The Rice (*Oryza Sativa* L.) *Rc* Gene, Which Imparts Resistance To Pre-Harvest Sprouting, Retains Seed and Milled Rice Quality

Min Wu
Zhejiang A and F University

Shan Xu
Zhejiang A and F University

Yuexin Fei
Zhejiang A and F University

Yujie Cao
Zhejiang A and F University

Hongkai Wu
Zhejiang A&F University

wuhk@zafu.edu.cn*Corresponding Author*
ORCiD: https://orcid.org/0000-0001-6433-2838

10.21203/rs.3.rs-26374/v1

SUBJECT AREAS

Plant Molecular Biology and Genetics *Plant Physiology and Morphology*

KEYWORDS

Pre-harvest sprouting (PHS), Rc gene/qSD7-1, Rice quality, Seed dormancy, NIL-derived population

Abstract

Pre-harvest sprouting (PHS) in cereal crops, including rice (Oryza sativa L.), causes substantial yield and end-use quality losses worldwide. These losses could be prevented through introgression of dormancy-related genes into commercial varieties. Rc gene, which, in the absence of Rd , results in rice with brown pericarp, controls seed dormancy. Through reciprocal cross analysis, we established that Rc has a completely dominant maternal effect on pericarp color, which may confer an advantage in PHS resistance to hybrid rice over inbred rice. To investigate the effect of Rc on PHS resistance and other traits, we developed two sets of NIL-derived populations targeting the Rc locus in japonica and indica rice. In japonica rice, the sprouting percentage (SP) of seeds produced by maternal plants with an Rcrc or RcRc genotype was significantly (~43%) lower than that produced by maternal plants with an rcrc genotype. Furthermore, there was no significant difference between the SP of seeds produced by maternal plants with Rcrc and RcRc genotypes; therefore, the SP of seeds produced by maternal plants with the Rcrc genotype showed no genetic segregation, indicating that Rc has a dominant maternal effect on PHS resistance. The SP of brown hybrid seed was significantly (~50%) lower than that of white hybrid seed, indicating that the effect of Rc on PHS resistance failed to be counteracted by gibberellic acid application in hybrid seed production. In indica hybrid rice, the SP of brown hybrid rice was significantly (~48%) lower than that of white hybrid rice. Thus, Rc significantly affects PHS resistance in both japonica and indica rice. Moreover, there was no difference between the RcRc , Rcrc , and rcrc genotypes in germination percentage (GP) of after-ripened seeds and no difference between brown and white hybrid seeds in seedling establishment, indicating that releasing Rc -controlled dormancy retains seed quality and does not negatively affect the next agricultural production cycle. Further investigation showed that there was no significant difference between the milled rice qualities of brown- and white-pericarp rice, including total amylose content, hot-water-insoluble amylose content, gel consistency, alkali spreading value, rapid viscosity analyzer profile properties, crude protein content, and crude fat content. DPPH• inhibition percentage, an indicator of antioxidative capacity, of Rcrc and RcRc genotypes bran was nearly double that of rcrc . Therefore, Wide application of the Rc gene would not only protect against PHS, but would also enhance the

production of naturally occurring antioxidants that could make a significant contribution to human health.

Description of key terms

NIL-derived population: a randomly segregated population (such as F2) developed by crossing a near-isogenic lines (NILs) with its background parent.

Hybrid seed production: for rice, the use of a fertile breeding line (male parent) to pollinate a male sterile line (female parent) to produce hybrid seeds (F1), which are harvested from the male sterile line and sold to farmers to produce hybrid rice (F2 seeds as food). In the hybrid seed production, gibberellic acid (GA) application is required to increase hybrid seed yield.

Highlights

- Compared with a NIL, a NIL-derived population can further eliminate not only the genetic background effect but also the environmental effect.
- Rc gene has a significant effect on pre-harvest sprout (PHS) resistance and Rc-controlled dormancy does not negatively affect next agricultural production cycle.
- Rc gene has a completely dominant maternal effect on PHS resistance, which confers an advantage to hybrid rice over inbred rice, and the effect fails to be counteracted by GA application in hybrid seed production.
- Rc gene retains the same milled rice qualities of brown-pericarp rice as that of white-pericarp rice and the brown-pericarp rice possesses a higher antioxidative capacity than the white-pericarp rice.
- Wide application of the Rc gene would not only protect against PHS, but would also enhance the production of naturally occurring antioxidants that could make a significant contribution to human health.

Background

Rice (*Oryza sativa* L.), a staple food crop, is widely cultivated around the world, especially in China, where ~ 30 million hectares of rice per year has been cultivated since 1991. The seeds of many rice varieties tend to germinate on the intact spike at maturity before harvest (pre-harvest sprouting (PHS)) when prolonged rainfall occurs, reducing grain yield and end-use quality (Fang et al. 2008).

Moreover, PHS tends to degrade the quality of seed for planting, resulting in severe economic losses (Hu et al. 2017).

Hybrid rice is grown in more than half of the rice planting areas of China because of its improved yield due to heterosis; however, PHS is more severe in both hybrid seed production and hybrid rice than in inbred rice (Hu et al. 2017). PHS is also reported to occur in other plants, including *Arabidopsis thaliana*, rapeseed (*Brassica napus*), barley (*Hordeum vulgare*), rye (*Loleum perenne*), wheat (*Triticum aestivum*) (Wang et al. 2018), and sorghum (*Sorghum bicolor*) (Benech-Arnold and Rodríguez 2018). PHS remains a severe agricultural problem.

Four chemical combinations of maleic hydrazide, eugenol, and uniconazole were applied to decrease PHS damage during hybrid seed production (Hu et al. 2016), and only eugenol delayed PHS (Hu et al. 2017). However, the most economically efficient method to decrease PHS is to breed PHS-resistant varieties. PHS resistance is associated with seed dormancy (Barrero et al. 2015; Gerjets et al. 2010; Torada et al. 2008). Seed dormancy is a complex adaptive trait of higher plants that allows embryos to survive extended periods of unfavorable environmental conditions (Koornneef et al. 2002). Over the course of evolution, plant seeds have evolved diverse dormancy habits in response to various climates to maintain viability (Finch-Savage and Leubner-Metzger 2006). However, strong seed dormancy negatively affects seed germinability in field cultivation (Jennings and Jesus 1964). This trait was selectively abandoned to achieve uniform germination and high germination percentage during the long history of domestication and breeding (Shu et al. 2016; Sugimoto et al. 2010). The introgression of dormancy-related genes/quantitative trait loci (QTLs) into commercial varieties remains a principal strategy for providing protection against PHS (Barrero et al. 2015).

The dormancy that seeds possess at the time of dispersal from the maternal plant is called ‘primary dormancy’ (Baskin and Baskin 2014). QTLs/genes regulating primary dormancy have been identified in rice and other crops. By 2016, over 165 QTLs associated with primary dormancy in rice had been identified (Magwa et al. 2016). Recently, (Mizuno et al. 2018) identified two QTLs, *qSDR9.1* and *qSDR9*.2, in cv. ‘Owarihatamochi’ within 4.1-Mb and 2.3-Mb intervals defined by the markers RM24039 ~ RM24260 and Indel-2 ~ RM24540, respectively, but these had relatively little effect on

PHS resistance. *Sdr4*, which promotes dormancy and inhibits germination under the control of a global regulator of seed maturation, *OsVP1*, was the first gene to be cloned and molecularly identified in rice cv. Kasalath (Sugimoto et al. 2010). *Sdr4* mRNA is expressed throughout the embryo (Sugimoto et al. 2010), indicating an embryo-imposed dormancy. *qSD1-2* from weedy rice is identical to a gibberellin-synthesis gene, *OsGA20ox2*, also known as *semidwarf1* (*sd1*), that regulates the development of endosperm-imposed dormancy (Ye et al. 2015). Gibberellic acid (GA) application accelerated the release of *qSD1-2*-controlled dormancy (Ye et al. 2015), indicating that GA application in hybrid seed production can counteract the effect of *qSD1-2* on PHS resistance.

In addition, seed dormancy has been associated with red grain color in cereal crops, an observation first reported in wheat (*Triticum aestivum* L.) (Nilsson-Ehle 1914). Red-kernel wheat is to be more resistant to PHS than white-kernel wheat; thus, red grain color has been used in wheat breeding programs as a morphological marker for PHS resistance (Flintham 2000; Himi 2002; Lin et al. 2016; Soper et al. 1989; Torada and Amano 2002; Zhou et al. 2017).

