

Genetic separation of chalkiness by hybrid rice of Huanghuazhan and CS197

LU GAN; XIAOSHU DENG; YAN LIU; ANCAI LUO; JIAO CHEN; JING XIANG; ZHENGWU ZHAO*

Chongqing Engineering Research Center of Specialty Crop Resources, Chongqing Normal University, Chongqing, China

Key words: Cluster analysis, Genetic segregation, Percentage of grains with chalkiness, Rice

Abstract: The present study focused on the segregation of the percentage of grains with chalkiness (PGWC), using Huanghuazhan as the female parent and CS197 as the male parent to construct the hybrid rice F₂ population. Molecular markers were used for genotype analysis among the extremely low and extremely high PGWC individuals from the F₂ population. The results revealed that the genotypes of 10 extremely low PGWC individuals were 80.00% and 3.33%, which is identical to Huanghuazhan and CS197, respectively. The heterozygotes accounted for 16.67%. On the contrary, the genotypes of 10 extremely high PGWC individuals were 37.78% and 32.22%, which is identical to Huanghuazhan and CS197, respectively. The heterozygotes accounted for 30.00%. The extremely low and extremely high PGWC individuals had high coincidence with the corresponding F_{2,3} family lines in phenotype and genotype. Scanning electron microscopy (SEM) images showed that the shape and spacing of starch granules in the extreme low and high PGWC individuals corresponded to Huanghuazhan and CS197, respectively. Correlation analysis showed that the F₂ individuals showed a normal distribution trending towards Huanghuazhan. The broad heritability sense was 26.31%, and the narrow heritability sense was 17.21%. The results of cluster analysis showed that the range of PGWC variations of group III based on phenotype varied from 5.66% to 50.00% with an average of 25.46%, and group II results based on genotype varied from 14.34% to 60.67% with an average of 32.33%. Groups II and III shared 28 identical individuals.

Introduction

Over the past half-century, rice production has made a second major leap to meet the increasing demand for food (Wan *et al.*, 2005). However, rice breeders ignored the improvement of rice quality, which caused the poor quality of cultivated varieties (Yang *et al.*, 2004). With the improvement of people's living standards, the difference in demand and preference for good quality rice compared to poor quality hybrid varieties is becoming increasingly obvious. Imported rice with good quality has had a definite impact on China's rice industry. Therefore, improving the international competitiveness of China's rice quality is becoming the main goal of Chinese scientific research workers. High percentage of grains with chalkiness (PGWC) is one of the most important factors in determining poor rice quality (Bian *et al.*, 2013; Zhou *et al.*, 2009a). In the practice of hybrid rice breeding, the genetic separation of the F₂ generation of hybrids was the largest. The selection of strain was difficult, especially the quantitative traits. The PGWC is a quantitative trait controlled by

multiple genes. Therefore, it is important to study the genetic separation of PGWC for the breeding of new high-quality hybrid rice varieties and the creation of materials.

Rice chalkiness results from insufficient seed filling, which in turn, influences light transmission (Kang *et al.*, 2005; Ingh *et al.*, 2006; Fujita *et al.*, 2007; Yamakawa *et al.*, 2007; Cheng *et al.*, 2005; Lin *et al.*, 2017). This is classified by a white-back, white-heart, and white-belly (Satoh and Omura, 1981; Tan *et al.*, 2000; Li *et al.*, 2004). However, the aforementioned characteristics do not directly reflect the quality of rice. PGWC and degree of endosperm (EDC) are two indicators of rice chalkiness. Studies have shown that PGWC is a quantitative trait controlled by multiple genes (Wan *et al.*, 2005). Kou and Liu (1986) revealed that additive and dominant effects significantly affected chalkiness, and the additive effect was more important than the dominant effect, based on a 6 × 6 complete double-row hybridization design. Huang and Zhou (1991) studied the compounding ability of indica rice. The results showed that the general combined effect influenced the chalkiness and grain strength. In other words, the influence of the gene additive effect was small, whereas the influence of the special combining effect was greater. Chalkiness has some

*Address correspondence to: Zhengwu Zhao, zhaozhengwu513@sina.com
Received: 20 July 2019; Accepted: 05 December 2019



dominant effects on chalkiness. Its genetic behavior belongs to independent inheritance and linkage inheritance. The restorer line plays a dominant role in chalkiness formation. Yang *et al.* (1986) found that rice chalkiness-controlled genes had a significant dose effect. There were significant differences in the chalky chrysanthemums of the crosses and hybrids and the chalky chrysanthemum lines. The chalkiness of the F₂ chimney is characterized by the distortion of chalkiness. It is considered that two main pairs of recessive genes control chalkiness.

PGWC in the hybrid F₂ generation has a large separation. Traditional breeding only selects for the phenotypic traits and not from the genes, which causes the failure of the target lines obtained after the strain is stable. Its low selection efficiency hinders the genetic improvement of rice quality. It is possible to improve the selection efficiency of hybrid rice quality from the phenotypic and genotypic studies that hybrid progeny PGWC genetic separation. The genetic separation of PGWC in Huanghuazhan has not been reported.

The present study focused on the coincidence mechanism of individual phenotypes and genotypes of hybrid F₂ isolates, which were bred by the high-quality rice cultivar Huanghuazhan as the female parent and CS197 as the male parent, using phenotypic identification, molecular markers, and morphological observation of chalky starch by SEM. The aim is to lay the foundation for the breeding of high-quality hybrid varieties.

