Plant Omics Journal POJ

POJ 7(1):12-18 (2014) ISSN:1836-3644

Fine-mapping and candidate gene analysis of *BLACK HULL1* in rice (*Oryza sativa* L.)

Chao CHEN[1,2†], Wei WU[1†], Xingming SUN[1], Ben LI[1], Guanglong HU[1,3], Qiang ZHANG[1,4], Jinjie LI[1], Hongliang ZHANG[1], Zichao LI[1*]

[1]Key Laboratory of Crop Heterosis and Utilization (MOE)/Beijing Key Laboratory of Crop Genetic Improvment, China Agricultural University, Beijing 100193, China
[2]China National Seed Group Co., Ltd., Beijing 100045, China
[3]Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100093, China
[4]Rice Research Institute, Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China

[*]Corresponding author: Lizichao@cau.edu.cn
[†]These authors contributed equally to this work

Abstract

Black hull, as a kind of visual traits which can be easily identified after heading, plays a crucial role in the study of rice domestication. Previous studies have shown that the gene for phenol reaction may be responsible for the phenotype of black hull. To further explore the molecular mechanism of the formation of black hull, a near-isogenic line was constructed with black hull phenotype and by gene mapping and cloning from the cross between the *indica* rice 05048 and the *japonica* rice Nipponbare according to recombinant inbreeding method. The segregation ratio of F_6 population indicated that the black hull trait is controlled by a dominant gene, which is called *BLACK HULL1* (*BH1*). Through the map-based cloning method, *BH1* was fine-mapped in a 24.2-kb interval between SSR marker RM6629 and SNP marker SNP6-1 on the long arm of chromosome 4. In this region, 5 genes were annotated by the MSU Rice Genome Annotation Project Database. Sequence analysis and phenol color reaction test showed that *Phr1*, one of the two polyphenol oxidase gene in rice genome, has the high possibility for being the candidate gene of *BH1* and involved in the formation of black hull. These findings contribute to facilitate the illumination of molecular mechanism in rice domestication.

Keywords: Black hull phenotype; Fine mapping; *Phr1* gene; Rice (*Oryza sativa* L.).

Abbreviations: NIL_Near isogenic lines; bHLH_basic helix-loop-helix; DFR_dihydroflavonol 4-reductase.

Introduction

The origin and evolution of cultivated rice (*Oryza sativa*) is one of the hot topics in rice genetics and genomics research. Previous studies reveal that cultivated rice is domesticated from the wild rice species *Oryza rufipogon* and *Oryza nivara* (Khush 1997; Cheng et al., 2003; Kovach et al., 2007; Sang et al., 2007). Black hull is a wide-spread phenotype in the wild rice, and is discarded by artificial selection during the long time of domestication. Genetic analysis of genes involved in the formation of black hull has been carried out, but the molecular mechanism of black hull remains unknown. Elucidation of the genes for black hull will help us to understand the evolution of rice in molecular level and provide evidences of rice domestication.

The hull color of wild rice has no difference with the cultivated rice in flowering period, but it then turns black with the ripening of seeds. It is believed by the scientists that the phenotype of black hull is controlled by one dominant gene (Kuang et al., 1946; Jodon 1964), two complementary genes (Chao 1928; Mitra 1937; Kuriyama et al., 1967), or three complementary genes (Nagao et al., 1954; Rao et al., 1973) based on classic genetics studies. Maekawa (1984) confirmed that black hull is controlled by three genes, symbolized as *Bh-a*, *Bh-b* and *Bh-c*, respectively. The *Ph* gene for phenol reaction was also found having this character (Kuriyama et al., 1967; Rao et al., 1973), but the molecular evidence has not been reported yet. With the development of molecular biology, studies on related genes have been reported successively. Using a near-isogenic line developed from a cross between the wild rice W1943 (black hull) and Guangluai 4 (straw-white hull), Zhu et al. (2011) cloned the *Bh4* gene, and found that a member of an amino acid transporter family is responsible for the black hull which exists on the short arm of chromosome 4. A 22-bp deletion in 3rd exon of *Bh4* leads to the straw-white hull phenotype in cultivated rice. Besides two loci *qHC4* and *qHC7*, which were responsible for black pigmentations on the hull, were identified using an F_2 population (Gu et al., 2005). Another gene for black hull segregation was mapped on chromosome 5 using 125 recombinant inbred lines derived from a cross between an *indica* cultivar of *O. sativa* and a strain of *O. rufipogon*, which carry some *japonica*-like characteristics (Cai and Morishima, 2002).