Three genes, *Tamyb10-A1* on 3A, *Tamyb10-B1* on 3B, and *Tamyb10-D1* on 3D in wheat, which encode MYB-type transcription factors involved in the flavonoid biosynthetic pathway in the testa, were identified as candidate genes that control grain color (Himi 2002; Himi et al. 2015; Himi et al. 2011; Himi and Noda 2005). Furthermore, polymerase chain reaction (PCR) markers for the *Tamyb10* genes have been developed to detect the alleles associated with red color to facilitate the breeding of PHS resistance varieties (Himi et al. 2011; Wang et al. 2014; Wang et al. 2016).

Red rice has strong seed dormancy (Cohn and Hughes 1981; Noldin et al. 2006). The *Rc* and *Rd* genes jointly regulate the production of red pericarp (Kato and Ishikawa 1921), whereas *Rc* in the absence of *Rd* regulates the production of brown pericarp (Sweeney et al. 2006). The *Rc* gene encodes a basic helix–loop–helix (bHLH) family transcription factor; the dominant red allele (*Rc*) differs from the recessive white allele (*rc*) by a 14-bp deletion within exon 6 that knocks out the bHLH domain of the protein (Sweeney et al. 2006). Genetic analysis has associated pericarp color with seed dormancy in red rice (Gu et al. 2005). The association was confirmed by the observation that *qSD7-1*, which controls seed dormancy, is identical to the *Rc* locus (Gu et al. 2011).

In this study, we aimed to (1) investigate the effect of the *Rc* gene on PHS resistance in inbred *japonica* rice and *indica* hybrid rice (the two main types of rice cultivated in China); (2) determine whether *Rc*-controlled dormancy influences the next agricultural production cycle; (3) compare the qualities of brown- and white-pericarp milled rice; and (4) explore the possibility of wide application of the *Rc* gene to provide protection against PHS.

Methods

Plant materials

Jinggangshanhongmi (JGSHM), an *indica* landrace with red pericarp grown in Jiangxi Province, China, harbors the *Rc* allele. A pair of primers (designated as the Rc-14 marker) were designed to anneal to the two flanks of the 14-bp deletion region at the *Rc* locus through an online tool (http://bioinfo.ut.ee/primer3-0.4.0/; Table 1). By Rc-14 marker-assisted selection, two sets of near-isogenic lines (NILs), $H5^{Rc}$/$H5^{rc}$ (background parent is Huaidao 5 (H5), with the *rc* allele) and $Y58S^{Rc}$/$Y58S^{rc}$ (background parent is Y58S, with the *rc* allele) were designed (Fig. 1). Their donor parents were both JGSHM with the *Rc* allele.

Table 1: Primer sequences of Rc-14 marker. * the amplified size of the *Rc* and *rc* allele is 169 bp and 155 bp, respectively.

Marker	Forward primer	Reward primer	Product size* (bp)
Rc-14	CAGGCACCACACAGAGAATG	TCTTTCAGCACATGGTTGGC	169(Rc); 155(rc)

Y58S, a thermosensitive genic male sterile (TGMS) *indica* line, has been used to produce numerous hybrid combinations (varieties) in China, but it is susceptible to PHS. During anther development, high temperatures (> 26 °C) cause Y58S to perform as a male sterile line; that is, Y58S can been used to produce hybrid seeds if the anthers develop before August in Hangzhou, whereas low temperatures (< 23 °C) convert Y58S into a partially or completely fertile line; that is, Y58S can reproduce as a ‘maintainer line’ if the anthers develop after September in Hangzhou (Deng 2005). To further ameliorate the effects of genetic background, based on the NIL, an F_2 plant population derived from a cross of $Y58S^{Rc} \times Y58S^{rc}$ (designated as the NIL-derived population) was developed and grown, one plant per hill, in the paddy field of the Zhejiang A&F University in Hangzhou, China, in 2016. In the NIL-derived population, ~ 200 sterile individual plants were selected and their genotypes at the *Rc* locus were determined using the Rc-14 marker. The homozygous sterile individual plants—brown-

pericarp *RcRc* and white-pericarp *rcrc*—were reproduced as maintainer lines through ratoons, and the seeds were harvested to be used as the female parent to produce hybrid seed for the next year. To eliminate the environmental effect, the brown-pericarp (*RcRc*) and white-pericarp (*rcrc*) homozygous seeds were thoroughly mixed to create a mixed female parent that was planted at a density of 25 cm × 15 cm in the same location in 2017. On August 15, when the plants were blooming, 0.075 L of 0.3 g/L aqueous gibberellic acid (GA3, Shanghai Tongrui Biotech Co., LTD) per square meter was applied. The following day, a male parent (*indica* breeding line LF183 (*rcrc*), with the same heading date as Y58S) was transplanted to a plot of the mixed female parent plants to induce production of hybrid seed by artificial supplementary pollination for only 1 day, followed by removal of the male parent plants. Approximately 120 panicles were randomly sampled every 5 days (on the 15th, 20th, 25th, 30th, and 35th days after pollination (DAPs)), and the panicles were evaluated for PHS resistance immediately after each harvest. On the 36th DAP, the rest of the panicles were harvested, threshed, and divided into two parts: one was stored under ambient conditions for 12 weeks to estimate the seed after-ripening effect; the other was used to produce hybrid rice (F_2 seeds as food) in the same location in 2018. Approximately 300 initially heading panicles (1 panicle per individual plant) were tagged on the heading date (August 21). Approximately 60 tagged panicles were randomly sampled every 5 days (on the 20th, 25th, 30th, 35th, and 40th days after heading (DAHs)) and evaluated for PHS resistance immediately after each harvest.

H5, an inbred *japonica* rice, is one of the most widely grown varieties in the middle and lower reaches of the Yangtze River in China, but it is susceptible to PHS. To ameliorate the effects of genetic background and environment, an F_2 plant population derived from a cross of $H5^{Rc} \times H5^{rc}$ (designated as the NIL-derived population) was developed and grown (one plant per hill) in the same location in 2018. In the NIL-derived population, ~600 initially heading panicles (one panicle per individual plant) were tagged on the heading date (August 17). From these panicles, ~60 tagged panicles, along with the corresponding flag leaves, were randomly sampled every 7 days (on the 21st, 28th, 35th, 42nd, and 49th DAHs) to extract DNA for determining the genotype at the *Rc* locus and to evaluate the

extent of PHS immediately after each harvest. On the 50th DAH, the rest of the tagged panicles, along with flag leaves, were harvested, threshed, classified into three genotypes (*RcRc*, *Rcrc*, and *rcrc*), and assessed for grain quality and seed after-ripening effect.

Genotyping of the *Rc* locus

Fresh leaves were used to extract genomic DNA samples for *Rc* genotyping. Genomic DNA was extracted by following standard methods. PCR was performed with initial denaturation at 99 °C for 3 min; followed by 30 cycles of amplification, with each cycle consisting of treatment at 95 °C for 30 s, followed by 55 °C for 45 s, and then 72 °C for 30 s; with a final extension at 72 °C for 8 min. PCR products were electrophoresed on an 8% polyacrylamide gel to determine the genotype at the *Rc* locus.

Evaluation of PHS resistance

PHS resistance was evaluated by measuring sprouting percentage (SP). The seed was considered to be sprouting when a visible protrusion of the radicle or coleoptile from the hull appeared. To ensure randomness of the experiment, it was conducted as a double-blind trial: the samples' genotypes were not determined before evaluation of PHS. PHS was evaluated under simulated environmental conditions (for October in Hangzhou). The freshly harvested, intact panicles were soaked in deionized water for 24 h at room temperature (25 °C) and then placed in an incubator set to a diurnal cycle of 12 h light, 28 °C/12 h dark, 20 °C for 7 days.

SP = number of sprouted seeds/total number of sampled seeds × 100%

To release *Rc*-controlled dormancy and estimate after-ripening effect

Freshly harvested seeds were air-dried to a moisture content of ~ 13.5% (as control) and then stored under one of two conditions: con1, the air-dried seeds were stored under simulated conditions in Southeast Asia (25 °C, 65% relative humidity (RH)) for 4 weeks; and con2, the air-dried seeds were stored under ambient conditions in Hangzhou for 12 weeks (from November 2018 to February 2019). The after-ripening effect was evaluated by measuring germination percentage (GP). A seed was considered to be germinating when the radicle reached the length of the seed and the coleoptile reached half the seed length. Six hundred seeds of each genotype (*RcRc*, *Rcrc*, and *rcrc*) were distributed equally into six germination boxes (12 cm × 12 cm × 6 cm, one genotype per box)

containing three layers of moistened filter paper, which were placed in an incubator set to a diurnal cycle of 14 h light, 30 °C/10 h dark, 20 °C for 7 days.