Materials and Methods

Plant materials and field experiment

Huanghuazhan, characterized as low PGWC from the Rice Research Institute of Guangdong Academy of Agricultural Sciences, was used as the female parent. The high quality, high yield, and late-maturing conventional rice Huangxinzhuan variety (F₁ of Luxinzhuan21/8072-2 X Texianzhuan 13) was used as the female parent and Fenghuazhan as the male parent. CS197, characterized as high PGWC from Chongqing Normal University, was used as the male parent, which was bred from the female parent of rice 89-1 and hybridized with Fuhui 838, and then with glutinous rice 89-1 for the reincarnation of parents back to self, and the stable lines were selected from the BC₃F₅ population and crossed with Shuhui527, which was bred

through pedigree selection. Glutinous rice 89-1 has a number of excellent traits. For example, its axillary buds could be dormant in winter against low temperatures and can germinate and regenerate for many years. The cold tolerance of the bud stage and seedling stage reached a Grade 1 cold tolerance standard (Zhao *et al.*, 2006, 2012). The F₂ hybrid population with 120 individuals was constructed from Huanghuazhan and CS197. In the winter of 2013, the group of hybrid combinations was constructed in Hainan. In the spring of 2014–2016, the F₁, F₂, and F_{2,3} generations were planted at the Bishan Mountain Experimental Base of Chongqing Normal University.

Trait measurement of PGWC

Trait measurement of PGWC of rice followed the “High-quality Rice” of People’s Republic of China National Standard (GB/T17981-2017). One hundred samples (N) were randomly selected from the whole milled rice samples and then placed on a Top Yunon TPMZ-A rice appearance quality detector to count chalky rice grains(n) and to calculate PGWC. PGWC (%) = n/N × 100. After three parallels, the average value was used as the trait value.

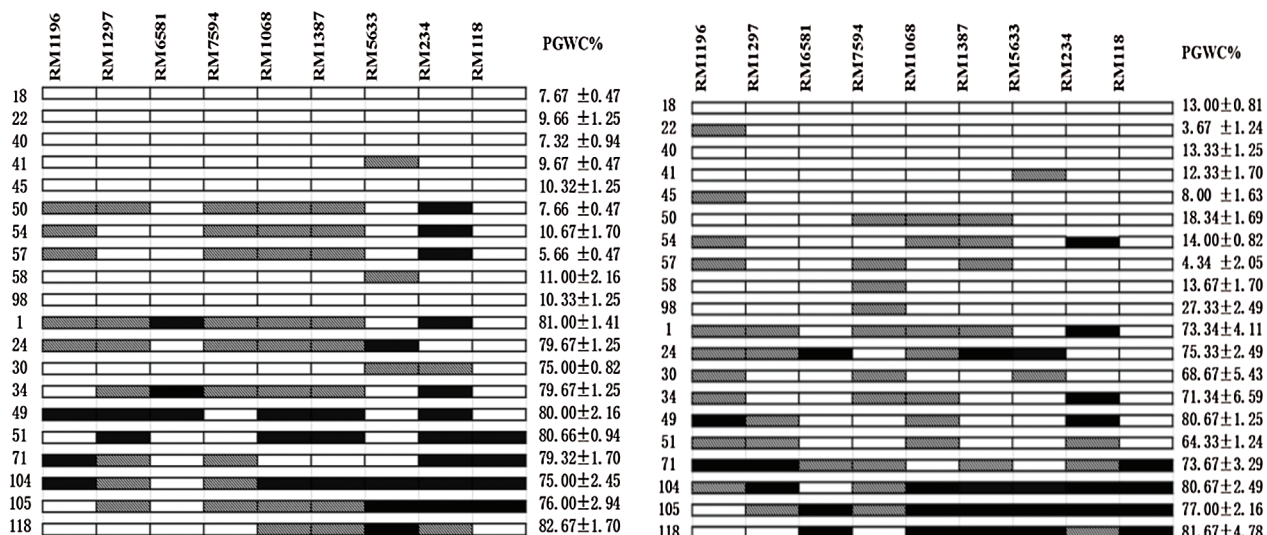
DNA extraction and SSR markers

DNA from seedling samples was extracted by CTAB (Edwards *et al.*, 1991). According to the literature and website information [<http://archive.Gramene.Org/ql/>], 46 molecular markers derived from Commission Sangon Biotech Shanghai Co., Ltd., synthesis were selected to test the polymorphism between the parents, including 36 of them near qPGWC1.1, qPGWC1.2 and qPGWC4 (Bian *et al.*, 2013) and 10 of them (Zhou *et al.*, 2009a) near qPGWC-7. 9 primers of them have good polymorphism (Tab. 1). These molecular markers that determine polymorphism were then used to test the 20 individuals with extremely high and low PGWC in the F₂ population. PCR amplification was performed on 120 individuals of the F₂ population. PCR amplification was carried out in 20 µL reaction mixtures that contained ~10 ng genomic DNA, 1 µL of each primer (10 µmol/L) and 10 µL Taq enzyme mixture (Takara, Dalian, China). The PCR reaction procedure was 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 1 min, and 72°C for 10 min.

TABLE 1

Chalkiness gene markers

No	Primer	Forward primer (5'–3')	Reverse primer (5'–3')	Chromosome	Gene name
1.	RM1196	AGCTGCCGTGAGCCTCAAG	TCCAAAACGCTCTCTTCGTC	1	qPGWC1.1
2.	RM1297	GTGCCTTACAACCTCAACGAC	CACTCCCAGTTCAGTACGTC	1	qPGWC1.1
3.	RM6581	GATCCAGAAGAAGCGGCAC	GCCTTCTCAACGATGGAGT	1	qPGWC1.1
4.	RM7594	CAACAGGCCCTGCATGAG	TCACTCCCCTCGCTCGATC	1	qPGWC1.2
5.	RM1068	GCTGTACAGTGTCTTCGTTTC	TGCCTATTGCCTACTCACTC	1	qPGWC1.2
6.	RM1387	GTGGCTGGCTGATCGATC	AATCAACCCAGCTACCATGC	1	qPGWC1.2
7.	RM5633	GTGTAGCTGCTAGGCCGAAC	TTCCTTTCGCTACGTTGGAC	4	qPGWC 4
8.	RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	7	qPGWC 7
9.	RM118	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG	7	qPGWC 7



A Genotype and phenotype of extreme population of F₂ population B Genotype and phenotype of extreme population of F_{2:3} population
FIGURE 1. Genotypes and phenotypes of extreme individuals of F₂ and F_{2:3} family lines. The white, black, and diagonal rectangles denote the genotype of Huanghuazhan, CS 197, and heterozygote, respectively.