In this study, a near-isogenic line with black hull phenotype, derived from the cross between 05048 (*O. sativa indica* with straw-white hull) and Nipponbare (*O. sativa japonica* with straw-white hull), was used to map the target gene *BH1*. The results of this research build the foundation for elucidating the mechanism of transition from black hull of the wild rice to the straw-white hull of cultivated rice.

Table 1. Genetic analysis of black hull gene.

Generation	Total	Black hull	Straw-white hull	χ^2	P value
F_2	34	28	6	0.32	0.57
F_6	4500	3443	1057	0.019	0.89

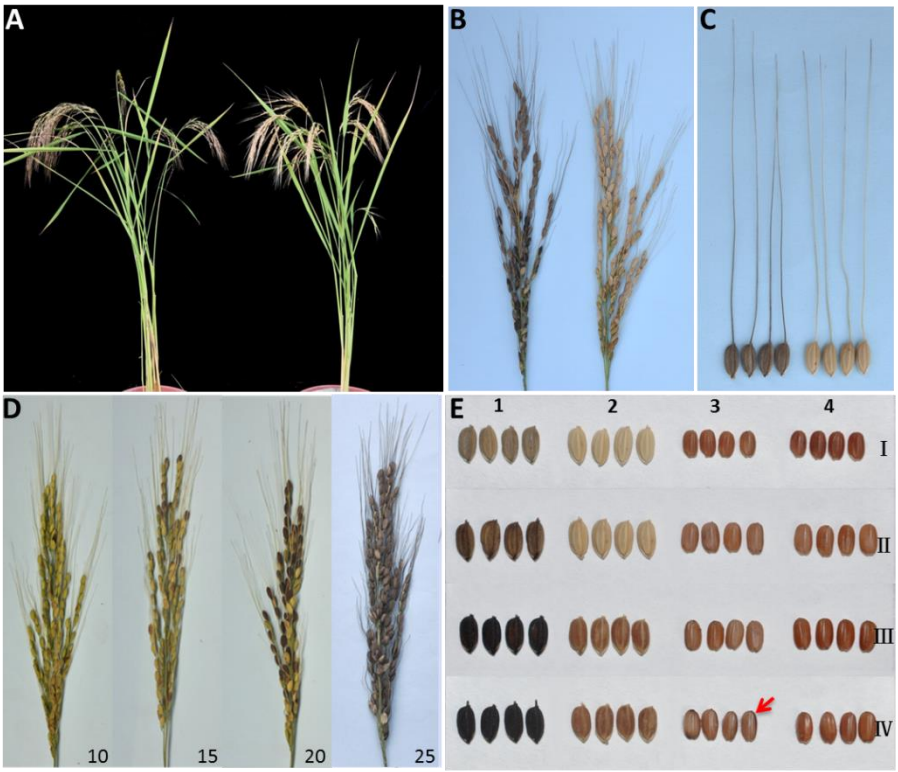

Fig 1. Phenotype of black hull in near-isogenic population. (A) Phenotype comparison of plant architecture between dominant and recessive plants in NIL; (B-C) Phenotype comparison of panicle and seeds between dominant and recessive plants; (D) The course of hull color change on maturing panicles. Period after heading is indicated in the lower right corner; (E) Analysis of phenol reaction. 1,2 represent seeds from black hull plant and straw-white hull plant, respectively; 3,4 represent dehulled seeds from black hull plant and straw-white hull plant, respectively; I-IV indicate the performance of seeds soaked in the phenol solution with 0d, 2d, 7d and 10d, respectively.