GP = number of germinated seeds/total number of sampled seeds × 100%

Evaluation of milled-rice qualities

Freshly harvested seeds were air-dried to a moisture content of ~ 13.5%, and stored under ambient condition in Hangzhou for 12 weeks to stabilize the physical and chemical characteristics for estimating grain qualities (Pang et al. 2018). The seeds were dehusked on a Satake Rice Machine (Satake Co., Tokyo, Japan) to obtain wholegrain rice. An equally weighted wholegrain (10 g) was milled on a Satake mill (Satake Corp., Tokyo, Japan) for the same time (60 s) every time. The milled rice was ground on a Cyclone sample mill (UDY Corporation, Fort Collins, CO). The flour was passed through an 80-mesh sieve for the following grain quality test, and the bran was passed through a 60-mesh sieve for antioxidative capacity assay.

Determination of total amylose content (TAC) and hot-water-insoluble amylose content (WIAC)

TAC was determined by method of BS EN ISO 6647-2-2015. The WIAC was given by subtracting the hot-water-soluble amylose content (WSAC) from TAC (Shanthy et al. 1980). The WSAC was measured by method of BS EN ISO 6647-2-2015, except for extraction of hot-water-soluble amylose as follows. Accurately weigh 100.00 mg milled rice flour sample into 50 ml centrifuge tube, pipette 1 ml of 95% ethanol, wash down any of test portion adhering to the tube side, shake slightly to wet all the sample, and then add 25 ml of distilled water instead of dilute alkali as solvent, vortex, incubate at 100 °C (in boiling water) for 45 min, shake slightly every 5 min. Cool to room temperature and centrifuge at 5000 × g for 10 min. Transfer the supernatant to a 100 ml volumetric flask, followed by the same process as determination of TAC. Experiments were done in sextuplicate.

Determination of gel consistency (GC)

GC was evaluated as described by (Cagampang et al. 1973) and China National Standard GB/T 22294 − 2008 based on the consistency of a cold 4.4% milled-rice paste in 0.20 N KOH. Briefly, 100.00 mg of milled-rice flour was accurately weighed into 13 × 150 mm glass test tubes, wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) thymol blue, followed by adding 2 ml of 0.2 N KOH, and mixed

vigorously. The tubes were covered with glass marbles, cooked in vigorously boiling water for 8 min, cooled to room temperature, and kept in an ice-water mixture for 20 min. Finally, the tubes were laid horizontally on a ruled graphing paper at 25 °C. Gel length was measured after 1 h. Sextuplicate measurements were performed for each sample.

Determination of alkali spreading value (ASV)

ASV was determined by incubating six milled grains in 10 ml of 1.7% KOH at 28 °C for 23 h with three replicates (Bhattacharya and Sowbhagya 1972; Mariotti et al. 2010; Peng and Li 2018). The degree of spreading was rated using the following 7-point semi-quantitative criteria: (1) grain not affected; (2) grain swollen; (3) grain swollen, collar incomplete and narrow; (4) grain swollen, collar complete and wide; (5) grain split, collar complete and wide; (6) grain dispersed, merging with collar; (7) grain completely dispersed and intermingled.

Determination of paste viscosity properties

Paste viscosity properties of the samples were determined on Rapid Viscosity Analyzer (Newport Scientific model 3D) following the approved method 61 − 02 (AACC 2000), and derived 7 characteristic parameters (CPs): peak, trough, final viscosity (FinalVisc), breakdown, setback, initial gelatinization temperature (PastingTemp), and peaktime. Their definitions refer to (Champagne et al. 2004; Champagne et al. 1999).

Determination of crude protein content (CPC)

CPC was measured by Kjeldahl method on Kjeltec 2300 Autoanalyser (Foss AB, Sweden) in sextuplicate according to (Wu et al. 2009a). A factor of 5.95 was used for converting nitrogen content to protein content.

Determination of crude fat content (CFC)

CFC is determined by Soxhlet extraction method on FOSS Tecator Soxtec 2050 according to (Wu et al. 2009b).

Extraction of bran pigments and its antioxidantive capacity assay

Accurately weighed 1.000 g bran was placed in 10 ml of 70% aqueous ethanol by shaking overnight at 37 °C under dark condition, and then centrifuged at 5000 × g for 15 min at room temperature (Nam et al. 2006). The supernatant was collected and the extraction was repeated one time with 5 ml 70% aqueous ethanol. Both supernatants were pooled. The solvent was then removed from the extract by

rotary evaporation at room temperature. The dried extracts were dissolved in 5 ml of 50% methanol (Pang et al. 2018).

Antioxidantive capacity of the extracts was evaluated by 2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging activity assay (Brand-Williams et al. 1995; Pang et al. 2018) with some modification. In brief, 100 μmol/L of DPPH• solution was prepared in methanol. The 3 ml of the DPPH• solution was mixed with 100 μL of the sample. The mixture was kept at room temperature for 30 min in the dark, and then the absorbance at 517 nm was measured with a spectrophotometer (UV2550, Shimadzu, Japan). All assays were performed in sextuplicate. The DPPH• inhibition percentage (DIP) of the samples was calculated as an indicator of DPPH• scavenging activity: $DIP=(A_0-(A_2-A_1))/A_0 \times 100\%$. Where A_0 is the absorbance of DPPH• solution, A_1 is the absorbance of the sample in solvent, and A_2 is the absorbance of the mixture of the sample and DPPH• solution.

Data analysis

Data of SP and GP was analyzed by chi-square test. Data of grain qualities and DIP was analyzed by analysis of variance (ANOVA). The differences between genotypic mean were separated using the least significance difference (LSD) test. Data analysis was performed using SPSS11.5 program at the 0.01 probability level.

Results

Verification of the inheritance pattern of rice pericarp color

The *Rc*-controlled pigments accumulate in the lower-epidermal cell layer of the pericarp, which is maternal tissue (Gu et al. 2011), suggesting that the pericarp color is dependent on the maternal genotype (Sweeney et al. 2006). To verify the inheritance pattern of rice pericarp color, we conducted a forward cross and reverse cross between $H5^{Rc}$ (brown pericarp) and $H5^{rc}$ (white pericarp) (Fig. 2). If the mother plant had a white pericarp (*rcrc*), all the F_1 seeds produced white pericarp; if the mother plant had a brown pericarp (*RcRc*), all the F_1 seeds produced brown pericarp. Thus, pericarp color of the F_1 seeds is dependent on the mother's genotype rather than on the genotype of the F_1 seeds. All of the F_2 seeds that the F_1 plants (*Rcrc*) produced were brown, showing no genetic segregation, irrespective of whether the female parent's pericarp was white or brown, indicating that the pericarp

color of the F_2 seeds was dependent on the maternal (F_1 plant) genotype rather than on that of the F_2 seeds. The F_2 plants produced two types of F_3 seeds: brown and white, showing that the pericarp color of the F_3 seeds was dependent on the maternal (F_2 plants) genotype rather than that of the F_3 seeds. Therefore, *Rc* has a maternal effect on pericarp color.

An investigation of 576 F_2 plants (that produced two types of F_3 seeds: brown and white) showed that the brown:white segregation ratio of 437:139 ($\approx 3.14{:}1$) corresponded to the 3:1 Mendelian expectation ($\chi^2 = 0.1875$; $P > 0.833$), further confirming that pericarp color is controlled by a single completely dominant gene, *Rc*.

These observations indicate that *Rc* has a completely dominant maternal effect on pericarp color; thus, we speculate that *Rc* has a completely dominant maternal effect on PHS resistance.

