Grain Quality Characteristics and RVA Profile Analysis of Yuenongsimiao, a High Yield and Disease-resistant Rice Variety

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Abstract

Background: Rice quality is one of the main targets for rice breeding, and is a complex trait involving grain appearance, milling, cooking, eating and nutritional quality. For a long time, rice breeding is facing the imbalance of yield, quality, disease resistance and lodging.

Result: Here, the grain quality characters (milling and appearance quality, cooking quality, starch RVA profile, and nutritional quality) of Yuenongsimiao (YNSM), a new *indica* rice variety with high yield, high quality and disease resistance, were performed. It showed that YNSM has excellent appearance and quality with low amylose content, long gel consistency, and had significant correlation with the HPU, CPV, SB, and CS. Moreover, 5 genes related to grain size (*GS3*, *GS9*, *GW5*, *GW7*, and *GW8*) and *Wx* gene were selected to detect the main quality genotype of YNSM. The results showed YNSM was a middle-long grains with relative high brown rice rate, white rice rate and whole white rice rate, and lower chalkiness. The results indicated the grain size and food quality of YNSM might related to *gs3*, *gw7* and Wx^b. This research also reported the quality characters of hybrid rice using YNSM as a restorer lines.

Conclusions: The quality characters and genotype of grain quality gene analysis in YNSM may facilitate the breeding of new rice varieties achieving a balance between grain yield, resistance and quality.

Background

Rice (*Oryza sativa* L.) grain quality is one of the main targets for rice breeding. It involves several components such as grain appearance, milling quality, cooking, eating and nutritional quality (Birla et al. 2017, Butardo et al. 2019). In particular, cooking and eating quality traits, such as amylose content (AC), gelatinization temperature (GT), gel consistency (GC) and pasting viscosity, and aroma are important factors in determining the quality of cooked rice (Hori et al. 2016).

Starch is the main component of rice grains accounting for about 80% of grain chemical components, including amylose and amylopectin. Starch characteristics directly affect the cooking and eating quality of rice (ref). AC has always been considered as the most important factor to determine the quality of rice (Fasahat et al. 2014). *Waxy*, the main gene that controls amylose synthesis, encodes granule-bound starch synthase (GBSS)and affects the AC in rice endosperm and pollen directly. In non-glutinous rice varieties, *Wx* gene differentiated into Wx^a and Wx^b alleles, among which wild rice and most *indica* rice were Wx^a with high amylose content (Zhou et al. 2016). The vast majority of *Japonic*a rice is Wx^b with relative low amylose content. Additionally, *Wx* can also control the gel consistency (Su et al. 2011).

Grain size is an important factor affecting rice yield and is also important for rice appearance quality. *GS3*, a major quantitative trait locus (QTL) for grain size, functions as a negative regulator of grain size and organ size (Fan et al. 2009, Mao et al. 2010). *GW7/GL7* is a main QTL for grain length and width. Over-expression of *GW7* can increase the longitudinal cell division of grain and reduce the transverse cell division, resulting in elongated grains (Wang et al. 2012, Wang et al. 2015). Additionally, *GW7* expression is directly regulated by OsSPL16, a transcription factor encoded by the grain-width locus *GW8* (Wang et al. 2015). *GS9* negatively regulates glumes division by GS9-OFP8/14-GSK2 pathway (Li et al. 2018).

Starch viscosity profile, named as Rapid viscosity-analyser profile (RVA profile), refers to the curve of starch viscosity varying with temperature during heating, high temperature and cooling, including peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), peak time (PeT), paste temperature (PaT), breakdown viscosity (BDV), consistency (CS, and setback viscosity (SBV) (Tong et al. 2014, Nakamura et al. 2016). It has shown that the RVA profile characteristic values of rice starch are closely related to rice cooking quality and food quality, especially the BDV, CS and SBV can reflect rice cooking and food quality(Yan et al. 2011). The higher the amylose content was, the smaller the BDV was, and the greater SBV and CS were. The characteristic values of RVA profile of rice starch could distinguish the difference of cooking and eating quality of rice varieties with similar apparent amylose content.

Rice breeding in China is faced with the imbalance of high yield quality, disease resistance and lodging. Yuenongsimiao (YNSM) is a new rice variety with high yield, high quality and disease resistance bred in Rice Research Institute of Guangdong Academy of Agricultral Sciences (GDAAS). In order to further reveal its quality characteristics, the milling quality, appearance quality, cooking and eating quality, nutritional quality, as well as the RVA profile of rice starch was performed. These results will provide a theoretical basis for the breeding of new rice varieties with high quality eating taste.