Results

Morphological characteristics and genetic analysis of BH1

All seed hulls of the NIL panicles stayed green from 1st to 14th day after flowering. On the 15th day after flowering, black spots appeared on the hulls of the first developing grains. All the hulls become completely black on the 25th day after flowering until ripened (Fig 1. A-D). All F_1 plants from the cross between 05048 and Nipponbare exhibited black color in seed hulls, indicating dominant inheritance of the trait. Among F_2 population, the segregation ratio showed the tendency that the black hull is controlled by one dominant gene (Table 1), but the identification of black hull is affected easily by the segregation of other traits. Cultivation to F_6 generation based on phenotype selection of other traits, except for black hull, were stable (Fig 1A). In F_6 population, 4500 plants were grown. The segregation ratio was investigated after being ripened. There were 3443 plants showing black hull and 1057 plants revealed straw-white hull, and the segregation showed a good fit to the 3:1 ratio (χ^2=0.019 <$\chi^2_{0.05}$=3.84)(Table 1). All these results demonstrate that the black hull trait in NIL is controlled by a single dominant gene, which is nominated *BH1*.

Genetic and physical mapping of the BH1

To map the *BH1* gene on rice chromosome, 312 SSR markers distributed in 12 chromosomes, were screened for polymorphism between dominant and recessive DNA pools, and two SSR markers on the long arm of chromosome 4 reveled differences. Therefore, the linkage analysis was carried out using two polymorphic markers, RM3687 and RM6748, and 90 DNA samples of plants with straw-white hull from F_6 population. Among 90 individuals, 2 and 6 recombinants were identified, based on the genotype at RM3687 and RM6748 locus, respectively. These results confirm that the target gene is located on the long arm of chromosome 4.

To identify more polymorphic SSR markers on the long arm of chromosome 4, 144 new SSR markers were synthesized according to available information in the Gramene database (www.gramene.org). Among them, 5 SSR markers showed polymorphism between two DNA pools. They were RM3843, RM6629, RM3836, RM5611 and RM3648.

To fine map the *BH1*, 2 polymorphic markers, RM6748 and RM3648, were used to screen recombinants from 1057 recessive plants of F_6 population. 87 and 155 recombinants were identified based on the genotypes at RM6748 and RM3648 locus, respectively. Among 87 recombinants at RM6748 locus, 24, 2 and 1 recombinants showed hetero-genotypes at RM3687, RM3843 and RM6629 locus, respectively. Besides, 78 and 8

Table 2. Some brief information on predicted genes.

No.	Gene Locus	Description
1	LOC_Os04g53300	Polyphenol oxidase, putative, expressed
2	LOC_Os04g53310	Soluble starch synthase 3, chloroplast precursor, putative , expressed
3	LOC_Os04g53320	Expressed protein
4	LOC_Os04g53330	RNA recognition motif containing protein, putative, expressed
5	LOC_Os04g53340	Expressed protein

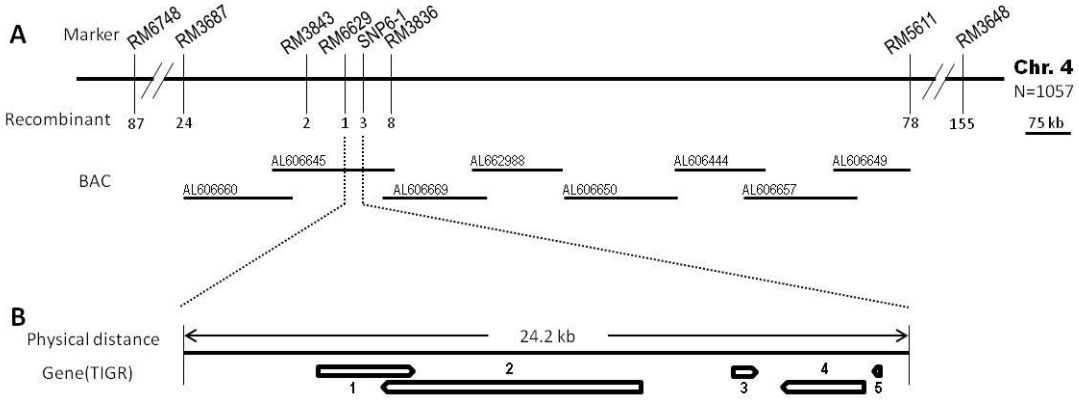

Fig 2. Fine mapping of *BH1* gene and gene prediction. (A) Fine mapping of *BH1* gene. (B) 5 ORFs predicted on TIGR database within the fine-mapped region (Table 2).

individuals out of 155 recombinants at RM3648 locus are identified to have hetero-genotypes at RM5611 and RM3836 locus, respectively.