Observation of seed cross-section by SEM

The parents, the 10 extremely low, and 10 extremely high PGWC F₂ individuals were selected. Ten seeds derived from each individual were de-hulled with a brown rice machine. Three representative brown rice samples were selected. A single-sided knife was gently tapped in the middle of the brown rice to make it laterally fracture in its natural state and both ends were removed to make a 3 mm thick sample. The sample was then adhered to a copper table with double-sided tape, and gold was sprayed across. The morphological structure of starch granules of the brown rice was examined by a Hitachi SU3500 scanning electron microscope.

Data analysis

SPSS19.0 software was used for cluster and statistical analysis and calculation of generalized heritability and narrow heritability. The phenotypic data and molecular data of extreme high and low PGWC individuals in the F₂ population were used to compare the phenotype and genotype.

Results

PGWC molecular genetic separation

Nine of the 46 primer pairs with polymorphism between the parents (i.e., Huanghuazhan and CS197) were finally screened (Tab. 1). The markers were consistent with the genotype of Huanghuazhan (white bars, Fig. 1A), CS197 (black bars; Fig. 1A), and the heterozygous genotype (diagonal bars; Fig. 1A). The genotypes of 10 extremely low PGWC individuals were 80.00% and 3.33%, which is identical to Huanghuazhan and CS197, respectively. The heterozygotes accounted for 16.67%. On the contrary, the genotypes of 10 extremely high PGWC individuals were 37.78% and 32.22%, which is identical to Huanghuazhan and CS197, respectively. The heterozygotes accounted for 30.00%. RM6581 and RM118 were detected on the 10 extremely low PGWC individuals. However, RM234 was detected on 7 of the 10 extremely low PGWC individuals. The low PGWC of the individuals was 5.66–11.00%, with an average of 9.00%, which was consistent with the rate of Huanghuazhan at 10.32%. The high PGWC of the individuals was 75.00–82.67%, with an average of 78.90%, which was close to the parental CS197 at 81.67%. The F_{2:3}

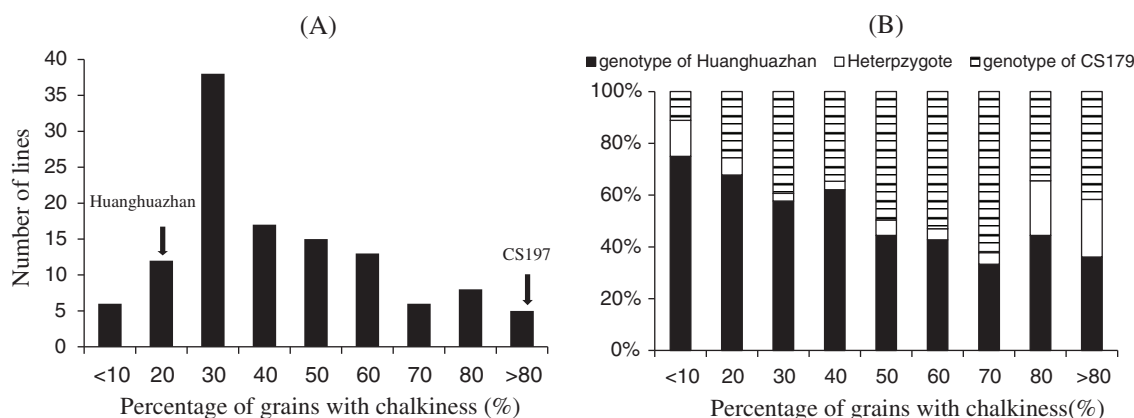


FIGURE 2. PGWC of F₂ population. (A) Frequency distribution for PGWC phenotype in F₂. (B) Genotypic ratio of PGWC frequency distribution in F₂.