Results

The breeding process of YNSM

YNSM was bred using Yuetai13, a high-yield and disease-resistant variety, as male parent, and good quality variety Huanghuazhan as female parent. The F_1 seeds were planted and harvest for F_2 seeds. Pedigree selection was performed to select the target line, and quality and resistance identification were carried out throughout the whole breeding process. Natural identification in the blast nurseries were used for blast resistance, artificial inoculation method was adopted for bacterial blight resistance, and appearance identification and cooking and tasting screening were used for rice quality. Through 6 generations pedigree selection, comparative tests were carried out and regional tests and production tests were conducted. So far, YNSM has been certificated in Guangdong province (2011), Hainan province (2013), Jiangxi province (2017) and Hubei province (2017), China.

The milling and appearance quality of YNSM

The grain size of YNSM is between YXZ and XYXZ, the grain length is 9.28 mm, grain width is 2.3 mm, and the length/width is 3.2-3.4, which is different from the small size variety YXZ and the thin-long grain variety XYXZ (Fig. 1A-D). The grain size is mainly controlled by genetic factors rather than environmental factors. In order to reveal the regulatory factors of YNSM grain size, we used the known molecular markers to detect. The results showed that all of YNSM, YXZ and XYXZ were *GS9, GW5,* and *GW8*. For the *GS3* gene, long grain variety YNSM and XYXZ is *gs3*, small variety YXZ is *GS3*. Moreover, compared to YNSM and YXZ, XYXZ is controlled by *GW7* (Fig. 1E).

For the milling quality was different in different seasons. In the early season, brown rice rate, white rice rate and whole white rice rate of YNSM was similar to YXZ and XYXZ, about 70%, 60% and 55%, respectively. However, in the late season, all of the milling quality of YNSM was much better than YXZ and XYXZ (Fig. 2A, B).

Generally, the white grain rate and chalkiness rate were greatly affected by seasons, and the rice quality is better in the late season than in the early season. The transparency level of YNSM was 3, which was better than YXZ and XYXZ. The chalkiness rate and chalkiness degree in the early season was 11.84% and 1.61%, respectively. While in the late season, the chalkiness rate and chalkiness degree was 6.71% and 0.84%, which were significantly lower than YXZ, but equal or better than XYXZ (Fig. 2C).

Cooking quality characters of YNSM

The AC of YNSM in the early and late season was 17.05% and 17.24%, respectively, which were lower than in YXZ (25.05% and 26.47%), and similar to XYXZ (15.9% and 18.2%). The gel consistency (GC) of YNSM was 72.0 mm and 75.0 mm, which was in accordance with XYXZ, but significantly higher than YXZ (44 mm and 46 mm). These results showed that YNSM has low AC and long GC as the high quality varieties. The eating score of YNSM (85.6-87.1/100) was similar to XYXZ (85.7-88.1/100), significantly higher than XYZ(57.0-63.8/100).

For *Wx* is the main gene that controls amylose synthesis, the genotype of *Wx* was performed in the YNSM (Zhou, Wang et al. 2016). The results showed that the YNSM and XYXZ is Wx^b, while YXZ is Wx^a, which is conformed to the AC (Fig. 3A).

The RVA profile of YNSM

Both in early and late seasons, the starch paste of the detected rice varieties displayed a process “up-down-up”. In the early season, the PV of YNSM was 248.92 RVU, which was half of YXZ and similar to XYXZ (Table 1). The HV was 88.02 RVU, significantly less than YXZ (312.09 RVU), but slightly higher than XYXZ (52.38 RVU). The CPV of YNSM was 163.88 RVU, which was substantially lower than YXZ (446.25 RVU) and more than XYXZ (99.21 RVU). In the early season, the BD, CS and SB of YNSM was 160.84 RVU, -85.04 RVU and 75.8 RVU, respectively. These values were similar to XYXZ, but the BD was higher than YXZ, the BD and CS was lower than YXZ. In the late stage, HPV, CPV and SB of YNSM was similar to XYXZ, but the PV, HPV and PeT with no significant difference, which revealed CPV, SB and CS is significantly correlated to the taste quality.

Protein and fatty acid content of YNSM

Rice quality includes the nutritional quality such as storage protein, fatty acid, anthocyanin and mineral (Dyer and Mullen 2008, Wang et al. 2015). Rice grain contains a certain proportion of protein and fat, which are related to nutrition, luster and palatability. In the early season, YNSM had the highest content, nonetheless had no significant difference in the late season (Fig. 4). The protein contents of YNSM is 7.68% in early season and 7.52% in late season, which is similar to XYZ and higher than XYXZ. Interestingly, the content of fatty acids reduced greatly in the late season (Fig. 4).

Quality-yield breeding applications of YNSM

YNSM is not only excellent in rice quality, but also outstanding in comprehensive agronomic traits. It is highly resistant to blast and bacterial blight. Meanwhile, YNSM is also an excellent restorer line (R1212) of two-line and three-line hybrid rice. At present, more than 20 hybrid rice combinations have been prepared with YNSM as restorer line, and most of the rice quality is above grade 3, which has passed the national examination (Table 2, http://www.ricedata.cn/variety/). Among them, the whole polished rice rate of Guangtaiyou-YNSM, Jitianyou-YNSM, Huiliangyou-YNSM, Cliangyou-YNSM, Xinrongyou-YNSM, Zaoyou-YNSM was above 65%, the glue consistency was 61-80 mm, the AC was 14.6-19.2%, and the rice quality is up to grade 2. These results indicated that YNSM can availably improved the grain quality of hybrid rice.

