

Quality Evaluation and Important Quality Genes Genotyping of Introduced Rice Germplasm Resources

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Abstract High quality is one of the most objectives in rice breeding. Introduction and evaluation of the quality of rice germplasm resources is of great significance for selecting high-quality rice parents and breeding high-quality rice varieties. In this study, 28 rice germplasm resources were introduced from Africa and Brazil, and their quality was evaluated in Wuhan, Hubei Province. The results showed that the processing and appearance quality of these germplasm resources were excellent, but the cooking and eating quality were poor. Finally, 13 varieties with high quality were screened out. The correlation analysis of different quality traits showed that the correlation among processing quality, appearance quality, cooking and eating quality was low. The genotypes of *GS3*, *Wx* and *Alk* were identified. It was found that both *GS3* and *Wx* contained two genotypes, and *Alk* contained three genotypes. The phenotypes showed significantly difference between different genotypes for both *Wx* and *ALK*. Our results will give valuable germplasm resources, gene resources and marker resources for breeding high quality rice varieties.

Keywords Germplasm; Quality; Genotype

Rice is the most important food crop in China. In recent years, with the improvement of people's living standard, people pay more and more attention to the quality of rice (Qiu et al., 2017a; 2017b; Qiu et al., 2020). Since the 13th five year plan, China has attached great importance to the improvement of rice quality (Xu et al., 2020). In 2018 and 2019, the national high-quality rice varieties eating quality evaluation was held for two consecutive years, and a number of high-quality rice varieties with good eating quality were identified. However, due to the fact that breeders have always taken improving yield as the primary breeding goal and neglected the improvement of rice quality, the quality of Chinese rice varieties, especially hybrid rice varieties, is poor, and the quality gap between Chinese and foreign high-quality rice became very large (Ou et al., 2016). Therefore, breeding high-quality rice varieties is of great significance to meet people's consumption demand (Zhang et al., 2010).

Rice quality includes processing quality, appearance quality, cooking and eating quality and nutritional quality. The processing quality includes brown rice rate, milled rice rate and head milled rice rate. The appearance quality mainly includes grain type and chalkiness. Cooking and eating quality mainly includes amylose content, gel consistency and gelatinization temperature. Nutritional quality mainly includes protein and fat content (Qiu et al., 2015). Head rice rate and chalkiness are the core indicators of rice quality, and amylose content is the limiting index of rice quality grading (Shen et al., 2019). At present, researchers have cloned several genes that affect rice quality, among them, *GS3* had the greatest effect on grain size (Fan et al., 2009), amylose content was mainly controlled by *Wx* gene (Wang et al., 1990), gelatinization temperature was mainly controlled by *ALK* gene (Gao et al., 2003). Each gene can be divided into several alleles. To clarify the phenotypic values of different alleles and their distribution in germplasm resources can help breeders select suitable parents and improve the efficiency of breeding high-quality rice.

Screening of high quality germplasm resources is the key to breeding high quality rice varieties. Therefore, it is of great significance to introduce, evaluate and screen high-quality rice germplasm resources (Chen et al., 2009). For example, high-quality rice variety "Meixiangzhan 2" contains the blood relationship of American high-quality rice variety "Lemon" (Zhou et al., 2005). Wuchang Rice Variety "Daohuaxiang No.2" and Japanese high quality rice variety "Yueguang" have part of the same blood relationship (<http://www.ricedata.cn/index.htm>). Research showed that in recent years, the genetic background of high-quality rice varieties in China has become more and more narrow, so it is urgent need to introduce new high-quality rice germplasm resources from abroad to expand the genetic background.

In this study, 28 rice germplasm resources were introduced from Africa and Brazil. The quality of these germplasm resources was investigated and identified in Wuhan, Hubei Province. The genotypes of important quality genes were identified by molecular markers, which provided parents and allele resources for high quality rice breeding.