The PHS resistance effect of *Rc* and release of*Rc*-controlled dormancy in *japonica* rice

To further ameliorate the effects of genetic background and environment, we developed a NIL-derived population. From this population, we randomly sampled the tagged panicles and evaluated PHS resistance immediately after each harvest. To ensure random sampling, we did not detect sample genotype before evaluating PHS resistance for each harvest. During the seed maturation period (from 21–49 DAH), the SPs of seeds produced by maternal plants of the RcRc, Rcrc, and rcrc genotypes all showed an increasing trend, with ranges of 25.8%–74.3%, 0–26.9%, and 0–23.8%, respectively (Fig. 3a). A Chi-square test showed that the SPs of seeds from maternal plants of the RcRc and Rcrc genotypes were both significantly lower than that of the rcrc genotype at each DAH by an average of ~43%, peaking at 55.0% (SP value of rcrc minus that of the RcRc genotype) and 53.3% (SP value of rcrc minus that of the Rcrc genotype), respectively, at 42 DAHs. By contrast, there was no significant difference between the Rcrc and RcRc genotypes at any DAH, indicating that Rc had a completely dominant effect over rc on PHS resistance and that the seeds from maternal plants of the Rcrc genotype showed no genetic segregation in PHS resistance. Therefore, Rc has a completely dominant maternal effect on PHS resistance. As Rc-controlled dormancy may reduce the germination percentage (GP) of seeds in the next agricultural production cycle, causing reductions in quality of

seeds for planting, we thus conducted dormancy-releasing experiments (Fig. 3b). In the control experiment, freshly harvested seeds were air-dried to a moisture content of ~13.5%. The GPs of seeds from maternal plants of the RcRc and Rcrc genotypes were significantly (~50%) lower than that of seeds from maternal plants of the rcrc genotype, further verifying that the Rc gene controls dormancy. There was no significant difference in GP between the Rcrc and RcRc genotypes, indicating that seeds from Rcrc plants showed no genetic segregation in dormancy.

For the con1 (treatment at 25 °C with 65% RH for 4 weeks) and the con2 (storage under ambient conditions in Hangzhou for 12 weeks from November 2018 to February 2019), the GPs of seeds from maternal plants of the *RcRc*, *Rcrc*, and *rcrc* genotypes were all greater than ~ 93%, with no significant difference, indicating that *RcRc* and *Rcrc* genotypes seeds can completely release dormancy within 12 weeks by after-ripening. Thus, releasing the dormancy retains the quality of the seed for planting, and *Rc*-controlled dormancy does not negatively affect the next agricultural production cycle. This is indirectly supported by the observation that red rice is cultivated widely around the world.

Effect of *Rc* on PHS resistance and release of *Rc*-controlled dormancy in *indica* hybrid rice

According to our results obtained in inbred *japonica* rice, all F_1 seeds that are produced with brown-pericarp rice as the female parent are brown, i.e., resistant to PHS; all F_2 seeds that F_1 plants (*Rcrc*) produced are brown, i.e., resistant to PHS, with no genetic segregation, which confers an advantage to hybrid rice. Thus, we investigated further the effect of the *Rc* gene on PHS resistance and the release of *Rc*-controlled dormancy in *indica* hybrid rice.

In our hybrid seed (F_1) production field where GA was applied, we randomly sampled tagged panicles (containing two types, brown- and white-pericarp rice) and evaluated PHS resistance for each harvest. During the seed maturation period (15–35 DAP), the SPs of brown and white hybrid seed both showed an increasing trend, ranging from 50.8–84.2% and 0–0.3%, respectively (Fig. 4a). A Chi-square test showed that the SP of brown hybrid seed was significantly lower than that of white hybrid seed at each DAP by an average of ~ 50%, peaking at 58.5% (the SP value of white hybrid seed minus that of brown hybrid seed) at 20 DAP. This result confirmed that GA application during hybrid seed

production failed to counteract the effect of the *Rc* gene on PHS resistance (Gu et al. 2011).

The freshly harvested seeds were air-dried to a moisture content of ~ 13.5% and then stored under ambient conditions for 12 weeks in Hangzhou from November 2018 to February 2019. Seedlings established from brown and white hybrid seed showed no visible difference (Fig. 4b), further confirming that *Rc*-controlled dormancy does not negatively affect the next agricultural production cycle.

In our hybrid rice production (F_2 seeds as food) field, we randomly sampled tagged panicles (containing two types, brown and white) and evaluated PHS resistance for each harvest. During the seed maturation period (20–40 DAHs), the SPs of brown and white hybrid rice both showed an increasing trend, ranging from 35.4–78.3% and 0–27.4%, respectively (Fig. 4c). A Chi-square test showed that the SP of brown hybrid rice was significantly lower than that of white hybrid rice by an average of ~ 48%, peaking at 59.1% (the SP value of white hybrid rice minus that of brown hybrid rice) at 30 DAHs. Therefore, the *Rc* gene has a significant effect on PHS resistance in *indica* hybrid rice.

Differences in milled rice qualities between the *RcRc*, *Rcrc*, and *rcrc* genotypes

Rice is generally consumed in the form of milled rice, from which the bran (seed coat and aleurone layer) has been removed. There is no visible difference between the appearances of milled rice of the *RcRc*, *Rcrc*, and *rcrc* genotypes (Fig. 5a), consistent with a report by (Gu et al. 2011) showing that the pigments accumulate in the lower epidermal cell layer of the pericarp tissue.

Starch, composed of amylose and amylopectin, is the major constituent of milled rice and accounts for 80–90% of its dry weight (Butardo et al. 2019). The cooking and eating quality of rice is routinely estimated, primarily on the basis of starch properties, using three main physicochemical tests: total amylose content (TAC), gel consistency (GC), and alkali spreading value (ASV) (Butardo et al. 2019). The results of our study showed no significant difference between the *RcRc*, *Rcrc*, and *rcrc* genotypes in TAC (Fig. 5b) or GC (Fig. 5d). The ASVs of the *RcRc*, *Rcrc*, and *rcrc* genotypes were all 7, meaning that all samples were completely dispersed and intermingled with no differences. The hot-water-insoluble amylose content (WIAC) differs markedly among different varieties with TAC in the same

range (Bhattacharya et al. 1978). WIAC correlates more strongly with textural quality than does TAC (Bhattacharya 2009). Therefore, we determined the WIACs of the *RcRc*, *Rcrc*, and *rcrc* genotypes. There was no significant difference in WIAC values between the genotypes (Fig. 5c). Thus, the starch properties, as quantified by TAC, WIAC, GC, and ASV, did not significantly differ between the *RcRc*, *Rcrc*, and *rcrc* genotypes.

The rapid viscosity analyzer (RVA) profile of rice flour is an important predictor of rice cooking, eating, and processing quality characteristics (Champagne et al. 2004; Champagne et al. 1999). The viscosity curves are the most useful tool available for assessing cooking and eating quality of rice, with the results associated with sensory or processing attributes (Butardo et al. 2019). The viscosity curves can derive seven characteristic parameters (CPs): peak, trough, final viscosity (FinalVisc), breakdown, setback, initial gelatinization temperature (PastingTemp), and peaktime (Champagne et al. 2004; Champagne et al. 1999). We conducted an RVA profile analysis of the *RcRc*, *Rcrc*, and *rcrc* genotypes in triplicate; viscosity curves expressed as the mean of three replicates showed no obvious difference among the genotypes (Fig. 5e). Further analysis showed that the seven CPs derived from the viscosity curves also had no significant difference among the genotypes (Table 2).

CPs	Genotypes	Mean	SD	95% CI	
				Upper	Lower
Peak (cP)	*RcRc*	3789.00 A*	140.82	3439.18	4138.82
	Rcrc	3671.67 A	251.96	3045.75	4297.58
	rcrc	3741.00 A	49.11	3619.00	3863.00
Trough (cP)	*RcRc*	2232.33 A	113.46	1950.49	2514.17
	Rcrc	2224.67 A	67.17	2057.80	2391.54
	rcrc	2276.67 A	96.26	2037.54	2515.79
Breakdown (cP)	*RcRc*	1556.67 A	47.93	1437.60	1675.73
	Rcrc	1447.00 A	213.70	916.14	1977.86
	rcrc	1464.33 A	120.56	1164.85	1763.82
FinalVisc (cP)	*RcRc*	3770.00 A	110.18	3496.30	4043.70
	Rcrc	3722.00 A	53.86	3588.20	3855.80
	rcrc	3772.00 A	75.15	3585.33	3958.67
Setback (cP)	*RcRc*	-19.00 A	45.43	-131.86	93.86
	Rcrc	50.33 A	222.27	-501.81	602.47
	rcrc	31.00 A	102.80	-224.36	286.36
PeakTime (min)	*RcRc*	5.78 A	0.04	5.68	5.88
	Rcrc	5.87 A	0.14	5.53	6.20
	rcrc	5.84 A	0.15	5.47	6.22
PastingTemp (°C)	*RcRc*	86.55 A	1.00	84.07	89.03
	Rcrc	86.55 A	0.98	84.11	88.99
	rcrc	87.23 A	0.06	87.09	87.38

Table 2: Summary of 7 characteristic parameters (CPs) derived from the viscosity curves, including the genotypic mean and its standard deviation (SD), and 95% credible intervals (CI). * The same characters (A and A) mean that their genotypic means have no significant difference at the $P > 0.1$.