Discussion

The influence of grain size on rice quality

Grain size is one of the most important factors of rice appearance quality and rice yield, and several genes have been cloned (Fan, Yu et al. 2009, Mao, Sun et al. 2010, Wang, Wu et al. 2012, Wang, Li et al. 2015, Wang, Xiong et al. 2015). As is known to all, slender rice like XYXZ is easy to break during milling, and the whole rice rate is very low, while large rice grains always bring chalkness. In this research, our research revealed the basis of high yield and appearance quality of YNSM. The main grain size controlling genes of YNSM were *gs3* and *GW7*, which result a middle-long grains with high whole white rice rate rather than YXZ and XYXZ. Therefore, rice yield and the whole white rice rate should be considered in breeding.

Effect of starch content on taste

Amylose is the main content of grain starch. Usually, rice with more AC is not good for eating (Tong, Chen et al. 2014). It has been clearly that *Wx* is the main gene that controls amylose synthesis (Zhou, Wang et al. 2016). For the most varieties of good taste, genotype of *Wx* was Wx^bcausing low AC. In this research, we revealed the low AC controlled by Wx^b in YNSM genome, which will helpful for molecular breeding of high quality rice (Fig. 3).

Effects of RVA profile on taste

The characteristics of rice starch RVA profile are an important factor affecting rice cooking and eating quality. The RVA profile can not only reflect the taste difference caused by the difference in amylose content among different varieties, but also reflect the different eating taste and palatability caused by the similar content of amylose (Tong, Chen et al. 2014). Generally, rice varieties with good taste had higher BD, lower SB and CS. RVA profile also related to gel consistency. Low amylose content variety usually bring long gel consistency, with higher BD, lower SB and CS (Nakamura, Katsura et al. 2016). In this research, YNSM and XYXZ with similar quality, the RVA profile is markedly different from high AC rice varieties YXZ. For RVA profile requires less sample and have good repeatability, should be effectively applied in early generation selection. Although YNSM and XYXZ are both with low starch content, there are still distinct differences in their taste quality, such as nutrient quality content, RVA profile etc. Therefore, YNSM/XYXZ recombinant inbred lines (RILs) have been constructed for further revealing the genetic network of food quality.

Improvement of rice varieties with high quality food and taste

The coordination of yield resistance and quality is an important goal in breeding. At present, with the huge genome-wide data, the continuous advancement of molecular marker-assisted selection and molecular design breeding, as well as the CRISPR/cas9 technology, it is possible to improve the yield, quality and resistance of rice in a precise and efficient manner. YNSM, which is not only good at quality but also good at yield, disease resistance and lodging, should be widely used to carry out genome-wide selective breeding for grain type, resistance and taste quality.

Conclusion

In this study, it revealed YNSM was a middle-long grains with relative high brown rice rate, white rice rate and whole white rice rate, and lower chalkiness. Low Amylose content, long gel consistency had significant correlation with the HPU, CPV, SB, and CS, resulting a good cooking quality of YNSM. Furthermore, 5 genes related to grain size (*GS3*, *GS9*, *GW5*, *GW7*, and *GW8*) and *Wx* gene were selected to detect the main quality genotype of YNSM, indicating the grain size and the cooking quality of YNSM might directly or indirectly regulated by *gs3* and *gw7*, and Wx^b, respectively. Additionally, YNSM as a restorer lines can improved the quality of hybrid rice facilitating the breeding of new rice varieties achieving a balance between grain yield, resistance and quality.

Methods

Materials

Rice varieties (*indica*), YNSM, high ACC Yuexiangzhan (YXZ), and low ACC with good taste variety Xiangyaxiangzhan (XYXZ) were used as control in this research. All varieties were planted in the experimental base of Rice Research Institute of GDAAS in Guangzhou city, Guangdong Province in the early and late season of 2019.

Rice quality determination

Seeds were harvested after maturity, and 100 g of air-dried rice, which was intact and free from pests and diseases, was stored in a ventilated and dry indoor environment for 3 months until the physicochemical properties of the seeds were stabilized. Before the determination, the samples to be tested were placed in a dry and ventilated place or an air-conditioned room for about 7 days to keep the moisture content of the samples below 14%.

Processing quality detection

Take out 30 g of rice grain. Using rice huller (JLG-III) sample of the processed into brown rice, then put in the brown rice polishing machine (JNM-III) processed into white rice, and then polished white rice into a diameter of 1.5 mm round hole in the screen to get white rice. Finally, sieved and isolated to get the whole white rice. Brown rice rate = brown rice weight (g)/30 (g)×100%, white rice rate = white rice weight (g)/30 (g)×100%, the whole white rice rate = the whole white rice weight (g)/30 (g)×100%.