TABLE 2

Quality traits of extreme individual in F₂ populations of Huanghuazhan/CS197

Traits	Material	PGWC/%	ACE/%	DEC/%	BR/%	MR/%	HR/%	AC/%	GL/ mm	GW/ mm	LWR	GS
Parents	Huanghuazhan	10.32 ± 2.33	2.31 ± 1.24	2.45 ± 0.47	77.17 ± 1.01	68.49 ± 0.59	58.87 ± 4.64	15.18 ± 0.01	6.66 ± 0.06	1.86 ± 0.03	3.57 ± 0.08	length
	CS197	81.67 ± 10.35	82.67 ± 0.94	65.51 ± 1.70	82.53 ± 2.13	71.34 ± 0.72	61.63 ± 2.59	7.33 ± 0.04	5.76 ± 0.08	2.79 ± 0.07	2.06 ± 0.04	Oval
Low PGWC lines	18	7.67 ± 0.47	15.66 ± 1.70	11.11 ± 1.24	77.36 ± 1.25	67.97 ± 1.69	56.36 ± 1.95	14.26 ± 0.03	6.78 ± 0.11	1.80 ± 0.06	3.76 ± 0.14	length
	22	9.66 ± 1.25	26.67 ± 0.47	26.76 ± 1.69	78.00 ± 1.60	68.53 ± 0.64	59.60 ± 1.26	10.26 ± 0.11	6.54 ± 0.23	1.76 ± 0.06	3.41 ± 0.03	length
	40	7.32 ± 0.94	6.33 ± 1.25	5.63 ± 1.24	75.24 ± 1.20	69.00 ± 0.93	58.36 ± 1.79	14.60 ± 0.08	6.62 ± 0.19	1.94 ± 0.07	3.06 ± 0.08	length
	41	9.67 ± 0.47	11.66 ± 1.24	10.28 ± 1.70	74.31 ± 2.77	68.21 ± 1.75	57.47 ± 0.97	12.72 ± 0.05	6.65 ± 0.26	2.17 ± 0.11	3.31 ± 0.09	length
	45	10.32 ± 1.25	8.67 ± 1.25	7.95 ± 0.94	75.78 ± 1.20	69.32 ± 0.73	57.70 ± 0.50	12.42 ± 0.04	6.73 ± 0.18	2.03 ± 0.10	3.29 ± 0.14	length
	50	7.66 ± 0.47	5.33 ± 2.05	4.08 ± 1.63	80.70 ± 2.39	66.73 ± 0.58	58.21 ± 0.86	14.58 ± 0.05	6.57 ± 0.20	2.00 ± 0.11	3.34 ± 0.16	length
	54	10.67 ± 1.70	5.64 ± 1.25	6.72 ± 2.16	76.72 ± 2.87	68.76 ± 1.13	59.06 ± 0.93	14.49 ± 0.04	6.48 ± 0.08	1.95 ± 0.08	3.52 ± 0.24	length
	57	5.66 ± 0.47	8.66 ± 1.70	5.31 ± 1.69	75.98 ± 2.86	68.27 ± 0.95	58.08 ± 0.86	15.50 ± 0.05	6.74 ± 0.30	1.98 ± 0.17	3.42 ± 0.15	length
	58	11.00 ± 2.16	6.32 ± 1.25	7.52 ± 1.88	80.19 ± 1.61	70.06 ± 0.90	56.78 ± 2.63	14.98 ± 0.03	6.23 ± 0.05	1.77 ± 0.12	3.52 ± 0.24	length
	98	10.33 ± 1.25	12.67 ± 1.24	14.09 ± 0.47	76.41 ± 0.43	69.59 ± 0.49	58.25 ± 1.10	14.09 ± 0.07	6.45 ± 0.16	1.94 ± 0.15	3.35 ± 0.29	length
Range		5.66–11.00	5.33–26.67	4.88–25.76	74.31–80.70	66.73–69.59	56.36–59.60	10.26–15.50	6.23–6.78	1.77–2.17	3.06–3.76	
Average		9.00	10.76	9.94	77.11	68.65	57.99	13.79	6.58	1.93	3.42	
CV/%		18.81	57.69	63.10	2.56	1.29	1.58	16.90	4.08	13.55	12.25	
High PGWC lines	1	81.00 ± 1.41	92.67 ± 8.99	78.32 ± 1.24	81.91 ± 1.12	71.23 ± 0.48	63.05 ± 0.48	7.27 ± 0.03	5.44 ± 0.16	2.85 ± 0.04	1.92 ± 0.07	Oval
	24	79.67 ± 1.25	31.33 ± 1.25	23.66 ± 0.47	81.79 ± 1.53	71.32 ± 1.14	61.96 ± 0.47	5.18 ± 0.02	5.76 ± 0.04	2.87 ± 0.07	2.01 ± 0.05	Oval
	30	75.00 ± 0.82	36.00 ± 0.82	26.67 ± 1.24	80.75 ± 0.63	70.05 ± 0.93	63.47 ± 0.79	9.95 ± 0.04	5.90 ± 0.14	2.75 ± 0.06	2.14 ± 0.08	Oval
	34	79.67 ± 1.25	56.34 ± 1.69	47.00 ± 2.16	81.48 ± 0.75	72.61 ± 0.46	62.73 ± 0.77	14.10 ± 0.02	5.84 ± 0.16	2.83 ± 0.08	2.06 ± 0.01	Oval
	49	80.00 ± 2.16	25.00 ± 2.16	18.67 ± 1.69	82.09 ± 0.19	72.51 ± 0.58	62.46 ± 0.85	7.74 ± 0.03	5.95 ± 0.11	2.71 ± 0.07	2.20 ± 0.10	Oval
	51	80.66 ± 0.94	56.66 ± 4.03	44.33 ± 3.09	82.61 ± 0.44	71.69 ± 1.13	62.21 ± 0.75	9.27 ± 0.04	6.04 ± 0.21	3.01 ± 0.05	2.00 ± 0.06	Oval
	71	79.32 ± 1.70	72.67 ± 3.29	59.67 ± 2.05	82.12 ± 1.12	72.77 ± 0.36	62.71 ± 1.16	8.34 ± 0.02	6.43 ± 0.54	2.71 ± 0.05	2.37 ± 0.24	Oval
	104	75.00 ± 2.45	52.32 ± 2.49	40.00 ± 6.53	81.56 ± 0.89	71.45 ± 1.48	61.99 ± 0.34	11.59 ± 0.02	6.47 ± 0.69	2.86 ± 0.04	2.26 ± 0.25	Oval
	105	76.00 ± 2.94	66.00 ± 7.79	46.34 ± 2.05	80.89 ± 1.31	72.34 ± 1.51	62.00 ± 2.43	9.74 ± 0.03	6.33 ± 0.46	2.64 ± 0.12	2.40 ± 0.25	Oval
118	82.67 ± 1.70	73.67 ± 3.30	62.66 ± 2.05	81.84 ± 1.11	72.55 ± 0.57	62.32 ± 0.72	10.91 ± 0.04	6.43 ± 0.74	2.81 ± 0.20	2.28 ± 0.11	Oval	

Table 2 (continued).

Traits	Material	PGWC/%	ACE/%	DEC/%	BR/%	MR/%	HR/%	AC/%	GL/ mm	GW/ mm	LWR	GS
Range		75.00– 82.67	31.33– 92.67	18.67– 78.32	80.75– 82.61	70.05– 72.77	61.96– 63.47	5.18– 14.10	5.44– 6.47	2.64– 3.01	1.92– 2.40	
Average		78.90	56.27	44.73	81.71	71.85	62.49	9.41	6.06	2.81	2.17	
CV/%		3.18	35.62	39.69	0.65	1.34	0.76	25.11	5.38	3.54	7.16	

Note: ACE, area of chalky endosperm; BR, Brown rice percentage; HR, Head rice percentage; AC, Amylose content; GL, Grain length; GW, Grain width; LWR, length-width ratio; GS, grain shape.

family lines of the F_2 population were identified by a molecular marker and phenotypic identification. The results showed that $F_{2,3}$ family lines with low PGWC of the F_2 population were still expressed as a low PGWC of 3.67–27.33%, with an average of 12.80%. The PGWC of most family lines was slightly increased, and the few numbers of family lines were slightly decreased. The results of the two generations were similar. In particular, the 9 markers detected were consistent with the genotype of Huanghuazhan (Figs. 1A and 1B). Phenotype and genotype similarity of 10 high PGWC extreme individuals is higher in the F_2 population and $F_{2,3}$ family. The markers consistent with the genotype of the two extremes were similar to those in the F_2 and $F_{2,3}$ family lines. RM1297, RM1068, RM1378, RM234 ratio is greater than 3, which is more suitable for PGWC of molecular marker-assisted selection.