Definition of CPs refers to Champagne et al. (1999; 2004).

Aside from starch, the main macronutrients in rice are storage proteins and crude fat. The former, accounting for 6–8% of milled rice on a dry-weight basis, is a major source of food protein (Shih 2004) and plays an important role in nutritional quality, as well as in textural and sensory traits (Butardo and Sreenivasulu 2016). The latter, accounting for < 1% of milled rice on a dry-weight basis, plays a minor role in influencing pasting properties (Godber and Juliano 2004). We detected no significant difference in the crude protein content (CPC) (Fig. 5f) or crude fat content (CFC) (Fig. 5g) between the *RcRc*, *Rcrc*, and *rcrc* genotypes.

In conclusion, we detected no significant differences in milled rice qualities, including TAC, WIAC, GC, ASV, RVA profile properties, CPC, and CFC, between the *RcRc*, *Rcrc*, and *rcrc* genotypes. Thus, the *Rc* gene retains the same milled rice qualities in brown-pericarp rice as those in white-pericarp rice, indicating that the pigments accumulated in the pericarp do not negatively affect milled rice quality.

Differences between the *RcRc*, *Rcrc*, and *rcrc* genotypes in the antioxidative capacity of the bran

Red rice possesses a powerful antioxidative capacity that is derived mainly from its proanthocyanidins. Proanthocyanidin synthesis involves the *Rc* and *Rd* genes in red rice (*RcRd*); thus, proanthocyanidins are not detectable in brown-pericarp rice (*Rcrd*), which lack the *Rd* allele (Furukawa et al. 2006). We determined the antioxidative capacity of the *RcRc*, *Rcrc*, and *rcrc* genotypes using a 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay (Fig. 5h). The DPPH• inhibition percentage (DIP) of the samples was calculated as an indicator of antioxidative capacity. DIPs of *RcRc* and *Rcrc* rice were significantly higher (nearly double) than that of *rcrc* rice, whereas there was no significant difference in DIP between *Rcrc* and *RcRc* rice. Although proanthocyanidins were not detected in the brown-pericarp rice (Furukawa et al. 2006), the brown-pericarp rice possessed a higher antioxidative capacity than white-pericarp rice.

Discussions

The advantages and limitations of NILs

NILs are a pair of lines with an identical genetic background, except for at the target locus; thus, NILs are an ideal tool for evaluating phenotypic effects attributable to a particular locus because any

differences among the lines will result from the target locus (Wang et al. 2018). The development of NILs for a QTL/gene facilitates the exact characterization of the QTL/gene. However, the accuracy of the characterization depends on the isogenicity of the NIL to the background parent. In this study, we generated two sets of NIL-derived populations by crossing the NILs with their respective background parents (Fig. 1). In the NIL-derived population, the objective traits are randomly segregated genetically, and all individuals are randomly distributed in the field plot. So, compared with a NIL, a NIL-derived population can further eliminate not only the genetic background effect but also the environmental effect.

Red rice: not a noxious weed but a perfect crop

Pigmented rice is cultivated in some regions of the world. There are two main types of pigmented rice: red rice and black rice, with red and black pericarps, respectively. Almost all wild rice is red rice. Natural selection favored red rice not because of pericarp color *per se* but probably because of its seed dormancy, which enhances survival of seeds and distributes germination over time (Gu et al. 2011). By contrast, domestication and breeding favored white-pericarp rice, probably because the strong seed dormancy of red rice negatively affects seed germinability in field cultivation (Jennings and Jesus 1964). In this study, we showed that *Rc*-controlled dormancy can be released by storage under ambient conditions for 12 weeks or storage at 25 °C with 65% RH (similar to the climate in Southeast Asia) for 4 weeks, and that releasing the dormancy retains the quality of the seed for planting, meaning that the dormancy does not negatively affect the next agricultural production cycle.

Further research showed that there was no difference in the milled rice qualities between brown-pericarp rice and white-pericarp rice. Black rice has the same grain qualities as white-pericarp rice (Maeda et al. 2014). Thus, the pigments accumulated in the pericarp do not negatively affect the milled rice qualities.

Why do farmers prefer white-pericarp rice to red rice? Farmers grow red rice in some regions of the world, especially in China, for its ceremonial or medicinal value but lack awareness of its other advantages. In this study, we confirmed that the *Rc* gene has a dominant maternal effect that confers

an advantage to hybrid rice: hybrid seeds (F_1) that are produced with red- or brown-pericarp rice as the female parent are red or brown, and therefore resistant to PHS, and all hybrid rice (F_2 seeds as food) that is produced with the hybrid seed is also red or brown and also resistant to PHS, with no genetic segregation.

Moreover, GA application is required for hybrid seed production to increase hybrid seed yield, but it tends to cause more serious PHS than occurs in rice not treated with GA. Further field trials confirmed that the effect of the *Rc* gene on PHS resistance failed to be counteracted by GA application in hybrid seed production.

Rice is consumed mostly in the form of milled rice. Red- or brown-pericarp rice can be milled to produce rice that has the same qualities as that of white-pericarp rice, and the bran (red or brown pigment) is a rich source of bioactive compounds. Brown-pericarp rice, with no detectable proanthocyanidins (Furukawa et al. 2006), possesses a higher antioxidative capacity than white-pericarp rice. In red rice, proanthocyanidins are one of the main types of bioactive compounds; they are powerful antioxidants that provide numerous human health benefits upon consumption (Deng et al. 2013). Proanthocyanidins have been shown to exhibit anti-inflammatory activities (Ahmad et al. 2013; Chen et al. 2016; Lyu and Park 2005; Monagas et al. 2010; Ronchetti et al. 2009), to inhibit cancer cell invasion (Pintha et al. 2015; Pintha et al. 2014), and to reduce lipid accumulation in adipocytes, resulting in reduced obesity (Visioli 2016).

Rice is consumed by more than half of the world’s population. Wide application of the *Rc* gene would not only protect against PHS, but would also provide naturally occurring antioxidants that could make a significant contribution to human health.

Conclusions

This study highlights the definite advantages of the *Rc* gene over other dormancy-associated genes in protecting against rice PHS. *Rc* has a significant effect on PHS resistance and releasing *Rc*-controlled dormancy retains seed quality and does not negatively affect the next agricultural production cycle. Importantly, *Rc* has a completely dominant maternal effect on PHS resistance, which confers an advantage to hybrid rice over inbred rice (hybrid seeds that are produced with red- or brown-pericarp

rice as the female parent are red or brown, and therefore resistant to PHS, and all hybrid rice that is produced with the hybrid seeds is also red or brown and also resistant to PHS, with no genetic segregation), the effect failed to be counteracted by GA application in hybrid seed production. Moreover, *Rc* retains the same milled rice qualities of brown-pericarp rice as that of white-pericarp rice and brown-pericarp rice possesses a higher antioxidative capacity than white-pericarp rice. Therefore, wide application of the *Rc* gene to protect against PHS would retain seed and milled rice quality and enhance the production of naturally occurring antioxidants that could make a significant contribution to human health.

Declarations

Acknowledgement

We would like to thank China-IRRI Joint Research Center on Rice Quality and Nutrition, China National Rice Research Institute, for help with evaluating grain qualities.

Authors' contributions

HW and MW conceived and designed the experiments. MW, SX, YF, and YC performed the experiments. MW analyzed the data. MW and SX wrote original draft. HW and MW wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by key research and development program of Zhejiang province (grant #2016C02050-2).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Author details

College of Agriculture and Food Sciences, Zhejiang A&F University, Hangzhou 311300, China.

