represent the identification of the *qSTV11*<sup>KA</sup>, *fgr*, *SSII-3*, *AGPiso* and *SBE3* genes, respectively. Lanes 1 and 2 of figure A are Kasalath and Jinfeng respectively; Lanes 1 and 2 of figure B are 2009-211H and Nipponbare respectively. Lanes 3-18 of figure A and B are individual F<sub>2</sub> plants. Lanes 1, 2 and 3 of figure C, D and E are Jinfeng, 2009-382H and 2009-211H, respectively. Lanes 4-18 of figure C, D and E are individual F<sub>2</sub> plants. M represents the DNA ladder**

15 were similar to those in Jinfeng (Table 1). Although seed setting rate of Shangshida No. 15 was slightly lower than that of 'Jinfeng', grain yield of Shangshida No. 15 was not lower as compared to Jinfeng (Table 1). When both of them were sowed on 25 May, Shangshida No. 15 rice headed earlier (31 August) than Jinfeng (3 September), and matured far earlier (23 October) than that of Jinfeng (5 November). As a result, the whole growth duration of Shangshida No. 15 rice (151 days) was shorter than that of Jinfeng rice (164 days) by approximately 2 weeks.

Both Shangshida No. 15 and Jinfeng had relatively low AC, low GC and low GT (Table 2), and there were no significant differences between two varieties. The grain quality of Shangshida No. 15 rice was regarded as the grade 2 standard of high-quality Japonica rice by the Ministry of Agriculture, and its quality is similar to the grain quality of Jinfeng rice as reported by Lu et al. (2004). Above data indicated that the content of AC, GC and GT affecting rice eating quality in Shangshida No. 15 rice is equivalent to that of Jinfeng.

**Table 1.** Agronomic traits of Shangshida No. 15 rice and Jinfeng rice

Traits	Shangshida No. 15 rice		Jinfeng rice	
Plant height (cm) (n=10)	108.22 ±	0.37**	101.56 ±	0.77
Effective panicles per hectare (n=2)	2463000.00 ±	37500.00	2312250.00 ±	62250.00
Spikelet length (cm) (n=40)	19.90 ±	0.25	19.38 ±	0.27
Filled grains per panicle (n=40)	165.08 ±	5.61	172.06 ±	6.87
Total grains per panicle (n=40)	182.23 ±	5.71	175.23 ±	7.00
Rate of setting seeds (%) (n=40)	91.88 ±	0.48**	95.29 ±	0.58
1000-grain weight (g) (n=3)	26.20 ±	0.06**	24.10 ±	0.07
Grain yield (kg/ha) (n=3)	10168.00 ±	83.50	9154.00 ±	245.51

Note: Values are expressed as the mean±standard error; \*\*significant difference at 0.01 probability (P) levels

**Table 2.** Comparison of rice eating quality between Shangshida No. 15 and Jinfeng

Traits	Shangshida No. 15	Jinfeng
AC (%)	13.20±0.24	13.60±0.15
GC (mm)	71.40±1.74	75.40±3.46
GT and ASV (grade)	6.832±0.09	6.915±0.05

Note: Values are expressed as means ±standard error

Sensory test results for Shangshida No. 15 and Jinfeng shown in Table 3 indicated that there were no remarkable differences in outward appearance, hardness, stickiness, degree of balance or taste, which confirming the results of abovementioned eating quality analysis. Meanwhile, The characterization of fragrance showed that Shangshida No. 15 was fragrant rice, which was different from parent Jinfeng without fragrance.

## Discussion

Molecular marker-assisted selection is widely used in rice breeding. Luo and Yin, (2013) developed a new Thai fragrant rice, 'T5105', which showed a semi-dwarf phenotype with traits such as submergence tolerance and disease resistance to rice blast and bacterial blight. Jiang et al. (2012) improved the rice variety Jin32B with blast resistance via the pyramiding of *Pi1*, *Pi2* and *D12* genes by marker-assisted selection. Regarding rice eating quality, phenotypes cannot be observed in the field. As a result, it is necessary to use marker-assisted selection to achieve good eating quality rice through breeding. *Wx* is typically considered to play an important role in improving eating quality. Jin et al. (2010) improved rice eating and sensory quality by pyramiding of *fgr*, *Wx* and *SSII-3* genes through marker-assisted selection. However, the

