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Genotyping of 10 Grain Shape Genes in High Quality *Xian/indica* Varieties by Molecular Marker

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Abstract 12 representative high quality *xian/indica* rice varieties were selected as test materials. Seven grain length genes including *GS3*, *LGY3*, *qGL3*, *GL7*, *SLG7*, *TGW6* and *GS9*, and three grain width genes including *GW8*, *GW5* and *GS5* were tested by molecular marker. The results showed that: All 12 varieties have the *gs3* long-grain allele, Ezhong 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao and Super-Basimati have the *GL7* and *SLG7* long-grain allele, 11 varieties with the exception of Jianzhen 2 have the *gw8* slender-grain allele, Yujing 91, Yuzhenxiang, 9311, R835, Huangmaozhan, Yangxizao have the *GS5* Wide-grain allele. This study is helpful to establish an overall understanding of the genotypes of 10 grain-shape genes in high quality *xian/indica* varieties, and provides important genotype information in selecting and cultivating new high quality long-grain *indica* varieties via molecular marker-assisted selection in the future.

Keywords High quality *xian/indica* rice; Grain shape gene; Molecular marker; Genotype

Rice is the most important crop in China, the production and the consumption of which is the highest in the world. For a long period in the last century, owing to lack of food, people took yield as the only goal of rice breeding, but ignored its quality. Since the beginning of this century, the production of rice, wheat, corn and other grains has all improved. With the continuous improvement of people's living standards and the increasing competition in the international and domestic markets, the problem of poor quality of rice in China has become increasingly prominent, which is becoming the main bottleneck of the current rice industry (Wan and You, 2018).

High quality rice in China accounts for more than 10% of the market, and Hubei Province can plant at least 3 million mu. But the four varieties with the largest promotion area, Ezhong No. 5, Jianzhen No. 2, Yuzhenxiang, and Exiang No. 2, have planted less than 1 million mu totally. It can be seen that the supply of high quality rice varieties is far from meeting the demand for the development of production, which has become the bottleneck of industrialization development of high quality rice. After nearly 20 years' scientific research, some high quality rice varieties had bred by Chinese rice breeders and been approved, such as the high quality *xian/indica* rice, Ezhong No. 5, cultivated by our unit, whose chalky rice rate and chalkiness degree were is double 0. Except the amylose content, the other indexesall reached the first grade of National standard, the taste score remained above 85, and it was examined and approved in Hubei and Hainan Province (Li et al., 2005) The quality of Ezhong No. 5 is equivalent to that of Thai fragrant rice. It has become the first choice for large rice processing enterprises such as "Guobao Qiaomi" and "Wacang Dami" in Hubei Province to develop high quality rice and build famous brands.

At present, the generally recognized high quality *xian/indica* rice refers to a type that has crystal-clear appearance, slender grain, excellent physical and chemical indicator, and high taste score, which is comparable to Thai fragrant rice. High quality *xian/indica* rice requires not only the improvement of physical and chemical indicators, but also the appearance and taste. The grain shape traits not only affect the yield, but also affect the quality, mainly including grain length, grain width and L/W Ratio (Gao et al., 2011). The grain shape traits are closely related to

the appearance and milling quality of rice, and also affect the thousand-grain weight. Therefore, studying the heredity and molecular mechanism of rice grain shape and applying it to breeding practice will help to improve the yield and quality (Luo et al., 2004; Huang and Qian, 2017). In order to analyze the genetic basis of grain length traits of high quality rice, this study collected 12 high quality *xian/indica* rice varieties, and used molecular marker methods to detect and analyze 10 cloned rice grain-type genes. This study not only has a comprehensive understanding of the genotypes of long-grain *xian/indica* rice varieties, but also provides scientific basis to breed new high quality *xian/indica* rice varieties by using molecular marker methods in the future.

1 Results and Analysis

1.1 Analysis of grain shape traits of 12 high quality *xian/indica* rice

The grain length of 12 high quality *xian/indica* rice varieties is 7.36~11.93 mm, the grain width is 2.10~2.73 mm, and the L/W Ratio is 2.70~5.29 mm (Table 1). The t-test results showed that, contrasting 9311, the grain length and L/W Ratio of high quality rice, Ezhong No. 5, Yujing 91, Yuzhenxiang, Huangmaozhan and Super-Basmati are larger than 9311, and the difference is extremely significant ($P<0.01$); The grain length of Xiang 5, 15Q340, Yangxizao, Huarun 2 and Jianzhen 2 are not significantly different from 9311; The grain length and L/W Ratio of R835 are significantly less than 9311. The results showed that, according to the L/W Ratio, these 12 high quality *xian/indica* rice varieties can be divided into three types: super long grain (>4.0), general long grain (>3.0 and <4.0), and medium and short grain (<3.0). Ezhong No. 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao and Super-Basmati belong to super long grain. R835 belongs to medium and short grain. 9311 and other five varieties belong to general long grain.