Appearance quality detection

The SC-E rice appearance quality detector (Wanshen Testing Technology Co. LTD, Hangzhou, China) was used to measure the length/width ratio of whole brown rice and chalky grain rate, chalkiness degree and transparency of white rice. Finally, all the refined rice was ground with a miniature universal pulverizer and screened with a 100-mesh sieve. The rice flour was stored at 4°C for the physical and chemical properties analysis.

Evaluation of rice cooking and eating quality

The AC and gel consistency (GC) were measured using standard procedures as described previously. AC was determined using a colorimetric method with $KI\text{-}I_{2,}$ and measured by Continuous Flowing Analyzer (Futura-II) with the protocol, and GC was measured by gel length with longer gels considered softer than shorter gels (Yang et al. 2020). The paste viscosity of rice starch was measured using a Rapid Visco Analyzer (RVA) according to RVA-TecMaster (Perten, Sweden). Briefly, 3 g of rice flour mixed with 25 mL of water in the RVA sample can. An RVA-Super 3 Viscometer instrument operated using Thermocline Windows control and analysis software version 1.2 were used (Newport Scientific, Sydney, Australia). RVA profile is generally composed by five primary and two secondary parameters of the pasting curve: peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), peak time (PeT), paste temperature (PaT), breakdown viscosity (BDV, PKV minus HPV), consistency (CS, CPV minus HPV）, and setback viscosity (SBV, PV minus PKV).

The grain protein content in rice flour was determined by the Kjeldahl method using a Kjeltec 2300 Autoanalyser (Foss AB, Sweden). A nitrogen conversion factor of 6.25 was used to calculate the grain protein content (Yang et al. 2015).

The taste value was measured by SATAKE rice taste meter. Wash the rice sample with water for 3 times in the stewed cup and filter water, then add water to the stewed cup in a ratio of 1:1.4, after the rice is cooked and cooled, weigh 7±1 g of white rice with a molding tool and press it into a micro-cake shape, then put it into the rice taste tester to determine the rice taste value.

The protein content of the brown rice was determined according to NY/T3-1982, and the fatty acid content of the brown rice was determined according to GB/T5510-2017. The fatty acid content was expressed as the potassium hydroxide mass (mg) needed to neutralize the free fatty acids in the 100 g sample.

PCR-based genotyping

Plant genomic DNA was extracted from fresh leaves of the heading-stage plants using a previously described CTAB protocol. The molecular marker for genotyping *Wx*, *GS3*, *GS9*, *GW5*, *GW7* and *GW8* were listed in Table 3. PCR amplification was performed on a Bio-Rad C1000 Touch Thermal Cycler (USA) and the protocol for PCR amplification with the appropriate parameters was described previously. The PCR product were resolved on a 3.0% agarose gel in 1× TBE buffer.

Statistical analysis

Statistical analysis was performed with independent samples using the Student's *t*-test to test differences, and all data were represented as means ± standard deviation (means ± SD).

Declarations

Authors' Contributions

ZL wrote the paper; XH and ZL designed the research. ZL and ZF performed most of the experiments and analyzed the data; WL, DL, XW, SW and JX participated in the data preparation and analysis. XH oversaw and improved the manuscript.. The author(s) read and approved the final manuscript.

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Availability of Data and Materials

The datasets supporting the conclusions of this article are provided in the article.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing financial interests.