**Table 3.** Sensory test of Shangshida No. 15 rice and Jinfeng rice

Traits	Shangshida No. 15	Jinfeng
Outward appearance	7.30±0.06	6.10±0.38
Hardness	6.67±0.03	7.13±0.17
Stickiness	8.63±0.03	7.33±0.46
Degree of balance	7.60±0.06	6.27±0.40
Taste evaluation <sup>†</sup>	76.33±0.33	68.33±2.60

Note: Values are expressed as means ±standard error; <sup>†</sup>Taste evaluation: 100 point mean is best score

pyramiding of more than three genes in rice has not been reported to date. This study, to our knowledge, is the first to pyramid six genes to develop a new rice variety via marker-assisted selection. Currently, molecular markers of the *Stv-b<sup>1</sup>* gene are widely used in rice breeding for stripe disease resistant varieties (Chen et al. 2009; Li et al. 2009; Pan et al. 2009; Yao et al. 2009; Zhang et al. 2009), however, this gene has not been accurately mapped and hence it lacks accuracy in practical applications. Zhang et al. (2011) cloned a new rice stripe resistance gene, *qSTV11<sup>KAS</sup>*, which is associated positively with RSV resistance, however, its markers have not been used in rice breeding. This study is the first study to use *qSTV11<sup>KAS</sup>* marker in practical rice production. Shangshida No.15 harboured the *qSTV11<sup>KAS</sup>* gene and it expected to resist RSV. Nevertheless, further field tests are needed to draw the final conclusion.

Tian et al. (2009) reported that 10 genes influence AC, GC and GT in rice. *Wx* and *SSII-3* centrally affect AC, GC and GT and both *ISA* and *SBE3* affect GC and GT. AC, GC and GT were also shown to be affected by several minor genes, including *SSIII-2*, *AGP1ar*, *PUL* and *SSI* for AC, *AGPiso* for GC and

SSIV-2 for GT. The successful development of Shangshida No. 15, a good eating quality variety, from Jinfeng by molecular marker-assisted selection confirmed the findings of these genes. Fan et al. (2005) reported that although the major genes were usually the most important determinants of the quality traits, interactions between minor genes that did not have detectable effects based on changes in individual loci may collectively have large influences on quality traits. By pyramiding both major (*Wx* and *SSI-3*) and minor genes (*AGPiso* and *SBE3*), we have successfully improved the rice eating quality in Shangshida No. 15, leading to desired low AC, low GC and low GT as well as fragrance in this novel variety, which could provide a reference for the pyramiding of elite genes by molecular marker-assisted selection in rice and other staple crops.

When investigating agronomic traits, we found that although the seed setting rate in Shangshida No. 15 was slightly lower than that of Jinfeng, the grain yield of the two rice genotypes was not significantly different. Dai et al. (2008) reported the factors that influence grain yield namely, the average of filled grains per panicle > 1000-grain weight > effective panicles. In this study, the average of filled grains per panicle of Shangshida No. 15 rice and Jinfeng did not differ significantly, but the 1000-grain weight of Shangshida No. 15 rice was significantly higher than that of Jinfeng (Table 1). Higher 1000-grain weight, thus, may make Shangshida No. 15 maintain higher grain yields. On the other hand, the growth duration of Shangshida No. 15 was 13 days shorter than that of Jinfeng, which could facilitate for planting succeeding crops, such as wheat and barley, in the Middle and Lower Yangtze River areas of China. Taken together, Shangshida No. 15 could be potentially a commercial variety with good eating quality with RSV resistance and favourable agronomic traits.

Conclusively, this study has demonstrated the feasibility of molecular marker-assisted pyramiding of six genes in the development of a new rice variety, Shangshida No. 15. The resulted Shangshida No. 15 harboured one fragrance gene *fgr*, one RSV resistance gene *qSTV1<sup>KAS</sup>* and four eating quality genes for *Wx*, *SSI-3*, *AGPiso* and *SBE3*, representing a new parent rice variety with optimal quality for rice production and hybrid breeding.

#### Authors' contribution

Conceptualization of research (GCZ, JYL); Designing of the experiments (GCZ, MX, JYL); Contribution of

experimental materials (JYL); Execution of field/lab experiments and data collection (FYF, DMH, TZ); Analysis of data and interpretation (GCZ, MX, JYL); Preparation of manuscript (GCZ, MX, JYL).

#### Declaration

The authors declare no conflict of interest.