Table 1 Measurement of grain shape of 12 high quality *xian/indica* varieties

Accessions	Length (mm)	Width (mm)	L/W Ratio
9311(CK)	9.5	2.5	3.8
Ezhong 5	11.1	2.3	4.8
Yujing 91	11.9	2.6	4.6
Yuzhenxiang	11.7	2.4	4.9
Xiang 5	9.1	2.5	3.6
15Q340	9.7	2.6	3.7
R835	7.4	2.9	2.5
Huangmaozhan	11.7	2.2	5.3
Yangxizao	10.2	2.3	4.4
Huarun 2	9.3	2.4	3.9
Super-Basimati	10.9	2.2	5.0
Jianzhen 2	9.7	2.4	3.9

1.2 Genotype analysis

GS3 is the first gene to be cloned to regulate grain shape, which mainly regulates rice grain length and weight. When a C-A mutation in the second exon caused a loss-of-function mutation in the N-terminal domain that regulates organ size, the rice grains became longer. Fan et al. (2009) designed primers SF28F/SF28R (Table 2) based on this site to amplify the genomic DNA of rice material, and a 136 bp target fragment could be obtained. The fragment of short grain allele produced two segments, 110 bp and 26 bp, after *Pst* I digestion. While due to the C-A mutation, the 136 bp produced by the long-grain allele cannot be digested by *Pst* I. Only the genotypes of japonica rice, referring to Nipponbare, could be digested by *Pst* I (Figure 1), and 110 bp bands containing *GS3* gene appeared. None of the tested 12 *xian/indica* rice varieties could be recognized and digested by *Pst* I, and they all contained the long-grain *gs3* allele.

Table 2 Functions markers for rice grain shape gene

Marker	Primer Sequence	Type	Polymorphic site	PCR product size (bp)	Reference
SF28F SF28R	F:TGCCCATCTCCCTCGTTTAC R:GAAACAGCAGGCTGGCTTAC	CAPs-*Pst* I	C/A substitution in Exon-2	136/110	Fan et al., (2009)
lgy3F lgy3R	F:CCTGACAATAATTCGCCCAATA R:CGTAAGAGAGCACGCACGTA	InDel	InDel between Exon-8 and Intron-9	392	Liu et al., (2018)
GL3F GL3R	F:CGATTCTATCTGGTTCAGTGGTAGA R:CACCGCCGTGTAAGTTCAAC	dCAPs-*Acc* I	A/C substitution in Exon-10	139/118	Yi et al., (2016a)
NGSP11F 210QCF	F:TGACACGCCACAGTCCAAGACGAGCAGT R:AAGGGAGTTGAGAGTAGAAAAAA	InDel	17.1Kb duplication	1421	Wang et al., (2015b)
SLG7F SLG7R	F:CCATACCACATCTCATCTCAC R:GCTCACGCACATCCAACT	InDel	11bp delete in promoter at -104bp	109/98	Zhou et al., (2015)
CAPS6-1F CAPS6-1R	F:CCACAGCCACAACGAGAAT R:ACCGTTCGGGTAGGTTATGT	CAPs-*Bss*H II	1bp delete (G) at 313bp	590/372/217	Wang et al., (2014)
GS9F GS9R	F:CTCGCTTTCTTTACCTATGTTCAAGCCTTC R:GAAACTGTTGCCTTTGCTCTTGTCT	InDel	7Kb insert	250	Zhao et al., (2018)
GW5-1F GW5-1R	F:AGTACGACCATGATGTTTCCC R:GACCTAACCCATCTCATTCCA	InDel	1212bp InDel	775	Weng et al., (2008)
GW8-1F GW8-1R	F:AAAGAGACAGCCACGGAATC R:ATCTTGAGATCCCACTCCATG	InDel	10bp InDel in promoter	151/141	Yi et al., (2016b)
GW8-2F GW8-2R	F:AGGCGAGATCAGCTTCGTCA R:GCTACCGTCTTCAGAAGTGGC	dCAPs-*Hin*d II	A/C substitution in Exon-3	108/89	
GW8-3F GW8-3R	F:ATGTTCTCCGATGGTGGGTT R:TGAAGGCCAGAGATGAGAGG	CAPs-*Nco* I	G/T substitution in Exon-3	164/134	
GS5-1F GS5-1R	F:GCAAGACAAGGAGCAGCACTA R:AGAAGCCGACCCCAACAG	CAPs-*Dde* I	CTA/ACC substitution in Exon-2	225/196	Yi et al., (2016c)
GS5-2F GS5-2R	F:CAGTTCTCGGTACTGCGTCGA R:CACAAACCTCCCAGCAACC	dCAPs-*Sal* I	A/C substitution in Exon-9	188/169	