Genetic separation of phenotypes and cluster analysis of PGWC

The PGWC of CS197 and Huanghuazhan were 81.67% and 10.33%, respectively. There were 5.66–82.67% of the PGWC distribution in the F_2 population, 31.67% of the largest individuals in the 20–30% range of PGWC, and 3.33% of the smallest individuals in the interval of 80–90%, with an average value of 35.27%. This was lower than the F_1 average of 39.33%, and the coefficient of variation was 57.13%. The broad-sense heritability was 26.31%, while the narrow sense heritability was 17.21%. The environmental variance was 42.10%, and the average dominant degree was 0.78. F_2 individuals showed a homogeneous distribution trending to a small PGWC, and there was a one-way super-affinity phenomenon (Fig. 2A). The super-parent individuals' genotype Huanghuazhan accounted for 75%, CS197 was 11.11%, and heterozygosity was 13.89% (Fig. 2B).

The quality traits of 10 extremely low PGWC were similar to those of parental Huanghuazhan, while those of 10 extremely high PGWC were similar to those of parental CS197 (Tab. 2). The PGWC phenotypic and genotyping analysis of 120 individuals of the F_2 population were analyzed by cluster analysis. Phenotypic inheritance can be divided into three groups (Fig. 3A). In the group I, II, and III, the amplitudes of PGWC were 69.82–82.67%, 49.48–80.00%, and 5.66–50.00%, respectively, and the average values were 76.40%, 57.53%, and 25.46%, respectively. Those individuals with the highest PGWC were clustered in group I. According to the results of molecular marker detection of PGWC, genotype inheritance can be divided into four groups (Fig. 3B). The values of PGWC in groups I, II, III, and IV were 6.10–79.67%, 14.34–60.67%, 17.00–82.67%, and

5.66–82.67%, respectively, and the average values were 34.98%, 32.33%, 44.48%, and 37.59%, respectively. The PGWC of group IV was highest, and the amplitude was higher than the other three groups.

Morphological structure of starch granules of chalky rice

Magnification of 50× under SEM was used to observe the cross-section of Huanghuazhan, CS197, and the 10 extremely low and 10 high PGWC F_2 individuals of the Huanghuazhan×CS197 hybrid. The starch granules were observed under 2500×. The starch granules of CS197 are mainly round or oval and the gap between starch granules is large and loose (Fig. 4). White-heart and white-belly are large (Figs. 4A, 4E and 4I). The starch particle shape (Figs. 4C, 4G and 4K) of the individual 49 (rice field number) is similar to that of CS197, but the gap between the particles is relatively small. On the contrary, the starch granules of Huanghuazhan are mainly oval or long strip. The gap between starch granules is smaller and more closely arranged than those of CS197 (Figs. 4B, 4F and 4J). The starch particle shape of individual 18 (Figs. 4D, 4H and 4L) is similar to that of Huanghuazhan (Figs. 4J and 4L).

Discussion

The PGWC is one of the main appearance qualities of rice. Huanghuazhan has been approved in China by the Guangdong, Hunan, Hubei, Guangxi, Hainan, Jiangxi, Zhejiang, Chongqing, and Shanxi provinces (Zhou *et al.*, 2012). It is the first grade I rice variety based on the national standard to be promoted in a large rice area of southern China. PGWC of Huanghuazhan was very low, and the results from Guangdong and Hunan were 4%. Preparing the hybrid combinations based on Huanghuazhan to study the phenotypic and genotypic conformational mechanisms of PGWC in the F_2 population is important to improve the selection efficiency of low PGWC in hybrid offspring and to utilize Huanghuazhan and hybrid rice quality breeding.

With the development of genomics and molecular marker technology, the QTLs controlling the PGWC were located by various populations, which were distributed on 12 chromosomes in rice (Guo *et al.*, 2011; Peng *et al.*, 2014., Li *et al.*, 2003; Zhou *et al.*, 2009b). Wan *et al.* (2005) used the CSSL population to detect QTLs associated with chalky grain size on chromosomes 8 and 9. Liu *et al.* (2012) detected three major QTLs affecting the PGWC on the 5th, 8th, and 10th chromosomes using the RIL population. Tan