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**Supplementary Table 1.** Functional primers of PCR markers for breeding program

Gene	Primer Sequences(5'-3')	Type of marker	Reference
<i>qSTV11KAS</i>	F: TGTCACAACGACCACGG R: CGGCTCTCCTCCCAGTA	STS	Xu et al. (2013)
<i>fgr</i>	F: GATGGGTTCAAGCGGAGA R: GCACTGGTCACTGTTTTAC	CAPS ( <i>AluI</i> )	Shi et al. (2013)
<i>Wx</i>	F: GGGT AAAATGTGTTGCGGGA R: AGCCTAACCAACATAACGAACG	SSR	Guo et al. (2011)
<i>SSII-3</i>	F7: CTGGATCACTTCAAGCTGTACGAC F22: CAAGGAGAGCTGGAGGGGGC R1: GCCGGCCGTGCAGATCTTAAC R21: ACATGCCGCGCACCTGGAAA	STS	Bao et al. (2006)
<i>SBE3</i>	F: GTCTTGGACTCAGATGCTGGACTC R: ATGTATAACTGGCAGTTCTGAACGG	CAPS ( <i>SpeI</i> )	Han et al. (2004)
<i>AGPiso</i>	F: TGGAATGGGAACCTCTATTATTGGT R: TCCCAACCTCTACCTTCAAATG	CAPS ( <i>EcoRI</i> )	Tian et al. (2010)
<i>SSIV-2</i>	F: CTTGGTGGTGCCTGCTTGTATG R: CCGACAGTATCGATTTCTGATG	STS	Xie et al. (2013)
<i>ISA</i>	F: ACTTTTGCATGGGCATGGGC R: TAGACATATGGCCAGATGTCAC	SNP	Xie et al. (2013)
<i>SSI</i>	F: TCTGAAAATCTCCCTGCCTATG R: CAGCAGTTGTGACCTCCCAT	SNP	Xie et al. (2013)
<i>AGPlar</i>	F: CAAGGACAGGAAACACACATCG R: GACAGAAATGAATGAAACGAAGC	SNP	Xie et al. (2013)
<i>SSIII-2</i>	F: GAGCAGGCTGAAGGTCGTC R: CGTATGAAGGGAAATCGTCC	SNP	Xie et al. (2013)
<i>PUL</i>	F: TATGATTTCGATTGGACC R: CGACGGCAGATAAGGACA	SNP	Xie et al. (2013)

F= forward primer; R = reverse primer; CAPS = cleaved amplified polymorphic sequence; STS= sequence-tagged site; SSR= simple sequence repeat; SNP = single nucleotide polymorphism;

**Supplementary Table 2.** The PCR reaction system of *qSTV11KAS*, *fgr*, *SSII-3*, *AGPiso*, *SBE3* and *SSIV-2* genes in this study

Element	Volumes ( $\mu$ l)
Taq DNA polymerase	0.2
Genomic DNA	0.5
10 x buffer	2.0
dNTPs	0.5
Primers	0.5
H <sub>2</sub> O	16.3
Total	20.0

**Supplementary Table 3.** The PCR reaction conditions of *qSTV11KAS*, *fgr*, *SSII-3*, *AGPiso*, *SBE3* and *SSIV-2* genes in this study

Process	Genes	Temp. (°C)	Time
Pre-denaturation		94	5 mins
Denaturation		94	45 sec
	<i>qSTV11KAS and fgr</i>	53	
Annealing	<i>SSII-3 and SBE3</i>	63	45 sec
	<i>AGPiso</i>	55	
	<i>SSIV-2</i>	56	
Extension		72	45 sec
Total extension		72	10 mins

The cycles is 32



(ii)

**Supplementary Table 4.** PCR reaction system of *Wx*, *ISA*, *AGPlar*, *SSI* and *SSSIII-2* and *PUL* genes in this study

Element	Volumes ( $\mu$ l)
KOD-Plus-Neo (1 unit)	1.0
Genomic DNA	1.0
10 x buffer	5.0
dNTPs (2 mM)	5.0
MgSO <sub>4</sub> (25 mM)	3.0
DMSO	2.0
Primers	3.0
H <sub>2</sub> O	30.0
Total	50.0

**Supplementary Table 5.** PCR reaction conditions of *Wx*, *ISA*, *AGPlar*, *SSI* and *SSSIII-2* and *PUL* genes in this study

Process	Molecular markers	Temp. (°C)	Time
Pre-denaturation		94	5 mins
Denaturation		94	45 sec
Annealing	<i>Wx</i>	54	
	<i>ISA</i>	56	45 sec
	<i>AGPlar</i> , <i>SSI</i> & <i>SSSIII-2</i>	58	
Extension	<i>PUL</i>	51	
		72	45 sec
Total extension		72	10 mins

The cycles is 32