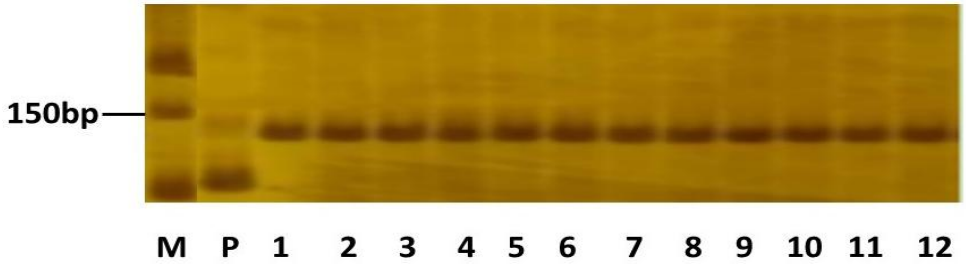

Figure 1 *GS3* genotypic analysis of high quality *xian/indica* varieties (after *Pst*I digestion)

Note: M: DL500 DNA Marker; P: Nipponbare; Lane 1~12: Ezhong 5, Yujing 91, Yuzhenxiang, Xiang5, 9311, 15Q340, R835, Huangmaozhan, Yangxizao, Huarun 2, Super-Basmati, Jianzhen 2

The *LGY3/OsLG3b* gene encodes a transcription factor OsMADS1 containing the MADS domain, which is a key effector of the downstream of the G protein βγ dimer (Liu et al., 2018; Yu et al., 2018). The *LGY3* allele has a deletion between the 8th intron and the 9th exon, and makes (TCCTTGGTGAAGGTA) insert (ATGTATATATACT), resulting in a loss-of-function mutation, which increases the grain length of rice. The primers lgy3F/lgy3R were designed according to the mutation site to amplify the selected rice material. If there is no mutation, a 392 bp sequence can be amplified. If it becomes a long-grain allele after mutation, no band will appear. The experimental results showed that all the 12 tested *xian/indica* rice varieties could amplify a 392 bp sequence, and all of them contained the *LGY3* short-grain allele.

1.3 Genotype analysis of *qGL3*/*GL3.1*/*qGL3-1*

qGL3/GL3.1/qGL3-1 is a gene that controls grain length on the third chromosome of rice. It was reported by three laboratories in China in 2012 that *qGL3/GL3.1/qGL3-1* plays a role as a negative regulator in the regulation of rice grain length (Yi et al., 2016a). In the 10th exon of this gene, there is an A/C mutation site, which is related to the grain length of rice. The average grain length of genotype A exceeds the average grain length of genotype Yi et al. (2016a) developed a molecular marker for *qGL3* based on this mutation site. If the genotype is A, the generated 139 bp target fragment cannot be recognized and digested by *Acc* I enzyme; The genotype C can be recognized and digested by *Acc* I enzyme, resulting in two bands, 118 bp and 21 bp. The results of PAGE gel electrophoresis showed that all the 12 tested *xian/indica* rice varieties could be recognized and digested by *Acc* I, and all were judged to be C genotypes, which belonged to the varieties without *qGL3/GL3.1/qGL3-1* long-grain allele.

GL7 is a gene that controls grain length on the 7th chromosome of rice. It encodes a protein homologous to Arabidopsis long leaf protein and regulates the longitudinal elongation of cells. The tandem duplication of a 17.1 kb fragment at the *GL7* site leads to up-regulation of *GL7* and the down-regulation of nearby negative regulators, resulting in the increase of grain length and the improvement of appearance quality. Wang et al. (2015b) designed primers based on this mutation site. If there is a 17.1 kb tandem repeat in the detected rice material, a 1.4kb fragment can be amplified during PCR; if not, no amplification products will appear after PCR. Ezhong No. 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao, and Super Basmati can amplify a 1.4 kb fragment, which are judged to contain the *GL7* long-grain allele. Xiang 5, 9311, 15Q340, R835, Huarun 2 and Jianzhen 2 have bands, which are judged to contain no *GL7* long-grain allele (Figure 2).