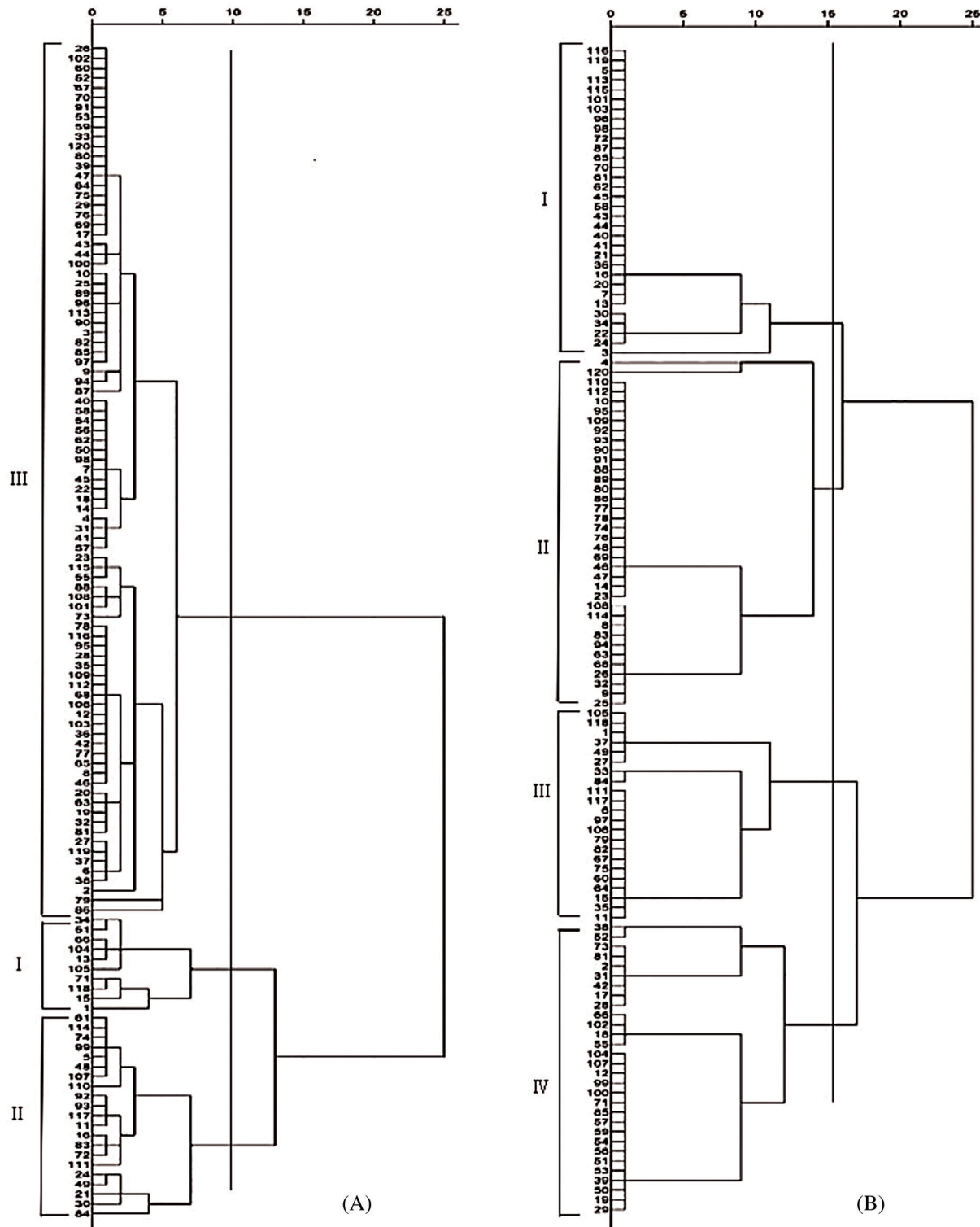


FIGURE 3. Cluster analysis of the PGWC (A) phenotype and (B) genotype, in F₂ populations.

et al. (2000) used an F₂ population to detect QTLs associated with PGWC on chromosomes 1, 3, 5, 6, and 7. In this study, 46 primers closely linked to the PGWC were selected. Nine of them were polymorphic between Huanghuazhan and CS197. The 10 extremely low and 10 extremely high PGWC F₂ individuals were detected by 9 polymorphic markers. The results showed that the genotypes of 10 extremely low PGWC individuals were 80.00% and 3.33%, which is identical to that of Huanghuazhan and CS197, respectively.

The heterozygotes accounted for 16.67%. The low PGWC genotype trends towards Huanghuazhan, which is consistent with the phenotypic value and the results of SEM. The genotypes of 10 extremely high PGWC individuals were 37.78% and 32.22%, which is identical to that of Huanghuazhan and CS197, respectively. The heterozygotes accounted for 30.00%. Using molecular marker detection and phenotypic identification in the extreme individuals of the F₂ population and the corresponding F_{2:3} family lines,

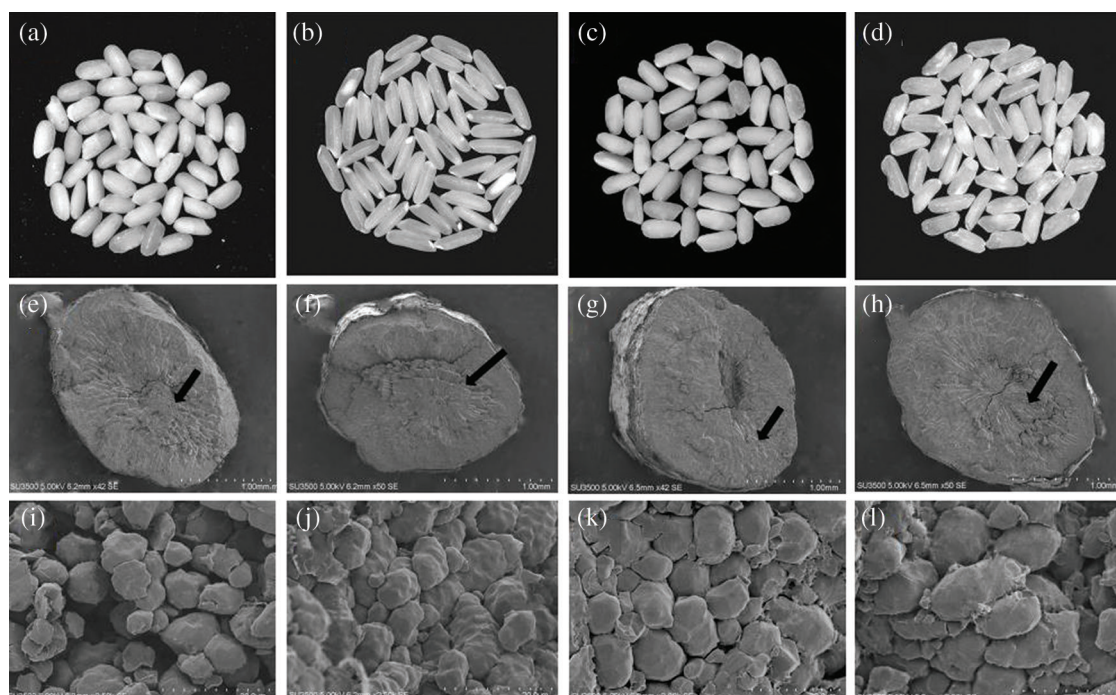


FIGURE 4. Phenotype of chalky rice and its morphological structure of starch granules.