1 Results and Analysis

1.1 Quality performance of 28 germplasm resources

The brown rice rate and milled rice rate of 28 germplasm resources had little difference among different introduced Germplasms (Table 1). The coefficient of variation was 3.38% and 4.07%, which was significantly lower than the head rice rate (10.47%). Among them, the head milled rice rate of 22 germplasm resources was more than 56% (national standard grade 1), which indicated that the processing quality of these germplasm resources was excellent. In the aspect of grain type, the grain length of 7 accessions from Africa was less than 9.0 mm, and the others were long grains. The grain width of 18 germplasm resources was less than 2.8 mm, and the others were narrow grains. Finally, except QG049 and QG046, the ratio of length to width of other germplasm resources was greater than 3.0, especially the aspect ratio of 5 germplasm resources from Brazil was more than 4.0. In addition, the chalkiness rate of 22 germplasm resources was lower than 20%, the chalkiness degree of 23 germplasm resources was lower than 5% (national standard grade 2), and the transparency of 19 germplasm resources was less than grade 3. The results of grain type, chalkiness and transparency showed that the appearance quality of these germplasm resources was excellent. Among the 28 germplasm resources, 15 germplasm resources showed poor performance with amylose content exceeding 14%~24% (high quality rice standard). The gel consistency of all germplasm resources was more than 60 mm, showing excellent performance. The alkali extinction value of 24 germplasm resources was lower than 5.0, indicating that most germplasm resources had low gelatinization temperature. The results showed that the cooking taste quality of 28 germplasm resources was poor. According to the national standard GB/T17891-2017, 13 germplasm resources are high-quality rice, of which 8 are grade I, 4 are grade II and 1 is grade III.

1.2 Correlation between different qualities

The correlation among different quality traits of 28 germplasm resources is shown in Table 2. The processing quality among three traits, only brown rice rate and milled rice rate had significant positive correlation. The correlation between 6 characters was not significant except for the correlation between grain length and grain width, transparency and other 5 appearance quality traits. There was significant positive or negative correlation among the other traits, which indicated that there was a strong correlation among different traits of appearance quality. There was no significant correlation between cooking and eating quality among the three traits. Results showed that the correlation between processing quality and cooking and eating quality in different traits was low, but the correlation between appearance quality was strong.