References

1. Birla, D. S., et al. (2017) Progress and challenges in improving the nutritional quality of rice (*Oryza sativa* L.). Crit Rev Food Sci Nutr 57(11): 2455-2481.
2. Butardo, V. M., Jr., et al. (2019) Improving Rice Grain Quality: State-of-the-Art and Future Prospects. Methods Mol Biol 1892: 19-55.
3. Dyer, J. M. and R. T. Mullen (2008) Engineering plant oils as high-value industrial feedstocks for biorefining: the need for underpinning cell biology research. Physiol Plant 132(1): 11-22.
4. Fan, C., et al. (2009) A causal C-A mutation in the second exon of *GS3* highly associated with rice grain length and validated as a functional marker. Theor Appl Genet 118(3): 465-472.
5. Fasahat, P., et al. (2014) Genetic controls on starch amylose content in wheat and rice grains. J Genet 93(1): 279-292.
6. Hori, K., et al. (2016) Variation in cooking and eating quality traits in Japanese rice germplasm accessions. Breed Sci 66(2): 309-318.
7. Li, N., et al. (2018) Control of grain size in rice. Plant Reprod 31(3): 237-251.
8. Li, Z., et al. (2009) Development and Utilization of SSR Primer in *Wx* Gene of Rice (*Oryza sativa* L.). Southwest China Journal of Agricultural Sciences 22(2): 231-235.
9. Liu, L., et al. (2019) Development of Functional Markers and Multiple PCR Detection of RiceGrain Width Genes *GW5* and *GW8*. Molecular Plant Breeding 17(13): 4280-4288.
10. Mao, H., et al. (2010) Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc Natl Acad Sci U S A 107(45): 19579-19584.
11. Nakamura, S., et al. (2016) Development of formulae for estimating amylose content and resistant starch content based on the pasting properties measured by RVA of Japonica polished rice and starch. Biosci Biotechnol Biochem 80(2): 329-340.
12. Su, Y., et al. (2011) Map-based cloning proves *qGC-6,* a major QTL for gel consistency of japonica/indica cross, responds by Waxy in rice (Oryza sativa L.). Theor Appl Genet 123(5): 859-867.
13. Tong, C., et al. (2014) Genetic diversity of amylose content and RVA pasting parameters in 20 rice accessions grown in Hainan, China. Food Chem 161: 239-245.
14. Wang, S., et al. (2015) The OsSPL16-GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat Genet 47(8): 949-954.
15. Wang, S., et al. (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet 44(8): 950-954.
16. Wang, X., et al. (2015) A lipid transfer protein, OsLTPL36, is essential for seed development and seed quality in rice. Plant Sci 239: 200-208.
17. Wang, Y., et al. (2015) Copy number variation at the *GL7* locus contributes to grain size diversity in rice. Nat Genet 47(8): 944-948.
18. Yan, C. J., et al. (2011) Genetic analysis of starch paste viscosity parameters in glutinous rice (*Oryza sativa* L.). Theor Appl Genet 122(1): 63-76.
19. Yang, Y., et al. (2015) Identification of quantitative trait loci responsible for rice grain protein content using chromosome segment substitution lines and fine mapping of qPC-1 in rice (Oryza sativa L.). Molecular Breeding 35(6): 130.

20. Yang, Y., et al. (2020) Genetic improvement of panicle-erectness japonica rice toward both yield and eating and cooking quality. Molecular Breeding 40(5): 51.
21. Zhang, C. (2018) Study on genetic interaction and breeding application of rice *GS9* gene with other grain shpe genes.
22. Zhou, H., et al. (2016) Critical roles of soluble starch synthase SSIIIa and granule-bound starch synthase Waxy in synthesizing resistant starch in rice Proc Natl Acad Sci U S A 113(45): 12844-12849.

Tables

Table 1 Starch physicochemical properties of this research.

Samples	PV(RVU)	HPU(RVU)	CPV(RVU)	BD(RVU)	SB(RVU)	CS(RVU)	PeT(min)	PaT(°C)
YXZ(ES)	426.92	312.09	446.25	114.84	134.17	19.33	5.97	73.98
XYXZ(ES)	205.29	52.38	99.21	152.92	46.83	-106.09	4.44	74.75
YNSM(ES)	248.92	88.08	163.88	160.84	75.80	-85.04	4.90	74.00
YXZ(LS)	402.04	260.04	440.25	142.00	180.21	38.21	5.90	69.88
XYXZ(LS)	431.13	163.21	275.17	267.92	111.97	-155.96	5.44	69.90
YNSM(LS)	410.54	185.00	304.13	225.55	119.13	-106.42	5.67	69.05

Note: ES, early season; LS, late season.

Table 2 Quality characters of hybrid rice with YNSM as the restorer.

No.	hybrid rice	CMS/PTGMS lines	quality level	whole white rice(%)	chalkiness rate(%)	chalkiness degree(%)	gel consistency (mm)	AC(%)	grain size (length/width)
1	Guang8you-YNSM	Guang8A	3	64.3	14	3.8	71	14.3	3.7
2	Hengfengyou-YNSM	HengfengA	3	63.6	9	2.4	65	4.8	3.5
3	Guangtaiyou-YNSM	GuangtaiA	2	65.4	7	1.6	61	15.2	3.3
4	Jitianyou-YNSM	JitianA	2	69.6	6	1.9	74	14.6	3.3
5	Changshengyou-YNSM	Changsheng843A	3	60.5	8	1.9	65	14.8	4
6	Taoyou-YNSM	Taonong1A	2	64.3	6	1.2	69	14.4	3.5
7	Huiliangyou-YNSM	1892S	2	67.9	10	2.8	80	14.6	3.4
8	Cliangyou-YNSM	C518S	2	68.4	8	2.7	72	15.2	3.2
9	Rongyou-YNSM	RongfengA	3	66.1	14	4.1	53	21.2	3.3
10	Xinrongyou-YNSM	XinrongA	2	70.1	9	2.3	75	14.4	2.8
11	Quanyou-YNSM	Quan9311A	3	65.2	18	4.7	79	15.2	3.1
12	Zaoyou-YNSM	ZaofengA	2	67.5	8	1.1	62	19.2	3.2
13	Long8you1212	Long8A	3	64.4	17	4.2	67	16.6	3.3
14	Jiuliangyou1212	33S	3	68.4	14	4.4	50	19.6	3.1
15	Longyou1212	Longxiang634A	3	65.2	20	4.9	72	14.3	3.5
16	Jingliangyou1212	Jin4128S	3	67.4	19	4.9	50	21.7	3.3
17	Longjingyou1212	Longjing4302A	3	61.1	16	3.1	52	15.7	3.4
18	Jingliangyou1212	Jing4155S	3	66.7	9.7	1.8	74	15.1	3.2
19	Longliangyou1212	Longke638S	2	60.1	4	0.4	62	15.3	3.2
20	Feiliangyou212	Longfei656S	3	57.6	9	2.2	51	17.7	3.3