(A), (E) and (I) from CS197; (B), (F) and (J) from Huanghuazhan; (C), (G) and (K) from F₂ individual plant 49; (D), (H) and (L) from F₂ individual plant 18. The arrowheads in (E), (F), (G) and (H) represent the position of SEM images in (I), (J), (K) and (L). Bar represents 1 mm in (A), (B), (C) and (D).

we found two generations of similar results, with a high degree of compliance. It is shown that the selection efficiency of PGWC can be improved by double selection of phenotype and molecular marker. The genotype of individuals with high PGWC was evenly distributed, which is different from the phenotypic value and the SEM results trending towards CS197, probably because PGWC is controlled by polygenes besides environmental impact. In the hybrid breeding of PGWC, the method involving a combination of molecular marker and phenotypic identification, while taking into account the effects of genes and the environment, can improve the efficiency of breeding selection. Leng and Hong (2004) argued that the PGWC is predominantly additive-dominated and dominated by dominance effects. When improving the chalky traits of rice, it is necessary to use the techniques of hybridization and backcrossing to polymerize as much chalkiness allele as possible. At the same time, it is necessary to identify the phenotype in a number of environments and then to select those that show low chalkiness in the new variety. The results from Leng and Hong (2004) were consistent with our study.

In the practice of hybrid rice breeding, the greater the separation of the F₂ generation, the greater there is a chance of selecting the target plants. Breeding selection is difficult, especially the low heritability trait. In this study, the PGWC of the hybrid F₂ population was 5.66–82.67% and had continuous distribution. The maximum value was close to 81.67% of CS197, and the lowest value is lower than the PGWC of the parent, Huanghuazhan, accounting for 10.32%. The largest PGWC distribution interval of 20–30% accounted for 31.67%, and the smallest PGWC interval of 80–90% accounted for 3.33%. PGWC distribution trended

towards the female parent Huanghuazhan with low PGWC. These results were consistent with direct genetic research from others. Kou and Liu (1986) used a 6 × 6 complete double-row hybridization design to observe significant differences in grain chalkiness between the positive and negative hybrids, showing significant maternal effects. Chol (1979) also found that the chalkiness of the hybrid variety was influenced by the maternal effect and showed a sense of inheritance. Qi *et al.* (1983) supported the opinion of the maternal effect of chalky inheritance. Based on the PGWC phenotype and molecular marker test results of the F₂ population, clustering analysis showed that the PGWC individuals of the phenotypic clustering was relatively concentrated, and the high PGWC individuals were clustered in group II of 70.00–82.67%. However, according to the results of molecular marker detection of PGWC, it was divided into four groups. The PGWC was highly dispersed, and the amplitude was larger. Comparing the PGWC phenotype and genotype, the distribution of PGWC phenotype III and genotype II were the closest, which were 5.66–50.00%, 14.34–60.67%, with an average value of 25.46%, 32.33%, respectively. The above two groups had 28 identical individuals. The low PGWC-individuals in the genotype clustering were mostly clustered in group I, and the high PGWC individuals were mostly clustered in group III. The low PGWC and high PGWC individuals of group I and IV were crossed.

Reducing PGWC is important for improving rice quality (Sun *et al.*, 2015). Although PGWC is affected by genetic and environmental effects (He *et al.*, 1997), it is important to explore the genetic mechanism of PGWC genes in its offspring by studying the genetic separation of PGWC,

which can play an important role in hybrid rice quality breeding. In this study, the PGWC phenotypic individuals of the F₂ population were analyzed. The starch granules of the low PGWC and high PGWC individuals in the F₂ and F_{2,3} family lines were observed by SEM to study the PGWC phenotypic and genotypic mechanisms. The application of phenotype and genotype in the prediction of heterosis by the effective selection of low PGWC lines will reduce blindness selection and will have an important role in chalkiness quality breeding.

Funding Statement: This research was supported by the National Natural Sciences Foundation (31670326), Technology Innovation and Application Development Program in Chongqing (cstc2019jscx-msxmX0353) The Program for Innovative Research Team in University, Chongqing (CXTDX201601018), Achievement Transfer Program of Institutions of Higher Education in Chongqing (KJZH17114), Key Natural Science Foundation of Chongqing (CSTC2015jcyjBX0091), Natural Science Fund of Chongqing (cstc2016jcyjA0031).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