To narrow down the region of *BH1* locus, 26 primer pairs were designed based on the divergences between *japonica* and *indica* rice genomes. An SNP marker was identified and used to screen the recombinants of RM3836 locus, and 3 individuals out of 8 recombinants were identified to have hetero-genotypes at SNP6-1 locus. Based on these results, the target gene *BH1* is finally mapped to a 24.2-kb interval between RM6629 and SNP6-1 markers (Fig 2).

Gene annotation and sequence analysis of the predicted genes

To screen the candidate gene responsible for black hull trait, we analyzed the 24.2-kb region through the Rice Genome Annotation Project Data (Release 7), and found 5 expressed genes in this region (Fig 2B, Table 2). Among the 5 genes, LOC_Os04g53300 encodes polyphenol oxidase, LOC_Os04g53310 encodes soluble starch synthase 3 and LOC_Os04g53330 encodes a protein containing RNA recognition motif. The other 2 genes encode proteins of unknown function (Table 2).

Five predicted genes from both black hull plants and straw-white hull plants were amplified by gene specific primers as described in Table 4 and the PCR products were used for sequence analysis. The results of sequence alignments showed that divergences are found in 2 genes, LOC_Os04g53300 and LOC_Os04g53310. Comparing with the sequence of straw-white hull plants, one SNP and an 18-bp insertion (Δ18) were identified in the predicted exon of LOC_Os04g53300 (Fig 3). The SNP in LOC_Os04g53310 comes from black hull plants.

Comparison of amino acid sequences which encoded by LOC_Os04g53300 and LOC_Os04g53310 between black hull plants and straw-white hull plants suggested that both SNPs in LOC_Os04g53300 and LOC_Os04g53310 were nonsense mutations.

According to Next Generation Sequencing Transcriptome Data in the Rice Genome Annotation Project (Wang et al., 2010), LOC_Os04g53300 has high expression level at palea/lemma and spikelet (Fig 4). In accordance with the process of black hull formation, this result infers that LOC_Os04g53300 may be the candidate gene of *BH1*.

Analysis of phenol reaction

To verify that *BH1* possesses the phenol reaction, phenol color reaction test was performed. Grains with hulls intact and dehulled were soaked in a 1.5% unbuffered aqueous phenol solution, respectively. After 2d, 7d and 10d, dried grains to assess color change compared with untreated grains from the same samples. The result of phenol reaction shows that the hulls and edge of dehulled grains from black hull plants turn deeply black after treated and wild type plants has no differences with untreated grains (Fig 1E).

Discussion

In the process of rice domestication, seed hull color is an important agronomic trait for phenotypic selection. Studies on the mechanism of transition from colored hull of the wild rice to the straw-white hull of cultivated rice contribute to further research on the topics of rice domestication. Rice seeds of wild type show purple, brown or black color. Both purple and brown colors result from the accumulation of flavonoids (Winkel-Shirley, 2001). *Rc* encodes a bHLH protein, which is respo-

Table 3. Information of markers used for gene mapping.

Markers	Forward Primer (5'-3')	Reverse Primer (5'-3')
RM6748	AGAGAAGCAGCTGGTGATTAGCC	CAACGATGTACCAGTTGAATACCC
RM3687	GGGCCCTACTAGTACAGCTAAAGG	AATTGGGAAGGAAGGAACAGG
RM3843	CCAGATCATCCAGGCATAACATCACC	CGGCGCTGGTAAACTCCATTCC
RM6629	CCGTAATGCCATTGTTGTCAGC	TTATTATGGGCGGTCGCTAACC
RM3836	CGGAATCACCAATTTCTCTCTCAGC	CGCAAGAAACGGAAACGAAACC
RM5611	GGAAGACACATCTTACACCGTTCC	CAGCCACTCTGATCTCGTGTCC
RM3648	CGAGAAGCCGAAGAGGAAGAGAGC	CCAACAGATCCATCTCAACCAACTCC
SNP6-1	GCTCCTCTTTACTTTCACACGGCA	GCTTTCAACGAACCCGGCCCAT