References

1. Ahmad SF, Zoheir KMA, Abdel-Hamied HE, Ashour AE, Bakheet SA, Attia SM, Abd-Allah ARA (2013) Grape seed proanthocyanidin extract has potent anti-arthritic effects on collagen-induced arthritis by modifying the T cell balance. International Immunopharmacology 17:79-87
2. Barrero JM, Cavanagh C, Verbyla KL, Tibbits JFG, Verbyla AP, Huang BE, Rosewarne GM, Stephen S, Wang P, Whan A, Rigault P, Hayden MJ, Gubler F (2015) Transcriptomic analysis of wheat near-isogenic lines identifies PM19-A1 and A2 as candidates for a major dormancy QTL. Genome Biology 16
3. Baskin CC, Baskin JM (2014) Types of Seeds and Kinds of Seed Dormancy:37-77
4. Benech-Arnold RL, Rodríguez MV (2018) Pre-harvest Sprouting and Grain Dormancy in Sorghum bicolor: What Have We Learned? Frontiers in Plant Science 9
5. Bhattacharya (2009) Physicochemical Basis of Eating Quality of Rice. Cereal Foods World
6. Bhattacharya KR, Sowbhagya CM (1972) An improved alkali reaction test for rice quality. International Journal of Food Science & Technology 7:323-331
7. Bhattacharya KR, Sowbhagya CM, Indudhara Swamy YM (1978) Importance of insoluble amylose as a determinant of rice quality. Journal of the Science of Food and Agriculture 29:359-364
8. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28:25-30
9. Butardo VM, Sreenivasulu N (2016) Tailoring Grain Storage Reserves for a Healthier Rice Diet and its Comparative Status with Other Cereals 323:31-70
10. Butardo VM, Sreenivasulu N, Juliano BO (2019) Improving Rice Grain Quality: State-of-the-Art and Future Prospects 1892:19-55
11. Cagampang GB, Perez CM, Juliano BO (1973) A gel consistency test for eating quality

of rice. Journal of the Science of Food and Agriculture 24:1589-1594

12. Champagne ET, Bett-Garber KL, McClung AM, Bergman C (2004) Sensory Characteristics of Diverse Rice Cultivars as Influenced by Genetic and Environmental Factors. Cereal Chemistry Journal 81:237-243

13. Champagne ET, Bett KL, Vinyard BT, McClung AM, Barton FE, Moldenhauer K, Linscombe S, McKenzie K (1999) Correlation Between Cooked Rice Texture and Rapid Visco Analyser Measurements. Cereal Chemistry Journal 76:764-771

14. Chen M-H, McClung AM, Bergman CJ (2016) Concentrations of oligomers and polymers of proanthocyanidins in red and purple rice bran and their relationships to total phenolics, flavonoids, antioxidant capacity and whole grain color. Food Chemistry 208:279-287

15. Cohn MA, Hughes JA (1981) Seed Dormancy in Red Rice (Oryza sativa) I. Effect of Temperature on Dry-Afterripening. Weed Science 29:402-404

16. Deng G-F, Xu X-R, Zhang Y, Li D, Gan R-Y, Li H-B (2013) Phenolic Compounds and Bioactivities of Pigmented Rice. Critical Reviews in Food Science and Nutrition 53:296-306

17. Deng Q (2005) Breeding of the PTGMS Line, Y58S, with Wide Adaptability in Rice. Hybrid Rice 20:15-18

18. Fang J, Chai C, Qian Q, Li C, Tang J, Sun L, Huang Z, Guo X, Sun C, Liu M, Zhang Y, Lu Q, Wang Y, Lu C, Han B, Chen F, Cheng Z, Chu C (2008) Mutations of genes in synthesis of the carotenoid precursors of ABA lead to pre-harvest sprouting and photo-oxidation in rice. The Plant Journal 54:177-189

19. Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytologist 171:501-523

20. Flintham JE (2000) Different genetic components control coat-imposed and embryo-

imposeddormancy in wheat. Seed Science Research 10:43-50

21. Furukawa T, Maekawa M, Oki T, Suda I, Iida S, Shimada H, Takamure I, Kadowaki K-i (2006) The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. The Plant Journal 49:91-102

22. Gerjets T, Scholefield D, Foulkes MJ, Lenton JR, Holdsworth MJ (2010) An analysis of dormancy, ABA responsiveness, after-ripening and pre-harvest sprouting in hexaploid wheat (Triticum aestivum L.) caryopses. Journal of Experimental Botany 61:597-607

23. Godber JS, Juliano BO (2004) Rice lipids. In: Champagne ET(ed) Rice: chemistry and technology, 3rd edn. American Association of Cereal Chemistry, St Paul, MN:163-190

24. Gu X-Y, Foley ME, Horvath DP, Anderson JV, Feng J, Zhang L, Mowry CR, Ye H, Suttle JC, Kadowaki K-i, Chen Z (2011) Association Between Seed Dormancy and Pericarp Color Is Controlled by a Pleiotropic Gene That Regulates Abscisic Acid and Flavonoid Synthesis in Weedy Red Rice. Genetics 189:1515-1524

25. Gu X-Y, Kianian SF, Hareland GA, Hoffer BL, Foley ME (2005) Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (Oryza sativa). Theoretical and Applied Genetics 110:1108-1118

26. Himi E (2002) Effect of grain colour gene (R) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. Journal of Experimental Botany 53:1569-1574

27. Himi E, Maekawa M, Matsuura T, Taketa S (2015) Real-time PCR-mediated diagnosis of hemizygosity at the Tamyb10-D1 locus controlling grain color in wheat. Molecular Breeding 35

28. Himi E, Maekawa M, Miura H, Noda K (2011) Development of PCR markers for Tamyb10 related to R-1, red grain color gene in wheat. Theoretical and Applied Genetics 122:1561-1576

29. Himi E, Noda K (2005) Red grain colour gene (R) of wheat is a Myb-type transcription

factor. Euphytica 143:239-242

30. Hu Q, Fu Y, Guan Y, Lin C, Cao D, Hu W, Sheteiwy M, Hu J (2016) Inhibitory effect of chemical combinations on seed germination and pre-harvest sprouting in hybrid rice. Plant Growth Regulation 80:281-289

31. Hu Q, Lin C, Guan Y, Sheteiwy MS, Hu W, Hu J (2017) Inhibitory effect of eugenol on seed germination and pre-harvest sprouting of hybrid rice (Oryza sativa L.). Scientific Reports 7

32. Jennings PR, Jesus J (1964) Effect of Heat on Breaking Seed Dormancy in Rice. Crop Science 4:530-533

33. Kato H, Ishikawa J (1921) On the inheritance of pigment of rice. Jap J Genet (Supp. 1):1-7

34. Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Current Opinion in Plant Biology 5:33-36

35. Lin M, Zhang D, Liu S, Zhang G, Yu J, Fritz AK, Bai G (2016) Genome-wide association analysis on pre-harvest sprouting resistance and grain color in U.S. winter wheat. BMC Genomics 17

36. Lyu S-Y, Park W-B (2005) Production of Cytokine and NO by RAW 264.7 Macrophages and PBMC In Vitro Incubation with Flavonoids. Archives of Pharmacal Research 28:573-581

37. Maeda H, Yamaguchi T, Omoteno M, Takarada T, Fujita K, Murata K, Iyama Y, Kojima Y, Morikawa M, Ozaki H, Mukaino N, Kidani Y, Ebitani T (2014) Genetic dissection of black grain rice by the development of a near isogenic line. Breeding Science 64:134-141

38. Magwa RA, Zhao H, Xing Y (2016) Genome-wide association mapping revealed a diverse genetic basis of seed dormancy across subpopulations in rice (Oryza sativa

L.). BMC Genetics 17

39. Mariotti M, Fongaro L, Catenacci F (2010) Alkali spreading value and Image Analysis. Journal of Cereal Science 52:227-235

40. Mizuno Y, Yamanouchi U, Hoshino T, Nonoue Y, Nagata K, Fukuoka S, Ando T, Yano M, Sugimoto K (2018) Genetic dissection of pre-harvest sprouting resistance in an upland rice cultivar. Breeding Science 68:200-209

41. Monagas M, Urpi-Sarda M, Sánchez-Patán F, Llorach R, Garrido I, Gómez-Cordovés C, Andres-Lacueva C, Bartolomé B (2010) Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. Food & Function 1:233

42. Nam SH, Choi SP, Kang MY, Koh HJ, Kozukue N, Friedman M (2006) Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chemistry 94:613-620

43. Nilsson-Ehle H (1914) Zur Kenntnis der mit Keimungsphysiologie des Weizens in Zusammenhang stehenden innerern Faktoren. Z Pflanzenzucht 2:5

44. Noldin JA, Chandler JM, McCauley GN (2006) Seed longevity of red rice ecotypes buried in soil. Planta Daninha 24:611-620

45. Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y, Bao J (2018) Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chemistry 240:212-221