Table 3 Primers used in this work

Gene name	Primer name	Forword	Reverse	Fregment size(bp)	Tm(°C)	References
Wx	RA19	TACAAATAGCCACCCACACC	TTGCAGATGTTCTTCCTGATG	157	56	(Li et al. 2009)
GS3	Chr301	TATTTATTGGCTTGATTTCCTGTG	GCTGGTTTTTTACTTTCATTTGCC	511	56	(Zhang 2018)
GS9	In0919	CGTTTAGGCTGGCTGC	CAGTTGGTGGTTTCGTAGAG	192	56	(Zhang 2018)
GW7/GL7	CHR701	AGGGCTGGGACTGAACTTTGT	ATGGACCCAGGCAAACACC	138	56	(Zhang 2018)
GW5/qSW5	CHR525	AAGAAAGCCCAAAACAACACA	CTTCCACCCTCAGTGTCGC	206	56	(Zhang 2018)
GW8/OsSPL16	GW8	AAAGAGACAGCCACGGAATC	ATCTTGAGATCCCACTCCAT	191/181	55	(Liu et al. 2019)

Figures

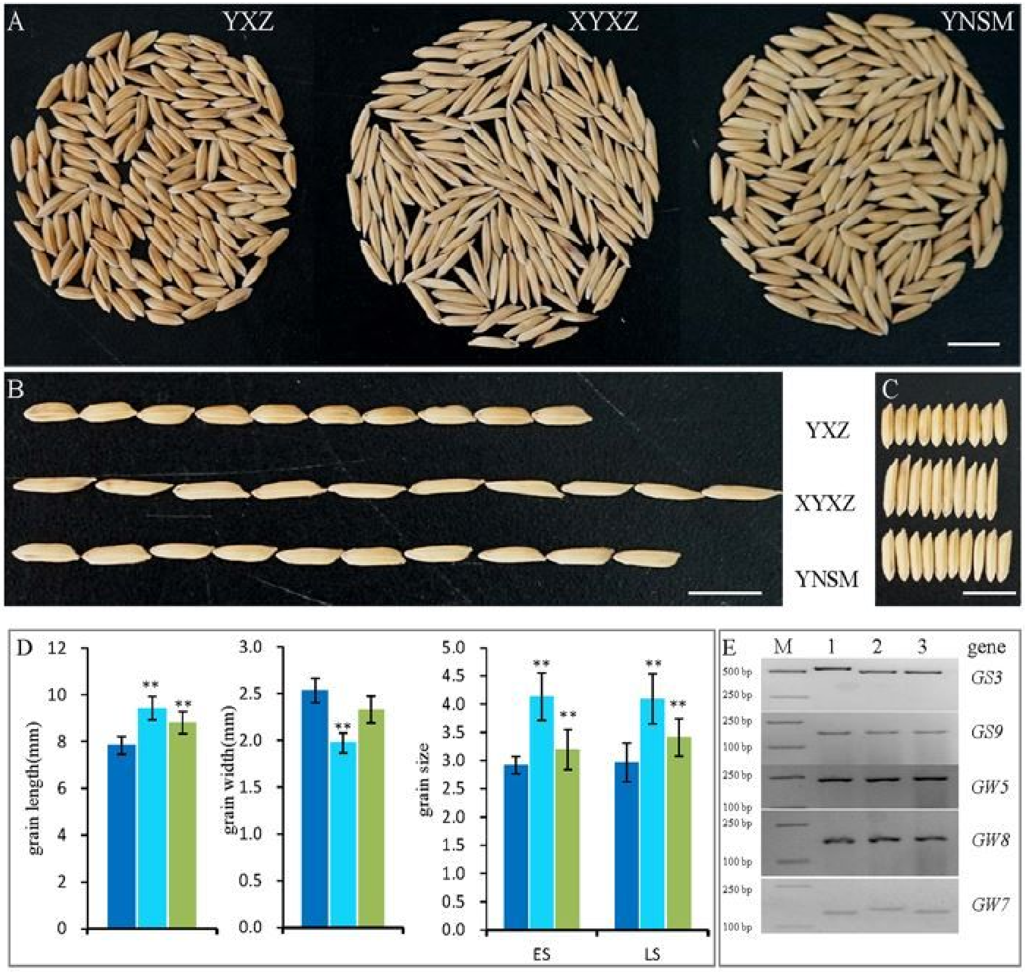

Figure 1

The appearance of grain A, the appearance of grain (n=150 grains); B, 10-grain length of 3 varieties; C, 10-grain width of 3 varieties; D, Statistical analysis of grain length, grain width and grain size (length/width), ES, early season, LS, late season; E, genotype of grain shape genes. ES, early season; LS, late season; Bar=1 cm