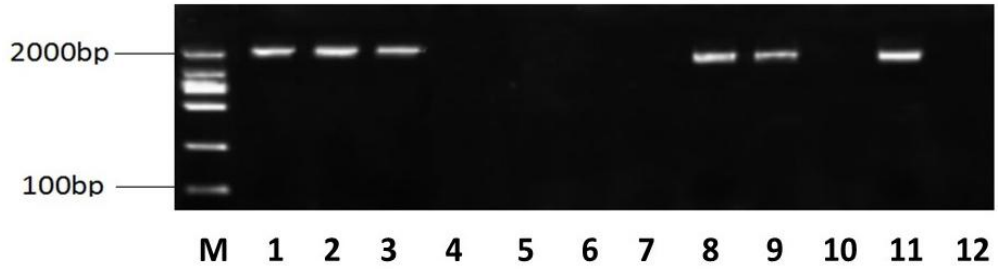

Figure 2 GL7 genotypic analysis of high quality *xian/indica* varieties

Note: M: DL500 DNA Marker; Lane 1~12: Ezhong 5, Yujing 91, Yuzhenxiang, Xiang5, 9311, 15Q340, R835, Huangmaozhan, Yangxizao, Huarun 2, Super-Basmati, Jianzhen 2

SLG7/GW7 is a gene that controls grain length on the 7th chromosome of rice (Wang et al., 2015a; Zhou et al., 2015). By comparison, it was found that the deletion of 11 bp at the long-grain allele promoter -104 bp is closely linked to the grain shape, and the InDel molecular marker M-SLG7 was developed based on this site. We using the marker to amplify rice material, the insertion and deletion of 11bp could be identified by PAGE electrophoresis. The material containing 11 bp can amplify a 109 bp DNA fragment, while the material without 11 bp can amplify a 98 bp fragment. The results showed (Figure 3), Ezhong 5, Yujing 91, Yuzhixiang, Huangmaozhan, Yangxizao and Super Basmati amplified 98 bp fragments, indicating that they contain the *SLG7* long-grain allele and other varieties amplified 109 bp fragments, indicating that they do not contain the *SLG7* long-grain allele.

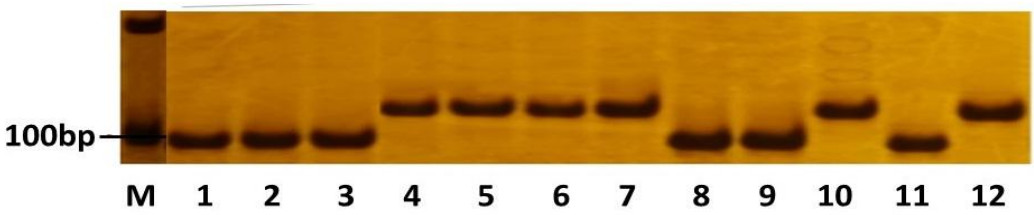

Figure 3 SLG7 genotypic analysis of high quality *xian/indica* varieties

Note: M, DL500 DNA Marker; Lane 1~12: Ezhong 5, Yujing 91, Yuzhenxiang, Xiang5, 9311, 15Q340, R835, Huangmaozhan, Yangxizao, Huarun 2, Super-Basmati, Jianzhen 2

TGW6 is a gene that controls grain length and thousand-grain weight on the 6th chromosome of rice. It encodes IAA-glucose hydrolase and has only one exon but no introns. Wang et al. (2014) designed a CAPS-*Bss*H II marker and amplified the rice genome with this marker to obtain a 590 bp target fragment. The long-grain allele can be recognized by *Bss*H II and digested to produce two bands, 372 bp and 217 bp, while the PCR product of the short-grain allele cannot be digested by the *Bss*H II restriction endonuclease. The results showed that the 12 tested *xian/indica* rice varieties cannot be digested by *Bss*H II, and it was judged that none of them contains the *TGW6* long-grain allele.

GS9 is a gene that controls grain length on the 9th chromosome of rice and encodes a protein with no known conserved functional domains. It is a transcriptional activator that regulates rice grain shape and appearance quality. Zhao et al. (2018) developed the InDel molecular marker based on the insertion of 7 kb at the second exon of the polymorphic site. *GS9* short-grain allele has no insertion of 7 kb fragment and about 250 bp fragment can be obtained by PCR. The *GS9* long-grain allelic has insertion of 7 kb fragment and will not produce about 250 bp fragment. The products were detected by agarose electrophoresis, and the results showed that the products were all about 250 bp, indicating that the 12 tested *xian/indica* rice varieties don't contain *GS9* long-grain allele.