Table 1 quality performance of 28 rice germplasm

Variety	Brown rice rate (%)	Milled rice rate (%)	Head milled rice rate (%)	Grain length (mm)	Grain width (mm)	Length to width ratio	Percentage of grains with chalkiness (%)	Degree of endosperm with chalkiness (%)	Clarity	Amylose content (%)	Gel consistence (mm)	Alkali spreading value	Degree
QG017	79.87	67.76	51.50	9.25	2.53	3.66	9.35	1.81	2	20.78	114.70	5.80	2
QG018	78.08	66.53	56.03	9.31	2.58	3.61	3.15	0.86	1	19.38	130.70	5.20	1
QG020	79.86	68.80	61.02	8.75	2.39	3.63	5.87	1.05	2	27.30	125.70	2.00	Substandard
QG039	80.19	68.79	58.47	9.59	2.62	3.47	20.32	2.26	3	27.13	138.70	2.30	Substandard
QG040	79.14	69.27	57.70	9.47	2.59	3.66	7.76	1.48	2	20.06	95.30	2.20	1
QG041	77.77	68.31	46.55	9.17	2.91	3.16	22.36	5.71	2	27.62	65.00	5.80	Substandard
QG044	79.30	71.47	67.02	8.10	2.38	3.41	11.52	1.89	3	28.32	133.00	2.20	Substandard
QG046	78.44	67.51	57.03	7.81	2.94	2.68	81.96	21.05	2	28.18	92.50	2.20	Substandard
QG049	80.13	72.43	63.39	8.12	3.18	2.56	81.40	15.56	4	26.63	130.50	2.30	Substandard
QG052	78.41	66.09	58.28	9.14	2.85	3.22	13.96	5.43	3	17.39	122.00	4.30	3
QG053	77.82	67.10	59.22	9.54	2.70	3.55	30.00	4.12	2	17.20	124.70	4.50	2
QG061	77.69	66.11	61.08	9.19	2.75	3.34	6.06	0.50	3	13.18	114.00	2.70	Substandard
QG064	79.16	70.14	63.15	8.64	2.92	3.00	35.29	5.13	2	27.02	66.70	4.20	Substandard
QG068	81.25	69.20	56.92	9.29	2.82	3.30	7.69	3.58	3	23.78	81.00	2.30	2
Sahel 108	79.79	68.07	46.49	8.67	2.38	3.66	10.00	2.41	2	25.91	134.50	2.20	Substandard
Sahel 134	79.29	69.10	42.52	8.95	2.30	3.90	19.23	2.07	4	29.38	142.00	2.30	Substandard
ZY-1	81.22	67.98	62.53	9.71	2.19	4.46	5.43	1.70	2	23.20	68.00	2.00	1
ZY-2	79.66	67.61	63.54	9.14	2.32	3.97	5.46	1.17	2	21.53	88.50	2.50	1
ZY-3	79.72	66.90	48.42	10.56	2.47	4.30	5.35	1.26	2	26.68	81.30	6.00	Substandard
ZY-4	80.67	63.53	59.90	9.89	2.55	3.89	3.63	0.56	2	13.16	73.70	2.00	Substandard
ZY-5	79.75	66.03	59.83	9.70	3.10	3.14	18.47	3.20	3	16.88	102.30	4.50	2
ZY-7	84.91	68.11	62.32	9.15	2.23	4.13	7.30	1.67	2	22.90	109.30	2.00	1
ZY-8	77.28	62.99	59.24	9.41	2.99	3.16	6.09	0.94	2	15.91	81.30	4.30	1
ZY-9	78.56	67.22	57.31	9.20	2.29	4.05	5.02	1.17	2	22.72	71.50	2.00	1
ZY-16	78.03	64.82	60.13	9.47	3.02	3.13	6.25	0.84	2	15.49	64.30	4.50	1
ZY-17	79.16	67.50	63.06	9.52	2.33	4.11	7.38	1.33	2	26.00	108.50	2.00	Substandard
ZY-25	76.76	65.81	60.52	9.31	2.91	3.21	5.38	1.20	3	25.48	104.70	2.00	Substandard

Note: a: The classification of quality grade shall refer to the national standard GB/T17891-2017

Table 2 Correlation coefficients of different quality traits in the 28 rice germplasm

Index of quality	Brown rice rate (%)	Milled rice rate (%)	Head milled rice rate (%)	Grain length (mm)	Grain width (mm)	Length to width ratio	Percentage of grains with chalkiness (%)	Degree of endosperm with chalkiness (%)	Clarity	Amylose content	Gel consistence
Brown rice rate	0.68**	-	-	-	-	-	-	-	-	-	-
Head milled rice rate	0.28	0.21	-	-	-	-	-	-	-	-	-
Grain length	0.05	-0.40*	-0.15	-	-	-	-	-	-	-	-
Grain width	-0.11	0.01	0.14	-0.15	-	-	-	-	-	-	-
Length to width ratio	0.15	-0.17	-0.16	0.56**	-0.89**	-	-	-	-	-	-
Percentage of grains with chalkiness	0.03	0.37*	0.07	-0.63**	0.50**	-0.65**	-	-	-	-	-
Degree of endosperm with chalkiness	0.02	0.30	0.03	-0.63**	0.48**	-0.63**	0.96**	-	-	-	-
Clarity	0.10	0.35	0.02	-0.26	0.28	-0.35	0.33	0.24	-	-	-
Amylose content	-0.03	0.43*	-0.27	-0.44*	-0.26	0.03	0.33	0.32	0.20	--	-
Gel consistence	-0.08	0.17	-0.12	-0.33	-0.19	-0.05	0.12	0.04	0.39*	0.23	-
Alkali spreading value	-0.09	-0.11	-0.32	0.35	0.38*	-0.16	-0.05	-0.06	-0.29	-0.25	-0.20