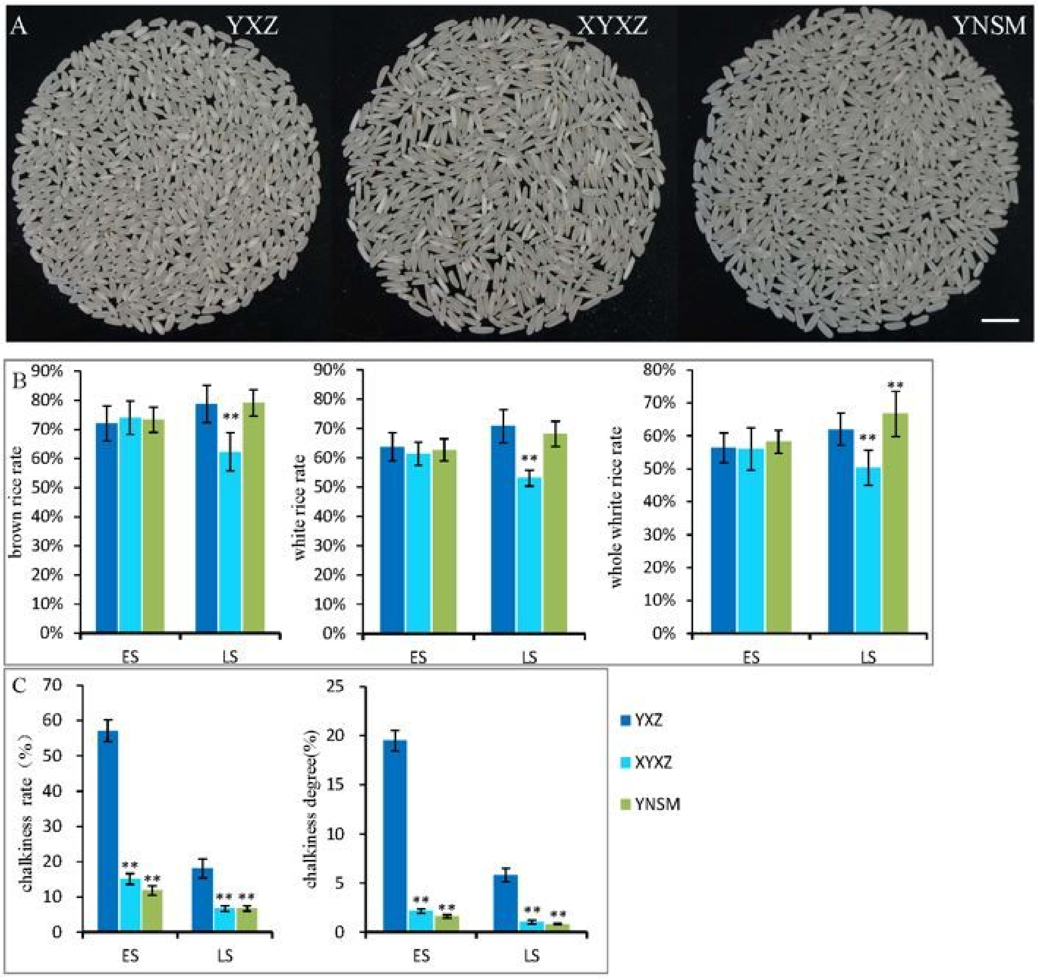

Figure 2

The milling quality analysis A, the appearance of white rice; B and C, The milling quality analysis. ES, early season, LS, late season. Bar=1 cm. ES, early season; LS, late season.