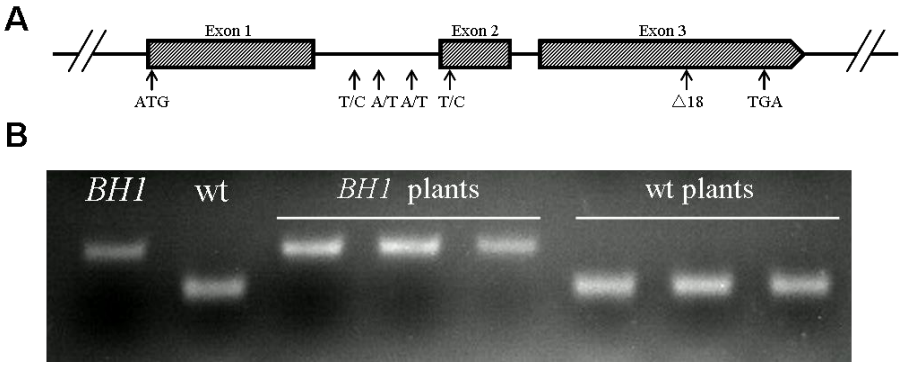

Fig 3. The structure of LOC_Os04g53300. A: The structure of LOC_Os04g53300. The start codon, stop codon, SNPs and InDel are signed by arrows. B: Size comparison of PCR products around Δ18 locus.

nsible for the formation of the brown pericarp (Sweeney et al., 2006; Furukawa et al., 2006). *Rd* encodes the DFR protein, controlling the variation of seed color from brown to red (Furukawa et al., 2006).

Black hull is a wide-spread phenotype in the wild rice, but only one gene related to black hull formation has been cloned yet. *BH4*, encoded an amnio acid transporter protein and involved in the formation of black hull, is functional in the ancestral wild rice and a 22bp deletion in exon 3 of cultivated rice disrupted its function which resulted in straw-white hull (Zhu et al., 2011). Based on this research, the molecular mechanism of the formation of black hull is still unclear.

In our study, a NIL with black hull was developed using two materials with straw-white hull which belonged to *indica* and *japonica* rice varieties, respectively. By a series of experiments, the *BH1* is fine mapped into a 24.2-kb interval on the long arm of chromosome 4, and *Phr1*, one of two phenol reaction genes in rice genome. It was identified as the candidate gene of *BH1*. These results will support that *Ph* gene involves in the formation of black hull and *Phr1*, as one of two polyphenol oxidase genes in rice genome, which might be responsible for that.

BH1 is a new allele of Phr1

In early classic genetics studies, researchers found that the *Ph* gene for phenol reaction was responsible for black hull (Kuriyama et al., 1967; Rao et al., 1973). But there is no molecular evidence providing that conclusion for such a long time. The completion of the sequencing of the *japonica* rice cultivar Nipponbare in 2005 and annotation of the 12 rice chromosomes provide the advantage of illuminating the molecular mechanism of black hull phenotype. Based on the latest database of the MSU Rice Genome Annotation Project, there are two polyphenol oxidase genes in rice genome which lie on chromosome 1 and 4.(Kawahara et al., 2013). *Phr1*, a gene localizing on the long arm of chromosome 4 and encoding a polyphenol oxidase. It was cloned by Yu et al. (2008) through map-based cloning method. Researchers have proved that the frequency of *Phr1* is high in the *indica* species and conversely low in the *japonica* species. For this reason, the grains, especially hulls of the *indica*-type cultivars show positive phenol reaction (PHR), in which color turn brown after soaked in phenol solution, whereas those of the *japonica* type are PHR-negative and their color remains unchanged.

The NIL used in our study derived from two cultivated varieties belonging to different subspecies of *Oryza sativa* and both varieties have straw-white hull. This phenomenon illustrates that the formation of black hull phenotype is influenced by the interaction among several genes, and related gene inflow into *indica* and *japonica* rice varieties separately over the long time of domestication through artificial selection. This is in accordance with the conclusion obtained by Yu et al. (2008) which showed *Phr1* is defective in all *japonica* lines but functional in nearly all *indica* and wild strains.