GW5 is a gene that controls grain width on the 5th chromosome of rice. Compared with narrow-grain rice, wide-grain varieties have a 1.2 kb deletion in nucleotides. Based on this 1.2 kb deletion, an Indel marker was designed (Weng et al., 2008). Using this primer to amplify the tested rice material, we found that there is no PCR product after PCR for wide-grain allele varieties, while the amplified product of narrow-grain allele is 775 bp fragment. The test results showed that none of the 12 *xian/indica* rice varieties had a 1.2 kb nucleotide deletion, indicating that they don't contain the *GW5* wide-grain allele.

GW8 is a gene that controls grain width on the 8th chromosome of rice. Yi et al. (2016b) developed functional markers (GW8-1), dCAPs (GW8-2) and CAPs (GW8-3) according to the 10 bp Indel in the gene promoter region and the 2 missense sites of the third exon A/C and T/G. And according to these 3 mutation sites, the gene is divided into 8 haplotype (called Hap for short). The grain lengths of Hap1, Hap2, Hap3, and Hap7 are longer than those of Hap4, Hap5, and Hap6. For GW8-1 primers, if there is a 10 bp sequence insert fragment in the tested rice material, a 151 bp fragment will be generated after PCR, while if there is no 10 bp sequence insert fragment, a 141 bp fragment will be generated. The result shows (Figure 4) that the three detection sites of Jianzhen No. 2 belong to 10 bp-, C, and G alleles, which belong to the wide-grain haplotype, Hap6. The three detection sites of 15Q340 and Super Bassi belong to 10 bp+, C and T alleles, which belong to the slender-grain haplotype, Hap1. And the

three detection sites of the other nine varieties belong to 10 bp+, A, and T alleles, which belong to the elongated-grain haplotype, Hap3.

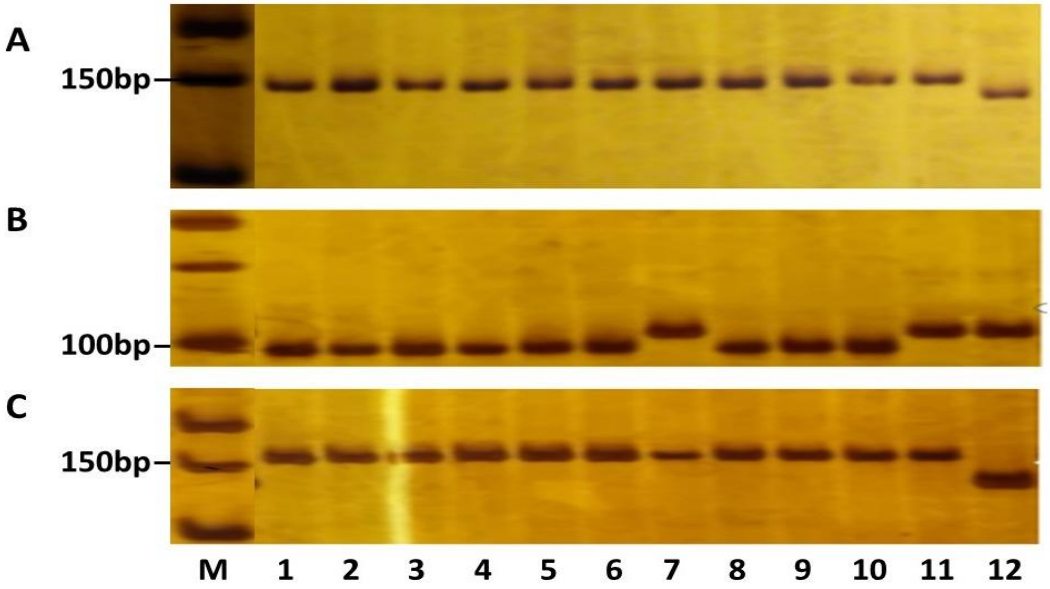

Figure 4 GW8 genotypic analysis of high quality *xian/indica* varieties

Note: M: DL500 DNA Marker; Lane 1~12: Ezhong 5, Yujing 91, Yuzhenxiang, Xiang5, 9311, 15Q340, R835, Huangmaozhan, Yangxizao, Huarun 2, Super-Basmati, Jianzhen 2; A: GW8-1; B:GW8-2; C: GW8-3