Note: * and ** represent $p < 0.05$ and 0.01 respectively

The correlation among different types of quality traits showed that milled rice rate, chalky grain rate had significant positive correlation with amylose content, and had significant negative correlation with grain length. There was no significant correlation between other processing quality traits and appearance quality, cooking and eating quality traits. There was a significant negative correlation between grain length and amylose content, a significant positive correlation between grain width and alkali extinction value, and a significant positive correlation between transparency and gel consistency. There was no significant correlation between other appearance quality and cooking and eating quality traits. The results showed that the correlation between processing quality, appearance quality and cooking and eating quality was low.

1.3 Genotypes of important genes in 28 germplasm resources

GS3、*Wx* and *ALK* are the most important quality genes that have been cloned. We identified the genotypes of these three genes in 28 germplasm resources by molecular markers or sequencing (Table 3). According to the C/A mutation in exon 2, *GS3* was divided into two genotypes: long grain and short grain, except QG041、QG044、QG053 and QG053 were short grain genotypes, others were long grain genotypes. However, among the four short grain varieties, the grain length of three varieties was more than 9.0 mm, 6 varieties had grain length of 9.0 mm in long grain genotypes. *Wx* was divided into two genotypes *Wx^a* and *Wx^b*, which contained 17 and 11 accessions respectively. The amylose content of *Wx^a* germplasm was 22.72% ~ 29.28%, which was significantly higher than that of *Wx^b* germplasm (13.16% ~ 21.53%). We sequenced *ALK* gene and compared it with Nipponbare as reference sequence, we found an A/G mutation at 3797, which resulted in the mutation of serine to glycine. Five African germplasm resources were mutated there. There was a T / G mutation at 3 901, but this mutation did not change the amino acid coding, and the mutation occurred in the same germplasm resources as 3797. A/G mutation was found at 4196, which resulted in the mutation of methionine to valine. All germplasm resources had mutation at this point. There were two GC/TT mutations at 4327 and 4328, which resulted in the mutation of glycine and leucine into glycine and phenylalanine, and 10 germplasms were mutated. Finally, we used mutations at 3797, 3901, 4327 and 4328 to classify *ALK* into three genotypes. There were 13 and 5 genotypes of ATGC and GCGC, respectively, with alkali extinction values of 2.48 and 2.18, which were significantly lower than those of ATTT (alkali consumption value was 4.53).

2 Discussion

With the development of China's economy and the improvement of people's living standard, rice quality has been paid more and more attention. However, for a long time, China's rice breeding has always taken high yield as the primary breeding goal, resulting in the current shortage of high-quality rice varieties (Ou et al., 2016). Although some high-quality rice varieties have been popularized on a large scale, it is still urgent for breeders to breed more high-quality rice varieties to meet different production needs. The breeding of high-quality rice depends on the exploration and utilization of high-quality germplasm resources, so the introduction and identification of high-quality germplasm resources is very important for the breeding of high-quality rice. Guangdong Academy of Agricultural Sciences (Zhou et al., 2005) started breeding high-quality hybrid rice earlier in China. Since the 1970s, the Institute has introduced a variety of high-quality germplasm resources as parents. After decades of efforts, a large number of high-quality rice varieties have been bred. However, at present, the genetic background of high-quality rice germplasm resources in China is very narrow, and the breeding of high-quality rice varieties is very close, so it is difficult to further improve the yield. Therefore, it is urgent to introduce new high-quality germplasm resources from abroad, broaden the genetic background of high-quality rice, and make the breeding of high-quality rice to a higher level.