In our study, we concluded that LOC_Os04g53300 may be the candidate gene of *BH1*. Based on the comparison of physical location on the chromosome 4 between *BH1* and *Phr1*, we find out that two genes possess the same locus. Because of the different phenotype and inconsistent sequences between *BH1* and *Phr1*, we believe that *BH1* is a new allele of *Phr1*.
These findings provide theoretical support for the conclusion that *Ph* gene involves in the formation of black hull and *Phr1*, as one of two polyphenol oxidase genes in rice genome, and may be responsible for that.

Other unknown genes involved in the formation of black hull

Previous study reported that the *Bh4* gene controlled the black hull phenotype in wild rice (*Oryza rufipogon* and *Oryza nivara*). After being transferred into the two subspecies of cultivated rice, *indica* (Guangluai 4, Kasalath) and *japonica* (Nipponbare), only one of them (*indica*) can be rescued with black hull phenotype (Zhu et al., 2011). In order to confirm whether the formation of black hull results from the interaction between *Bh4* and *Phr1*, we designed primers (Table 4) and sequenced the

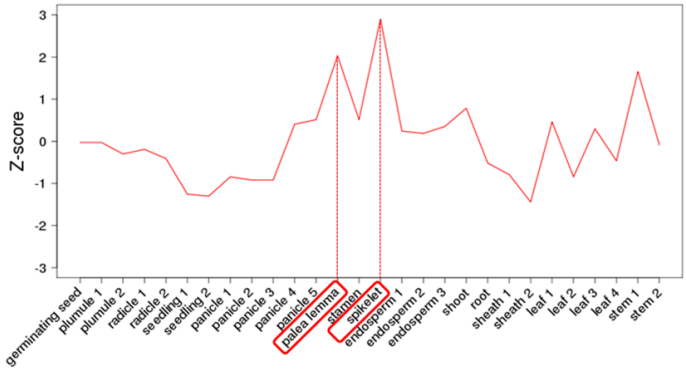

Fig 4. Tissue atlas of LOC_Os04g53300 from Minghui 63 rice show that the higher expression level of gene appear in tissues of spikelat and palea lemma. Experiment summary: Germinating seed harvested 72 hours post imbibition (germinating seed); light and dark grown plumules harvested 48 hours after germination (plumule 1, plumule 2); light and dark grown radicles harvested 48 hours after germination (radicle 1, radicle 2); 3 days old seedling (seedling 1); trefoil stage seedling (seedling 2); panicle (less than 1 mm) (panicle 1); panicle (3 to 5 mm) (panicle 2); panicle (10 to 15 mm) (panicle 3); panicle (40 to 50 mm) (panicle 4); heading panicle (panicle 5); palea/lemma 1 day before flowering (palea/lemma); stamen 1 day before flowering (stamen 1); spikelet 3 days post anthesis (spikelet); endosperm 7 days post anthesis (endosperm 1); endosperm 14 days post anthesis (endosperm 2); endosperm 21 days post anthesis (endosperm 3); shoot of seedling with three tillers (shoot); roots of seedling with three tillers (root); sheath tissues from plants with panicles less than 1 mm (sheath 1); sheath tissues from plants with panicles between 40 and 50 mm (sheath 2); leaf tissues from plants with panicles less than 1 mm (leaf 1); leaf tissues from plants with panicles between 40 and 50 mm (leaf 2); leaf tissues 5 days before heading (leaf 3); leaf tissues 14 days post anthesis (leaf 4); stem tissue 5 days before flowering (stem 1); stem tissue 14 days post anthesis (stem 2).

Bh4 gene in NIL. Sequence alignment showed that *Bh4* in NIL was nonfunctional. This result implies that new genes involved in the formation of black hull phenotype may be included in our NIL material except for *Bh4* and *Phr1*, and further study can identify the new genes and clarify the mechanism of black hull formation.

Materials and Methods

Plant materials

The near-isogenic line with black hull was developed from the cross between the *indica* rice 05048 and the *japonica* rice Nipponbare after the selection of phenotype and selfing for 6 generations. In addition, both materials are varieties with straw-white hull. All experimental materials were grown at China agricultural university's experimental farms in Beijing for the summer crop and Sanya, Hainan province for winter crop. The second top leaves of F_6 plants of segregate population were collected at heading stage for DNA extraction.