GS5 is a quantitative trait gene that controls grain width, fullness and thousand-grain weight on the 5th chromosome of rice, encoding a serine carboxypeptidase. The two mutation sites of *GS5* in ACC/CTA in the second exon and A/C in the ninth exon make a significant difference to the grain length, grain width and L/W Ratio of rice grains. Yi et al. (2016c) designed two CAPS markers, GS5-1 and GS5-2, based on these two sites. And according to these two mutation sites, the gene can be divided into 4 haploid genotype. GS5-1 can amplify a 225 bp PCR product, which be cut into 196 bp after *Dde* I digestion in CTA genotype, while cannot be cut in ACC genotype. And GS5-2 can amplify a 188 bp PCR product, which can be cut into 169 bp after *Sal* I digestion in C genotype, while cannot be cut in A genotype. The grain width of Hap2 of ACC-C in *xian/indica* subspecies is significantly larger than that of the other three haplotype. The results are shown in Figure 5. Among the 12 varieties, Yujing 91, Yuzhenxiang, 9311, R835, Huangmaozhan, and Yangxizao all have 225 bp and 169 bp bands, and the corresponding mutation sites are ACC and C, which belong to the wide-grain haplotypeHap2. while the other 6 varieties show 225 bp and 188 bp bands, and the corresponding mutation sites are ACC and T, which belong to the narrow-grain haplotype Hap1.

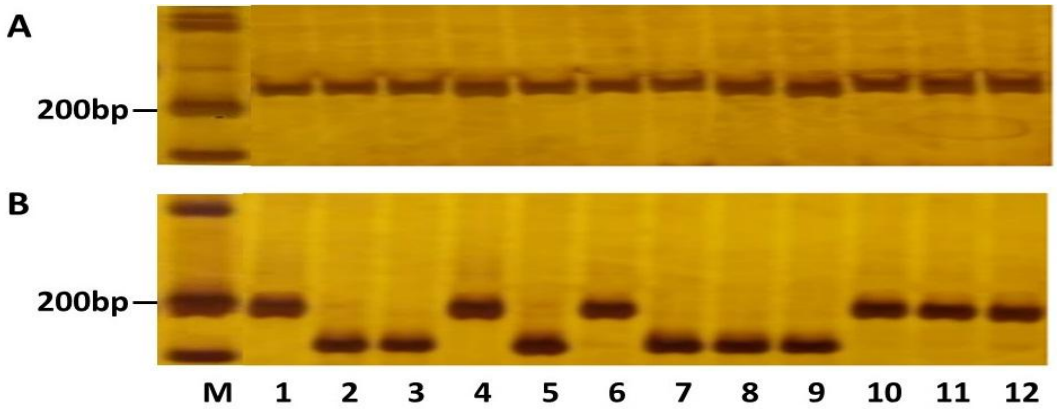

Figure 5 GS5 genotypic analysis of high quality *xian/indica* varieties(A:GS5-1；B:GS5-2)

Note: M, DL500 DNA Marker; Lane 1~12: Ezhong 5, Yujing 91, Yuzhenxiang, Xiang5, 9311, 15Q340, R835, Huangmaozhan, Yangxizao, Huarun 2, Super-Basmati, Jianzhen 2

1.4 Genotypic analysis of 10 grain shape genes in high quality *xian/indica* rice varieties

Table 3 shows the genotypic analysis of 10 grain shape genes in 12 high quality *xian/indica* rice varieties.

In this study, molecular marker technology was used to identify and analyze the selected 12 high quality *xian/indica* rice varieties with 10 cloned genes which related to grain length and grain width. The test results are shown in Table 3. The results show that 12 high quality *xian/indica* rices all contain *gs3* long-grain allele; Ezhong No. 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao, Super Basmati and other 6 varieties contain *GL7* and *SLG7* long-grain alleles; 11 varieties except Jianzhen 2 contain *gw8* slender-grain allele; Yujing 91, Yuzhenxiang, 9311, R835, Huangmaozhan, Yangxizao contain *GS5* wide-grain allele; The detected rice varieties don't contain *lgy3*, *qGL3*, *TGW6* and *gs9* long-grain allele and don't contain *GW5* wide-grain allele.