46. Peng B, Li Y (2018) Determination of Gelatinization Temperature of Rice Starch (Alkaline Digestion). Bio-protocol:e1010166

47. Pintha K, Yodkeeree S, Limtrakul P (2015) Proanthocyanidin in Red Rice Inhibits MDA-MB-231 Breast Cancer Cell Invasion via the Expression Control of Invasive Proteins. Biological & Pharmaceutical Bulletin 38:571-581

48. Pintha K, Yodkeeree S, Pitchakarn P, Limtrakul P (2014) Anti-invasive Activity against Cancer Cells of Phytochemicals in Red Jasmine Rice (Oryza sativa L.). Asian Pacific Journal of Cancer Prevention 15:4601-4607

49. Ronchetti D, Borghi V, Gaitan G, Herrero JF, Impagnatiello F (2009) NCX 2057, a novel NO-releasing derivative of ferulic acid, suppresses inflammatory and nociceptive responses in in vitro and in vivo models. British Journal of Pharmacology 158:569-579

50. Shanthy AP, Sowbhagya CM, Bhattacharya KR (1980) Simplified Determination of Water-Insoluble Amylose Content of Rice. Starch - Stärke 32:409-411

51. Shih FF (2004) Rice proteins. In: Champagne E T(ed) Rice: chemistry and technology, 3rd edn. American Association of Cereal Chemistry, Inc, St Paul, MN:143-162

52. Shu K, Liu X-d, Xie Q, He Z-h (2016) Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. Molecular Plant 9:34-45

53. Soper JF, Cantrell RG, Dick JW (1989) Sprouting Damage and Kernel Color Relationships in Durum Wheat. Crop Science 29:895-898

54. Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y, Hattori T, Yano M (2010) Molecular cloning of Sdr4, a regulator involved in seed dormancy and domestication of rice. Proceedings of the National Academy of Sciences 107:5792-5797

55. Sweeney MT, Thomson MJ, Pfeil BE, McCouch S (2006) Caught Red-Handed: Rc Encodes a Basic Helix-Loop-Helix Protein Conditioning Red Pericarp in Rice. The Plant Cell 18:283-294

56. Torada A, Amano Y (2002) Effect of seed coat color on seed dormancy in different environments. Euphytica 126:99-105

57. Torada A, Somers D, Koike M, Ikeguchi S, Tsutsui I (2008) Mapping of a major locus

controlling seed dormancy using backcrossed progenies in wheat (Triticum aestivum L.). Genome 51:426-432

58. Visioli F (2016) Nutraceuticals in the treatment of metabolic syndrome. In: Mahabir, S., Pathak, Y. (Eds.), Nutraceuticals and Health: Review of Human Evidence. Taylor & Francis, Boca Raton, FL:191-197

59. Wang G, Bi H, Sun Y, Wang C, Xia L (2014) Characterization of Tamyb10 Haplotypes and Their Association with Pre-harvest Sprouting Resistance in a Set of Chinese Red-grained Wheats. ACTA AGRONOMICA SINICA 40:984-993

60. Wang X, Liu H, Mia MS, Siddique KHM, Yan G (2018) Development of near-isogenic lines targeting a major QTL on 3AL for pre-harvest sprouting resistance in bread wheat. Crop and Pasture Science 69:864-872

61. Wang Y, Wang XL, Meng JY, Zhang YJ, He ZH, Yang Y (2016) Characterization of Tamyb10 allelic variants and development of STS marker for pre-harvest sprouting resistance in Chinese bread wheat. Molecular Breeding 36

62. Wu H, Liu S, Jiang L, Zhang W, Wang Y, Ren Y, Han X, Liu F (2009a) Relationship between protein composition and total protein content and starch RVA profile properties in rice. Chinese Journal of Rice Science 23:421-426

63. Wu H, Liu S, Zhang W, Liu L, Zhao Z (2009b) Relationship between crude fat and Flour Viscosity Characteristics in Rice (Oryza sativa L.). Jiangsu Jurnal of Agricultural Science 25:464-468

64. Ye H, Feng J, Zhang L, Zhang J, Mispan MS, Cao Z, Yang J, Beighley DH, Gu X (2015) Map-Based Cloning of qSD1-2 Identified a Gibberellin Synthesis Gene Regulating the Development of Endosperm-Imposed Dormancy in Rice. Plant Physiology:pp.01202.02015

65. Zhou Y, Tang H, Cheng M-P, Dankwa KO, Chen Z-X, Li Z-Y, Gao S, Liu Y-X, Jiang Q-T,

Lan X-J, Pu Z-E, Wei Y-M, Zheng Y-L, Hickey LT, Wang J-R (2017) Genome-Wide Association Study for Pre-harvest Sprouting Resistance in a Large Germplasm Collection of Chinese Wheat Landraces. Frontiers in Plant Science 08

Figures

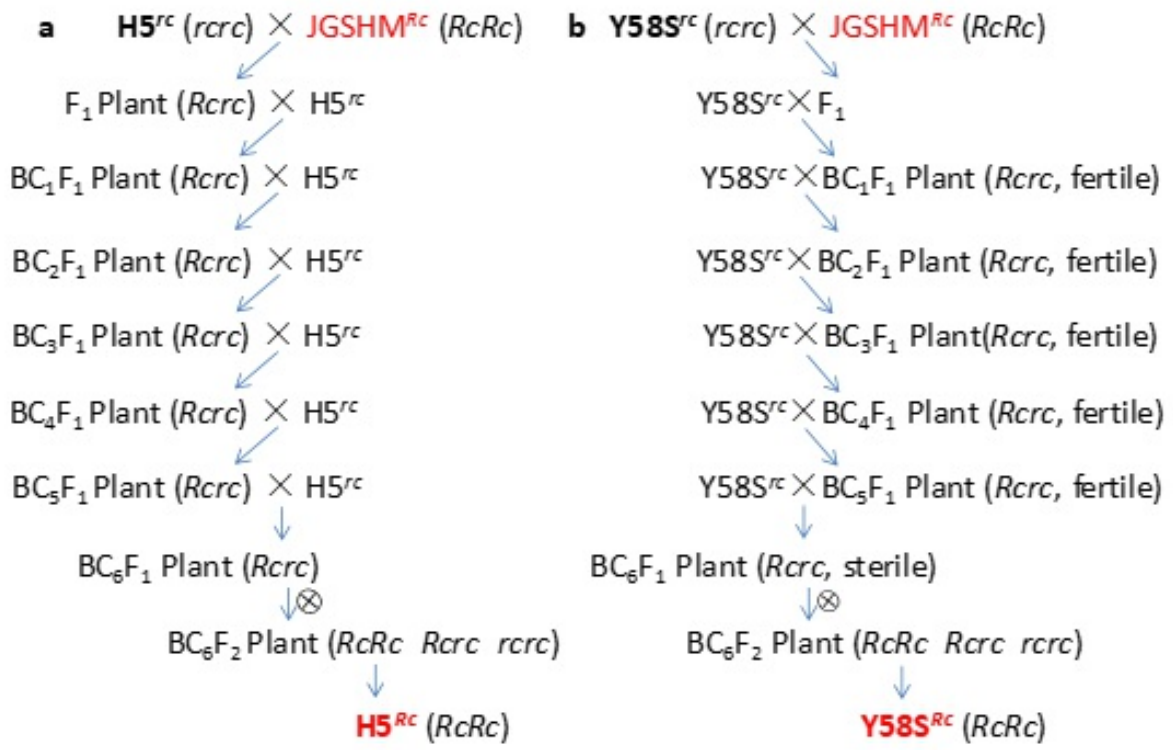

Figure 1

Illustration of development of near-isogenic lines (NILs), H5Rc/H5rc (a) and Y58SRc/Y58Src (b). Huaidao#5 with rc allele is named as H5rc and Y58S with rc allele is named as Y58Src. To begin with an F1 (Rcrc) produced by crossing JGSHM (RcRc) with H5rc (a) or Y58Src (b), using the Rc-14 marker-assisted selection of Rcrc genotype in the progeny, the Rcrc was backcrossed generation by generation with H5 (a) or Y58S (b) as recurrent parent to produce BC6F1 population. The BC6F1 selfed to produce BC6F2 population, in which the plants with an RcRc genotype produced NILs, H5Rc (a) or Y58SRc (b).