Phenotypic analysis

To record the process of formation of black hull, panicles of both dominant and recessive plants were collected and photos were taken every five days after heading. The ratio of segregation was investigated in the segregate population after seeds ripened, and phenotype of each plant was taken notes. All seeds of each plant were harvested separately, and the serial number of seeds was in accordance with leaves.

DNA extraction and molecular makers

Genomic DNA was extracted from leaf according to the cetyl-trimethyl-ammonium bromide method (Murray et al., 1980). DNA samples were quantified using ultraviolet spectrophotometer. Following the protocol of bulk segregation analysis, DNA pools were admixed using 6 DNA samples with black hull phenotype and 6 DNA samples with straw-white phenotype, respectively (Zhang et al., 1994). 312 SSR markers (Chen et al., 1997; McCouch et al., 1998; Temnykh et al., 2000; McCouch et al., 2002) distributed in 12 chromosomes evenly were used to screen out polymorphic markers which showed polymorphism between two DNA pools.

PCRs were performed in 20μL volumes including approximately 50ng genomic DNA, 1×PCR buffer, 400nmol/L each primer, 200μmol/L each dNTP, 2mmol/L $MgCl_2$ and 1U Taq enzyme. Amplification program of SSR markers were set following the protocol of Temnykh et al. (2001). The PCR products of SSR markers were run on 8% poly-acrylamide gels.

Preliminary and fine mapping

According to the results of polymorphic markers screening, linkage analysis was carried out using 90 DNA samples of plants with straw-white hull, and region of preliminary mapping was defined by means of linkage analysis. Based on the results of preliminary mapping, new SSR marker primers were synthetized and tested for their polymorphisms among DNA pools, and polymorphic markers were used for analyzing all DNA samples of straw-white hull plants. All primers used for gene mapping are listed in Table 3.

Analysis of the candidate genes

The annotation of genes located in the fine-mapped region was collected through the Rice Genome Annotation Project Data known as TIGR:
<http://rice.plantbiology.msu.edu/annotation_pseudo_current.shtml>. PCR primers for amplifying predicted genes were designed according to the genome sequence of Nipponbare by Primer-BLAST, an online tool for primer design (Table 4). The annotated genes of dominant and recessive plants were PCR amplified and sequenced with an ABI 3730XL DNA Analyzer provided by TSINGKE Biotech Co. Ltd. Sequences were assembled using Contig Express and aligned using DNAMAN (v6).

Phenol color reaction test

Grains, both wild type and mutant plants, were selected as test samples. 20 grains with hulls intact and dehulled were put

Table 4. PCR primers used for sequence analysis of the predicted genes.

Primer ID	Forward Primer (5'-3')	Reverse Primer (5'-3')	Gene
BG1-1	TCTCCCAAATTGTTAGTGCGCGGC	GGTCTTTCGGGATGTTCGTGTCGG	LOC_Os04g53300
BG1-2	GATTCTGTGAACGCTGGACGCA	TGGCATGTGACGGCCTGTGTA	LOC_Os04g53300
BG2-1	ATTTCGGGAGCTAGGAAGGCGGAG	TTGGCAGCGTGAACCAAACCATCA	LOC_Os04g53310
BG2-2	CGTCAGCATCGGCTACGCTAAGTG	GCATGGCGAATACCATGGTCGTGT	LOC_Os04g53310
BG2-3	GGAACAAGGATGAAGTCGGCACCA	TGGTCAAGTGCTCCAGTTGCTTGG	LOC_Os04g53310
BG2-4	AGCACCATGTCCTGACACTTCCCT	TCTGCTCTAGAGTTTCTCCGCCA	LOC_Os04g53310
BG2-5	GTGATGGTTCAAACGTTCAGCGA	CACAGGTTGGGGGTCTTGGTGA	LOC_Os04g53310
BG2-6	AAGGAAGACGCCAGGATGCCTT	ACATCCAGGAGGGCATCATTTGAC	LOC_Os04g53310
BG2-7	GAACATCCACGGTGGTTCCTGCAC	TTAACAGGGGATTGGTGGTGCTGC	LOC_Os04g53310
BG2-8	TCCTCCTTACGACGCTCCTCCTCA	GGACAAAGCGGCACAGATGAAGGT	LOC_Os04g53310
BG2-9	GCTCGACCTCTTCTTCAGTCTCAGC	ATGCGTGGTCGAGGCGTTTTGT	LOC_Os04g53310
BG2-10	ATCCCTGAAGCAGAACCGCACA	TTGAAACTGGGCAGTCGGCGAG	LOC_Os04g53310
BG3-1	GCTCCTCTTTACTTTCACACGGCA	GCTTTCAACGAACCCGGCCCAT	LOC_Os04g53320
BG3-2	AGGGAGAGCTAGATCCCCAAAT	TCGTTTCAAAACGTGAGCAACC	LOC_Os04g53320
BG4-1	GAAGTGCATGATGTTCCATAGGCCA	GCATCAGCTCACATCCACCGCA	LOC_Os04g53330
BG4-2	TGCAGAGCCAGCAGTAGAGACTGT	TGCATGTAATACTGGCACTCCCTCT	LOC_Os04g53330
BG4-3	ACTTGTCAGCAAGCGTTATCAATGG	GAGGCGGCCCAAGAGAAGAAGAAG	LOC_Os04g53330
BG5	CAGAGTGGTGGTGATGCCGAAACC	CGCGAGCTTGCTTCTTCTCCCAAC	LOC_Os04g53340
BH4-1	GATGGTCGATCAATATACTGACCA	ATGCACCCTTGCAATCTATGCA	*Bh4*