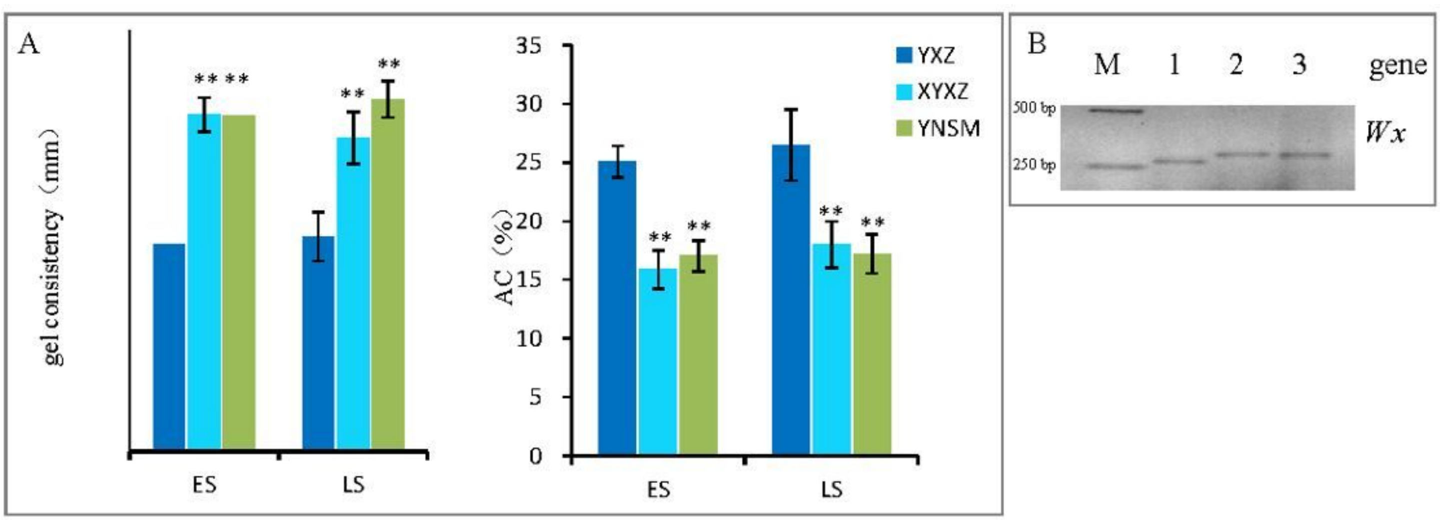

Figure 3

The cooking quality characters of YNSM A, GC and AC of 3 detected rice; B, genotype of cooking quality controlled genes. ES, early season; LS, late season.

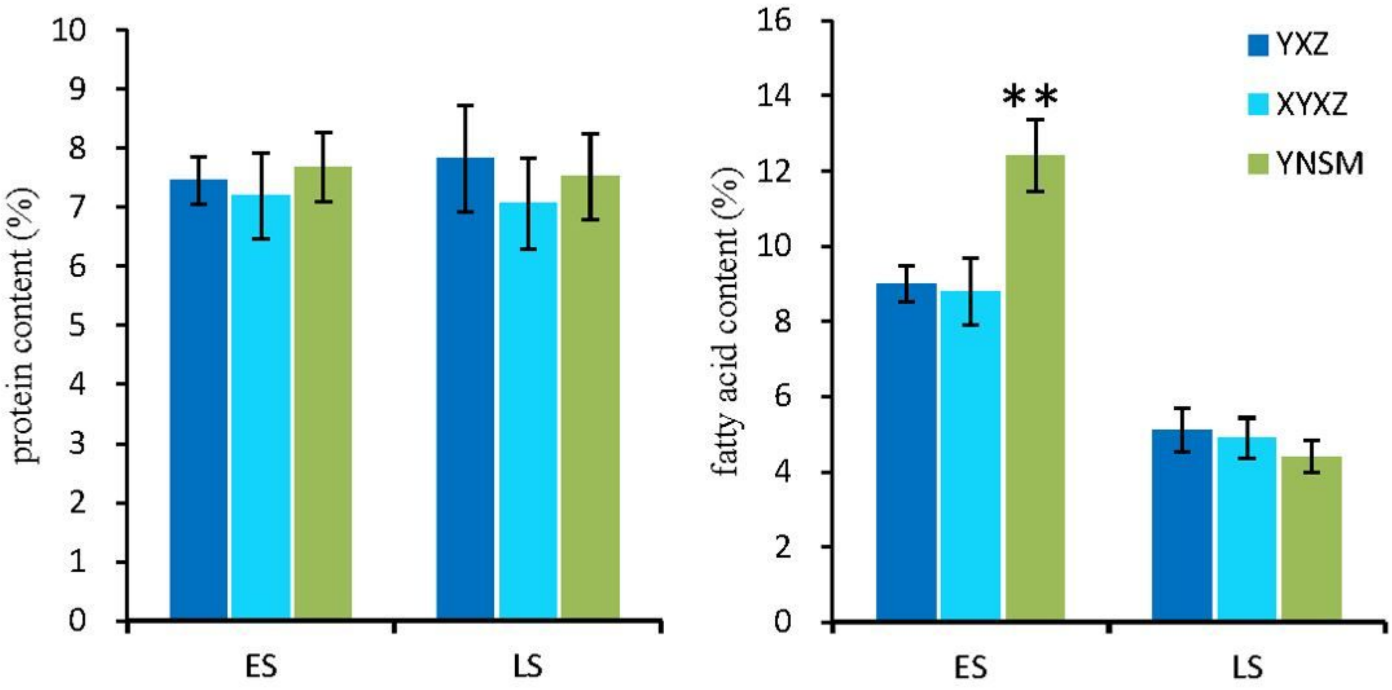

Figure 4

protein and fatty content analysis. ES, early season; LS, late season.