Table 3 12 detected genes in high quality *xian/indica* varieties

Accessions	*gs3*	*lgy3*	*qGL3*	*GL7*	*SLG7*	*TGW6*	*gs9*	*GW5*	*gw8*	*GS5*
9311(CK)	✓								✓	✓
Ezhong 5	✓			✓	✓				✓	
Yujing 91	✓			✓	✓				✓	✓
Yuzhenxiang	✓			✓	✓				✓	✓
Xiang 5	✓								✓	
15Q340	✓								✓	
R835	✓								✓	✓
Huangmaozhan	✓			✓	✓				✓	✓
Yangxizao	✓			✓	✓				✓	✓
Huarun 2	✓								✓	✓
Super-Basimati	✓			✓	✓				✓	
Jianzhen 2	✓									

2 Discussion

Traditional rice breeding methods are time-consuming, labor-intensive, and vulnerable to environmental influences. Molecular marker-assisted selection (MAS), as a new breeding method developed with the development of molecular biology and bioinformatics technology, has been widely used in major crop breeding practices because of its accuracy, speed, and no environmental influence. If conventional rice breeding technology is combined with MAS technology in the future, new high quality rice varieties can be cultivated more accurately and efficiently. Clarifying parent genotype and corresponding phenotype is the premise of using molecular marker-assisted selection. This study is helpful to establish an overall understanding of the genotypes of 10 grain shape genes in high quality *xian/indica* rice varieties, and provides important theoretical basis and genotype information in selecting and cultivating new high quality long-grain *indica* varieties via molecular marker-assisted selection in the future.

So far, according to incomplete statistics, more than 120 QTLs related to grain length of rice have been mapped. The statistical results show that the main QTLs controlling grain length are mainly located on the 3rd chromosome and the 7th chromosome (Han et al., 2016). The main genes controlling grain length on the 3rd chromosome are mainly *GS3* and *qGL3*. Ding et al. (2014) showed that when only one of these two genes exists, it can show a strong phenotypic effect, but when they exist at the same time, there is no gene cumulative effect. Due to the limited distribution of *qGL3* gene in germplasm resource, the gene controlling grain length on the third chromosome is mainly the first cloned grain length gene *GS3* in the existing bred varieties. Therefore, more studies are also focused on *GS3* gene. Yang et al. (2010) improved the grain shape of rice by using a single fragment substitution line carrying *gs3* allele, which effectively improved the appearance quality of Huajingxian 74. Wang et al. (2012) introduced *gs3* allele of 9311 into Zhenshan 97B. The grain length of Zhenshan 97B reached 9.2 mm, increased by 10.8%, and the thousand-grain weight was also increased. The appearance quality and yield were improved as well. Liu et al. (2018) found that the polymerization of *lgy3* and *gs3* alleles increased the grain yield (about 7%) and improved rice quality, providing a new strategy for breeding high-yield and high-quality rice. It can be seen from this study that 12 *xian/indica* rice varieties all contain the *gs3* allele, and the *gs3* allele has been widely used in the traditional empirical breeding of long-grain *xian/indica* rice. The reason is

that *gs3* gene is the main gene and its parents are widely distributed. Both long-grain tropical japonica rice and long-grain *xian/indica* rice contain *gs3* long-grain allele.

At present, the cloned grain shape genes not only cluster in a specific region of a chromosome, but also have more allelic forms at each locus, such as *qGL3/GL3.1/qGL3-1* on the third chromosome and *GL7/GW7/SLG7* on the 7th chromosome. Gene sequence analysis showed that *GL7* has two copies of *GL7* alleles compared with gl7, leading to the improvement of gene expression; The functional variation of *GW7* and *SLG7* is the same, but the difference of gene promoter leads to the difference of expression level; Cytological mechanism studies showed that *GL7* and *GW7* act in the same way and increasing the vertical division of cells and decreasing the lateral division leads to slender grains. Studies on *SLG7* showed that increasing the vertical length of cells and decreasing the lateral width leads to slender grains, which is not related to cell division (Wang et al., 2015a; Wang et al., 2015b; Zhou et al., 2015). Although the results of different research groups indicate that the functional haploid genotype sites and regulatory mechanisms of *GL7/GW7/SLG7* are not completely the same, these three alleles all improve the expression level of gene *Os07g0603300* and make the arrangement and structure of the starch grains of the rice grains changed. Moreover, these three alleles all lead to more slender rice grain, better color of rice grain, and improved appearance and taste of rice. If they aggregate *GL7+gw8* or *gs3+GW7*^{TFA}(*SLG7*), slender grains can be shown (Wang et al., 2015a; Wang et al., 2015b).