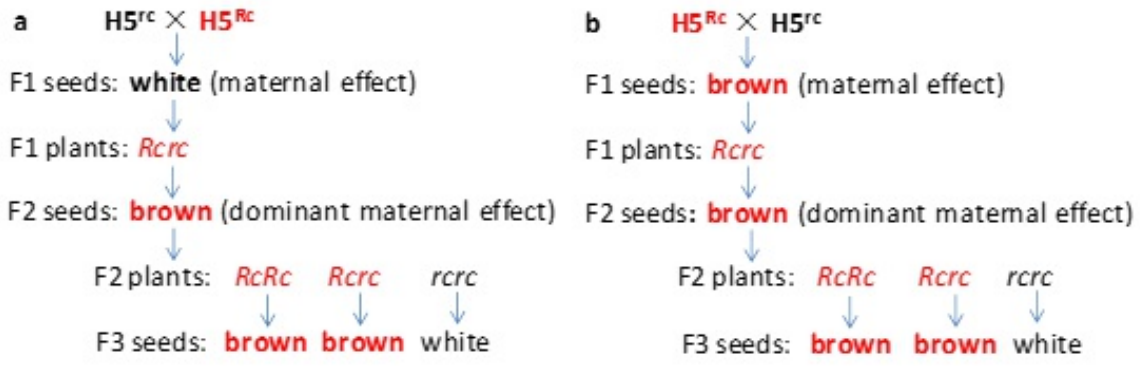

Figure 2

Illustration of analysis of inheritance pattern of rice pericarp color by forward cross (a) and reverse cross (b).

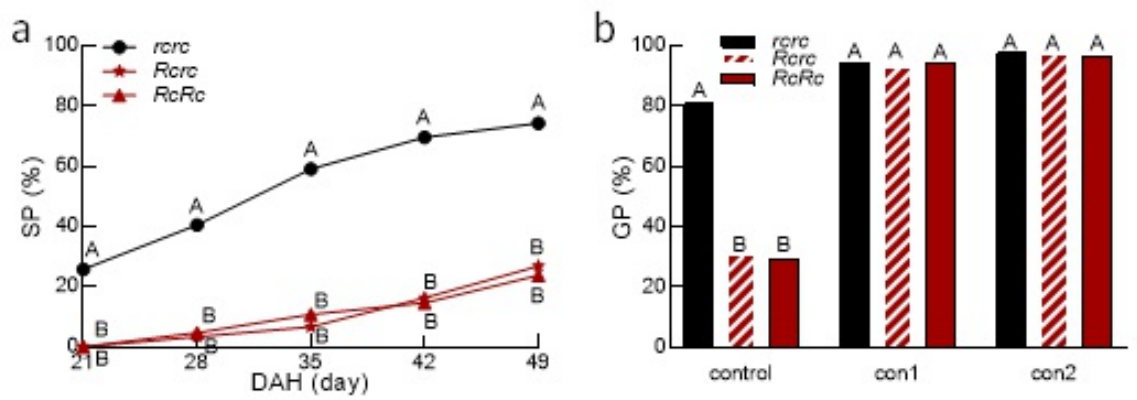

Figure 3

PHS resistance effect of Rc gene and release of the Rc-controlled dormancy in japonica rice. (a) Evaluation of PHS resistance by measuring sprouting percentage (SP). DAH, day after heading. (b) Evaluation of the seed after-ripening effect by measuring germination percentage (GP). Control, freshly harvested seeds were air-dried to a moisture content of ~13.5%; con1, the air-dried seeds were treated at 25°C with 65% relative humidity (RH) for 4 weeks; con2, the air-dried seed was stored under the ambient condition in Hangzhou for 12 weeks from November 2018 to February 2019. The different characters (A and B) mean that their genotypic means significantly differ at the $P < 0.01$; the same characters (A and A; B and B) mean that their genotypic means have no significant difference at the $P > 0.1$.

Figure 4

PHS resistance effect of the Rc gene and release of the Rc-controlled dormancy in indica hybrid rice. (a) Evaluation of PHS resistance of hybrid seeds by measuring sprouting percentage (SP). DAP, day after pollination. (b) Evaluation of after-ripening effect of the hybrid seeds by estimating seedling establishment. The hybrid seeds were treated by storage under the ambient condition in Hangzhou city for 12 weeks from November 2018 to February 2019. (c) Evaluation of PHS resistance of hybrid rice by measuring SP. The different characters (A and B) mean that their genotypic means significantly differ at the $P < 0.01$.

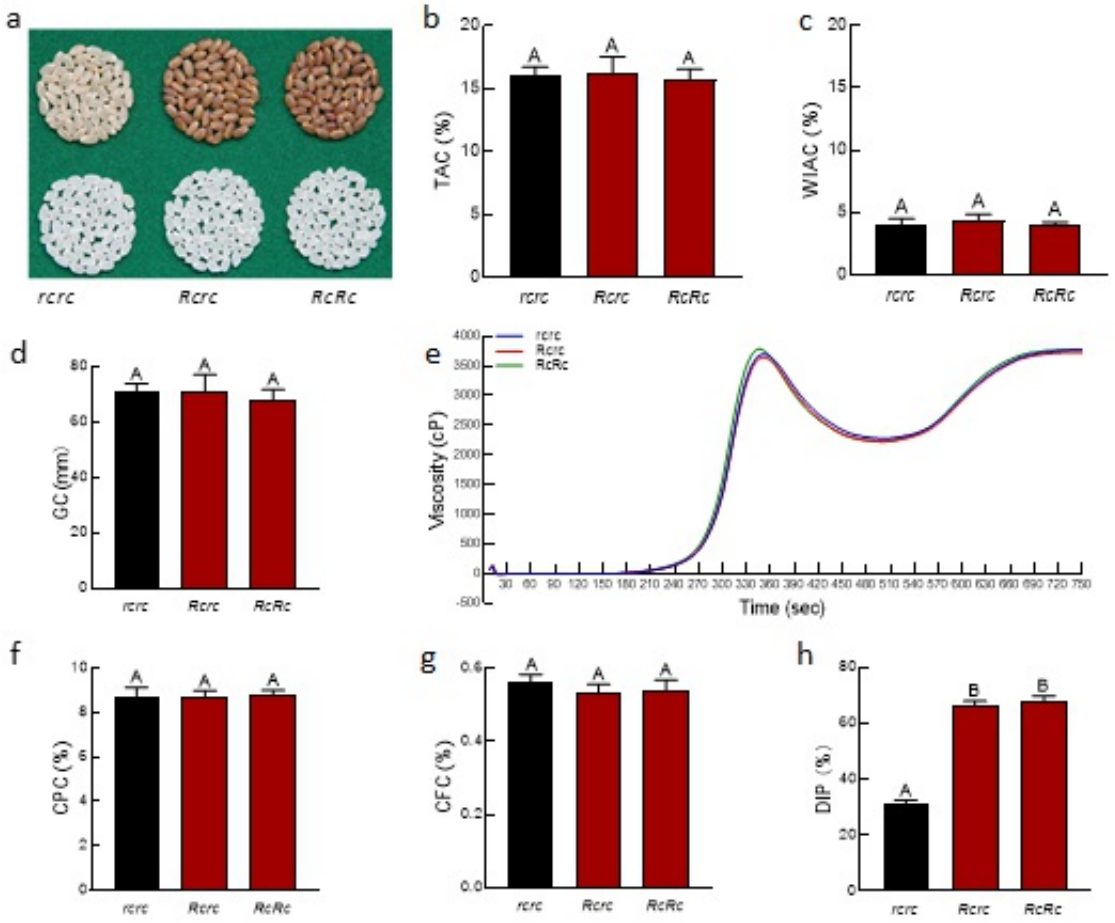

Figure 5

Evaluation of milled rice qualities (a-g) and the bran antioxidantive capacity (h). (a) Morphology of wholegrain (upper) and appearance quality of the milled rice (lower). (b) Total amylose content (TAC). (c) Hot-water-insoluble amylose content (WIAC). (d) Gel consistency (GC). (e) Viscosity curves expressed as mean of three replicates that were tested on Rapid Viscosity Analyzer. (f) Crude protein content (CPC). (g) Crude fat content (CFC). (h) Antioxidantive capacity of the bran, which was evaluated by measuring 2-diphenyl-1-picrylhydrazyl (DPPH•) inhibition percentage (DIP). The different characters (A and B) mean that their genotypic means significantly differ at the $P < 0.01$; the same characters(A and A; B and B) mean that their genotypic means have no significant difference at the $P > 0.1$.