- Bian JM, Shi H, Li CJ, Zhu CL, Yu QY, Peng XS, Fu JR, He XP, Chen XR, Hu LF, Ouyang LJ, He HH (2013). QTL mapping and correlation analysis for 1000-grain weight and percentage of grains with chalkiness in rice. *Journal of Genetics* **92**: 281–287. DOI 10.1007/s12041-013-0267-6.
- Cheng FM, Zhong LJ, Wang F, Zhang GP (2005). Differences in cooking and eating properties between chalky and translucent parts in rice grains. *Food Chemistry* **90**: 39–46. DOI 10.1016/j.foodchem.2004.03.018.
- Chol SJ (1979). Seoul National University. *College of Agriculture Bulletin* **4**: 247–276.
- Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* **19**: 1349. DOI 10.1093/nar/19.6.1349.
- Fujita N, Yoshida M, Kondo T, Saito K, Utsumi Y, Tokunaga T, Nishi A, Satoh H, Park JH, Jane JL, Miyao A, Hikonaga T, Nakamura Y (2007). Characterization of SSIIIa deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the endosperm. *Plant Physiology* **144**: 2009–2023. DOI 10.1104/pp.107.102533.
- Guo T, Liu XL, Wan XY, Weng JF, Liu SJ, Liu X, Chen MJ, Li JJ, Su N, Wu FQ, Cheng ZJ, Guo XP, Lei CL, Wang JL, Jiang L, Wan XY (2011). Identification of a stable quantitative trait locus for percentage grains with white chalkiness in rice (*Oryza sativa*). *Journal of Integrative Plant Biology* **53**: 598–607. DOI 10.1111/j.1744-7909.2011.01041.x.
- He SH, Peng XS, Liu YB (1997). Effect of environmental conditions on rice quality. *Acta Agriculturae Jiangxi* **9**: 66–72.
- Huang HQ, Zhou XY (1991). Analysis of combining ability of rice chalkiness. *Hunan Agricultural Sciences* **9**: 18–20.
- Ingh N, Kaur L, Sandhu KS, Kaur J, Nishinari K (2006). Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloids* **20**: 532–542. DOI 10.1016/j.foodhyd.2005.05.003.
- Kang HG, Park SH, Matsuoka M, An G (2005). White-core endosperm floury endosperm-4 in rice is generated by knockout mutations in the C4-type pyruvate orthophosphate dikinase gene (OsPPDKB). *Plant Journal* **42**: 901–911. DOI 10.1111/j.1365-313X.2005.02423.x.
- Kou YC, Liu C (1986). Inheritance of chalkiness of rice endosperm. *Journal of Agricultural Research of China* **35**: 129–138.
- Leng Y, Hong DL (2004). Quality characters of hybrid rice grain derived from different ecological types and their genetic analysis in Japonica rice (*Oryza sativa*). *Chinese Journal of Rice Science* **18**: 29–33.
- Li JM, Xiao JH, Grandillo S, Jiang L, Wan Y, Deng Q, Yuan L, McCouch SR (2004). QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. *Genome* **47**: 697–704.
- Li ZF, Wan JF, Xia JF, Zhai HQ (2003). Mapping quantitative trait loci underlying appearance quality of rice grains (*Oryza sativa* L.). *Acta Genetica Sinica* **30**: 251–259.
- Lin ZM, Zhang XC, Wang ZX, Jiang YT, Liu ZH, Alexander D, Li GH, Wang SH, Ding YF (2017). Metabolomic analysis of pathways related to rice grain chalkiness by a notched-belly mutant with high occurrence of white-belly grains. *BMC Plant Biology* **17**: 39–53. DOI 10.1186/s12870-017-0985-7.
- Liu X, Wang Y, Wang SW (2012). QTL analysis of percentage of grains with chalkiness in Japonica rice (*Oryza sativa*). *Genetics and Molecular Research* **11**: 717–724. DOI 10.4238/2012.March.22.1.
- Peng B, Wang LQ, Fan CC, Jiang GH, Luo LJ, Li YB, He YQ (2014). Comparative mapping of chalkiness components in rice using five populations across two environments. *BMC Genetics* **15**: 49–63. DOI 10.1186/1471-2156-15-49.
- Qi ZB, Li BJ, Yang WG, Wu XF (1983). A Study on the genetic of exterior quality and fat of the rice grains. *Acta Genetica Sinica* **10**: 452–458.
- Satoh H, Omura T (1981). New endosperm mutations induced by chemical mutagen in rice (*Oryza sativa* L.). *Japanese Journal of Breeding* **31**: 316–326. DOI 10.1270/jsbbs1951.31.316.
- Sun W, Zhou Q, Yao Y, Qiu X, Xie K, Yu S (2015). Identification of genomic regions and the isoamylase gene for reduced grain chalkiness in rice. *PLoS One* **10**: e0122013. DOI 10.1371/journal.pone.0122013.
- Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang QF (2000). Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. *Theoretical and Applied Genetics* **101**: 823–829. DOI 10.1007/s001220051549.
- Wan XY, Wan JM, Weng JF, Liang L, Bi JC, Wang CM, Zhai HQ (2005). Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. *Theoretical and Applied Genetics* **110**: 1334–1346. DOI 10.1007/s00122-005-1976-x.
- Wu SH, Li RS (2002). Food demand, ensure and countermeasures for China in the next 30 years. *Progress in Geography* **21**: 121–129.
- Yamakawa H, Hirose T, Kurofua M, Yamaguchi T (2007). Comprehensive expression profiling of rice grain filling related genes under high temperature using DNA microarray. *Plant Physiology* **144**: 258–277. DOI 10.1104/pp.107.098665.
- Yang RC, Liang KJ, Chen QH (1986). Xenia of chalkiness in rice and the genetic analysis of chalkiness of hybrid rice. *Journal of Fujian Agricultural college* **15**: 51–54.

- Yang SH, Cheng BY, Shen WF, Liao XY (2004). Progress and strategy of the improvement of Indica rice varieties in the Yangtse Valley of China. *Chinese Journal of Rice Science* **18**: 89–93.
- Zhao ZW, Luo AC, Le T, Li SG (2012). Identification of quantitative trait locus for overwintering germinability in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture* **11**: 1767–1774. DOI 10.1016/S2095-3119(12)60181-6.
- Zhao ZW, Li SG, Huang WZ, Lei SF (2006). Genetic variation of main agronomic traits in a new germplasm resource of perennial rice. *Chinese Journal of Rice Science* **20**: 481–486.
- Zhou LJ, Chen LM, Jiang L, Zhang WW, Liu LL, Liu X, Zhao ZG, Liu SJ, Zhang LJ, Wang JK, Wan JM (2009a). Fine mapping of the grain chalkiness QTL qPGWC-7 in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **118**: 581–590. DOI 10.1007/s00122-008-0922-0.
- Zhou LJ, Jiang L, Liu X, Chen H, Chen LM, Liu SJ, Wan JM (2009b). QTL mapping and interaction analysis for 1000-grain weight and percentage of grains with chalkiness in rice. *Acta Agronomica Sinica* **35**: 255–261.
- Zhou SC, Li H, Huang DQ, Lu DC, Lai HC, Wang ZD, Zhou DG, Li KH, Wang CR, Li H (2012). Breeding and application of Huanghuzhan—a new variety with 1st class rice quality of national standard. *Hubei Agricultural College* **51**: 1960–1964.