In this study, we found that Ezhong No. 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao, Super Basmati and other 6 super-long-grain high quality *xian/indica* rice varieties from different sources contain allelic variation sites of *GL7* and *SLG7*/*GW7* simultaneously. That is, they have two copies of the *GL7* gene and the functional site of the *SLG7* promoter at the same time, which showed that the functional loci of these three alleles are closely linked and may be the same allele in nature. Moreover, three long-grain gene loci (*gs3*, *GL7/GW7/SLG7* and *gw8*) are polymerized in the six super long-grain *xian/indica* varieties. Compared with general long-grain varieties such as 9311, only these super long-grain varieties have *GL7/GW7/SLG7* allele. *GL7/GW7/SLG7* gene is likely to be the main reason for these varieties to perform better than general ones. These six super long-grain *xian/indica* rice varieties not only taste good, but also show slender grains, good appearance and good physical and chemical quality. Among them, Ezhong No. 5, Yujing 91, and Yuzhenxiang have successively won the gold award of *xian/indica* rice group about the evaluation of eating quality of national high quality rice varieties. Super Basmati is the representative variety of high quality *xian/indica* rice in Southeast Asia. Huangmaozhan and Yangxizao are high quality local varieties in Hubei Province. Although the yield is low, due to the good appearance and eating quality, some enterprises still produce large areas at present. Although these varieties are not directly bred through MAS technology, they provide a theoretical and material basis for breeding high quality long-grain *xian/indica* rice by genotype selection of *GL7/SLG7/GW7* through MAS in the future. There are no long-grain alleles, *qGL3*, *TGW6*, and *gs9*, in 12 high quality *xian/indica* rice varieties. It may be because of the low distribution frequency of these genes in rice germplasm. Using molecular marker technology for targeted selection will be beneficial to the use of these genes in breeding.

3 Materials and Methods

3.1 Experimental materials and DNA extraction

The rice materials used in this study were 12 high quality *xian/indica* rice varieties, including eight high quality conventional *xian/indica* varieties Ezhong 5, Yujing 91, Yuzhenxiang, Huangmaozhan, Yangxizao, Huarun 2, Jianzhen 2, Super Basmati, and four high quality *xian/indica* rice restorer lines Xiang 5, 9311, 15Q340, R835.

All rice materials were planted in the Nanhu Rice Experimental Base of Hubei Academy of Agricultural Sciences in 2018, and young leaves were taken for genomic DNA extraction.

3.2 Determination of related data of rice grain shape

The main panicle of selected plants were examined the grain shape after maturity stage. After the seeds were dried, 50 full grains randomly selected from each individual plant to measure the grain length, grain width and L/W ratio of each variety with a seed test instrument. The obtained data was statistically analyzed by Microsoft Excel 2010.

3.3 Gene detection of rice grain shape

Referring to previous reports, the primers were synthesized to amplify 10 genes (Table 2), including *GS3*, *LGY3/OsLG3b*, *qGL3*, *GL7*, *SLG7/GW7*, *TGW6* and *GS9*, which control grain length, and including *GS5*, *GW5* and *GW8*, which control grain width. The primers were all synthesized by Wuhan Quintara Biological Company.

The PCR reaction system was 15 μL, containing 1.0 μL template DNA (50ng/μL), 0.2 μL *Tag* enzyme (5 u/μL), 1.50 μL 10×Buffer (Mg^{2+}plus), 0.70 μL each primer (66 ng/μL), 0.8 μL dNTPs (2.5 mM/μL), and making up to 15.00 μL with ddH_2O. The PCR amplification procedure was as follows. Pre-denaturation was set at 95 ℃ for 5 min, and then there were 35 cycles of denaturation at 95℃ for 30 s, annealing for 30 s and extension at 72 ℃. Finally, amplified product were extended for 5 min at 72℃ and stored at 4℃.

Enzyme digestion system: 5 μL of PCR amplified product was taken into the PCR tube. 1μL of the corresponding reaction buffer and 0.3 μL of restriction enzyme were added, and then ddH_2O was added to make up to 10 μL. Finally, we put it in 37℃ water to bath for 3 h and it stopped reaction at 60℃.

Electrophoresis detection: PAGE gel or agarose gel electrophoresis is used to detect the target band.

Authors' contributions

ZL and LEJ designed and executed the experiments, completed data analysis and wrote the first draft of the manuscript. XHS, CZJ, YGC and LK participated in designing the experiments and analyzing the results. ZL and YAQ conceived the project and was responsible for the project. And they directed the experiments, data analysis, paper writing and revision; All authors read and approved the final manuscript.

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