In this study, 28 rice germplasm resources were introduced from Africa and Brazil, and their quality was evaluated in Wuhan, Hubei Province. The results showed that most of these germplasm resources had excellent processing quality and appearance quality, but poor cooking and eating quality. In the end, 13 germplasm resources reached the standard of high-quality rice, among which there were 8 first-class, 4 second-class and 1 third-class germplasm resources. These high-quality germplasm resources can be used as important backbone parents for

breeding high-quality rice. Because the quality of rice is greatly affected by the environment, repeated identification is needed in different years and under different ecological conditions. In addition, other agronomic traits of these high-quality germplasm resources need to be investigated to provide the basis for future high-quality rice breeding.

Table 3 Genotypes of three important quality genes in the 28 rice germplasm

Variety	Gene		
	<i>GS3</i>	<i>Wx</i>	<i>ALK</i>
QG006	L	<i>Wx^a</i>	ATGC
QG017	L	<i>Wx^b</i>	ATTT
QG018	L	<i>Wx^b</i>	ATTT
QG020	L	<i>Wx^a</i>	GGGC
QG039	L	<i>Wx^a</i>	ATGC
QG040	L	<i>Wx^b</i>	ATTT
QG041	S	<i>Wx^a</i>	ATTT
QG044	S	<i>Wx^a</i>	GGGC
QG046	L	<i>Wx^a</i>	GGGC
QG049	L	<i>Wx^a</i>	ATGC
QG052	S	<i>Wx^b</i>	ATTT
QG053	S	<i>Wx^b</i>	ATTT
QG061	L	<i>Wx^b</i>	ATGC
QG064	L	<i>Wx^a</i>	ATGC
QG068	L	<i>Wx^a</i>	ATGC
Sahel 108	L	<i>Wx^a</i>	GGGC
Sahel 134	L	<i>Wx^a</i>	GGGC
ZY-1	L	<i>Wx^a</i>	ATGC
ZY-2	L	<i>Wx^b</i>	ATTT
ZY-3	L	<i>Wx^a</i>	ATTT
ZY-4	L	<i>Wx^b</i>	ATGC
ZY-5	L	<i>Wx^b</i>	ATTT
ZY-7	L	<i>Wx^a</i>	ATGC
ZY-8	L	<i>Wx^b</i>	ATGC
ZY-9	L	<i>Wx^a</i>	ATGC
ZY-16	L	<i>Wx^b</i>	ATTT
ZY-17	L	<i>Wx^a</i>	ATGC
ZY-25	L	<i>Wx^a</i>	ATGC

In addition, 15 of the 28 germplasm resources did not meet the quality standard. It was found that the amylose content of these germplasm resources did not meet the standard, which caused them to be out of grade. Among these germplasm resources, the head rice rate of 10 germplasm resources exceeded the national standard 1 (more than 56%), and the chalkiness degree of 8 germplasm resources reached the national standard level 1 (less than 2%), which indicated that these germplasm resources had important utilization value in some aspects. In the process of breeding high-quality rice with these germplasm resources, special attention should be paid to the fact that single quality can not be improved and other quality will be decreased. In addition, the correlation analysis of quality traits showed that the correlation between processing quality, appearance quality and cooking and eating quality was low. It shows that we can only improve one quality or one type of quality without causing the decline of other qualities.

We used molecular markers to identify the genotypes of *GS3*, *Wx* and *ALK*, three important quality genes in germplasm resources. *GS3* and *Wx* were divided into two genotypes, while *ALK* was divided into three genotypes. *GS3* encodes the β subunit of G signal protein (Mao et al., 2010), and a C/A mutation in exon 2 leads to the premature termination of the coding protein, resulting in the formation of a long grain phenotype (Fan et al., 2009). The marker was used to identify the mutation, but most of the germplasm resources with short grain genotype showed long grain, while 6 varieties with long grain genotype showed short grain. These results indicated that these materials also contain other genes that affect the grain size. In the follow-up study, genetic populations can be constructed to mine new grain type genes. *Wx* encodes granular starch synthase (Wang et al., 1990) and is the most important gene controlling amylose content in rice. *Wx* is mainly divided into two genotypes, *Wx^a* is the wild type, *Wx^b* is evolved from *Wx^a*, and the amylose content of *Wx^b* is significantly lower than that of *Wx^a* (Sano et al. 1986), which is consistent with the results of this study. *ALK* is the only gene that controls gelatinization temperature and encodes soluble starch synthase II (Gao et al., 2003). In this study, three genotypes of the gene were found in 28 germplasm resources. The alkali extinction value of ATGC and GCGC was significantly lower than that of ATTT. In the future, PCR based molecular markers can be developed to identify the two genotypes. In addition, it is difficult to identify the quality phenotype of rice, especially the low generation individual plant. Therefore, improving rice quality by molecular breeding is the best way to breed high quality rice. The genotype resources of the above three genes are important gene resources for improving rice quality by molecular breeding. Using these markers (or developing new molecular markers based on sequencing results) can provide important marker resources for molecular breeding for high quality rice breeding, which can greatly accelerate the breeding rate of high-quality rice and improve the efficiency of breeding.

3 Materials and Methods

3.1 Test materials

The rice germplasm resources used in this study came from Africa and Brazil. Among them, 17 germplasm resources with "QG" number and "Sahel" number are from Africa, and 11 germplasm resources with "ZY" number are from Brazil.

3.2 Material planting and quality inspection

All germplasm resources were planted in the base of Institute of food crops, Hubei Academy of Agricultural Sciences in summer of 2014. All the materials were sown on May 15. The randomized block design was adopted. Each variety was planted in 3 rows with 10 plants in each row. The spacing between plants and rows was 20 cm \times 25 cm, which was repeated twice. Field management is the same as conventional field management. The heading date of each variety was recorded, and the mixture was harvested 30 days after heading. After harvesting and threshing, they were spreaded out and dried in the shade of the Grain Crops Research Institute of Hubei Academy of Agricultural Sciences, and stored at room temperature for 3 months.

The appearance quality of various rice varieties, including grain length, grain width, length width ratio, chalky grain rate, chalkiness degree and transparency, was detected by Hangzhou Wanshen SC-E rice appearance quality analyzer. According to the national standards GB/T 5495-2008 and GB/T 21719-2008, the processing quality of rice varieties was determined, including brown rice rate, milled rice rate and head rice rate. Amylose content, gel consistency and alkali consumption value were determined according to the Ministry of agriculture industry standard NY147-88.

3.3 Genotyping of important quality genes

DNA extraction reference to the method of Murry and Thomson (1980). Three markers for three important quality genes were developed by Primer5.0 software (Table 4). Two of them were CAPS markers, which were digested with PST I and one was sequenced marker. The PCR reaction system was based on the system of Fan et al. (2009) and Lv (2018), and PCR products were identified by 1% agarose gel. The *ALK* gene was sequenced by Nanjing Kingsley Company, and the sequence variation of *ALK* gene of different varieties was compared with using Nipponbare as reference sequence.

Table 4 Information of markers for genotyping target genes (Sequences were provided by Liu Qiaoquan Lab in Yangzhou University)

Gene	Marker name	Primer sequence	Marker type
GS3	GS3PST-F	TATTTATTGGCTTGATTTCTGTG	CAPs
	GS3PST-R	GCTGGTTTTTACTTTCATTGCC	
Wx	pAg	GCTTCACTTCTCTGCTTGTG	CAPs
	pBg	ATGATTTAACGAGAGTTGAA	
ALK	ALK-F	CAGGGACACGATAAACTCTTCTT	Sequencing
	ALK-R	GCATCAATGGACATAACAAACAC	

Authors' contributions

Xu Huashan and He Wei are the experimental design and executor of this study. Xu Huashan, He Wei and Zhou Lei complete the data analysis and the writing of the first draft of the paper. Liu Kai and Yang Xiaolin participate in the experimental design and analysis of the experimental results. You Aiqing is the designer and person in charge of the project, guiding the experimental design, data analysis, assisting and revising the paper. All authors read and approved the final manuscript.

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