down on culture dishes with filter paper on the bottom, respectively. 10ml 1.5% unbuffered aqueous phenol solution was transfused and then covered the culture dish rapidly. Each treatment set 4 replicates. After 2d, 7d and 10d, the grains color change compared with untreated grains from the same samples. Every sample showing a negative response was phenotyped at least twice to verify the phenol reaction.

Conclusion

To explore the molecular mechanism of the formation of black hull by gene mapping and cloning, a near-isogenic line with black hull phenotype was constructed and then fine mapping and candidate gene analysis were carried out. According to the results, *BH1* locates on the long arm of chromosome 4 and *Phr1*, one of two polyphenol oxidase genes in rice genome, may be the candidate gene of *BH1*.

Acknowledgments

This work was funded by the Project of the Ministry of Sciences and Technology of China (2010CB1125904), the “863 Project” (2012AA020407) and the “948 Projects” (2011-G1(2)-25 and 2011-G2B).

References

Cai HW, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. Theor Appl Genet. 104:1217-1228

Chao LF (1928) Linkage studies in rice. Genetics. 13:133-169

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet. 95:553-567

Cheng C, Motohashi R, Tsuchimoto S Fukuta Y, Ohtsubo H, Ohtsubo E (2003) Polyphyletic origin of cultivated rice: based on the interspersion pattern of SINEs. Mol Biol Evol. 20:67-75

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

Gu XY, Kianian SF, Hareland GA, Hoffer BL, Foley ME (2005) Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (*Oryza sativa*). Theor Appl Genet. 110:1108-1118

Jodon NE (1964) Genetic segregation and linkage, important phases of rice research. In: IRRI (ed) Rice Genetics and Cytogenetics. Elsevier, Amsterdam

Kawahara Y, Bastide M, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Schwartz DC, Tanaka T, Wu J, Zhou S, Childs KL, Davidson RM, Lin H, Quesada-Ocampo L, Vaillancourt B, Sakai H, Lee SS, Kim J, Numa H, Itoh T, Buell CR, Matsumoto T (2013) Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice. 6:4-13

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol. 35:25-34

Kovach MJ, Sweeney MT, McCouch SR (2007) New insights into the history of rice domestication. Trends Genet. 23:578-587

Kuang HH, Tu DS, Chang YH (1946) Linkage studies of awn in cultivated rice (*Oryza sativa* L.). J Genet. 47:249-256

Kuriyama H, Kudo M (1967) Complementary genes Ph and Bh controlling ripening-black coloration of rice hulls and their geographical distribution. Jpn J Breed. 17:13-19

Maekawa M (1984) Geographical distribution of the genes for black hull coloration. Rice Genet Newsl. 1:104-105

McCouch SR, Kochert G, Yu ZH, Wang ZY, Kush GS, Coffman WR, Tanksley SD (1998) Molecular mapping of rice chromosomes. Theor Appl Genet. 76:815-829

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9:199-207

Mitra SKIAS, Ganguli PM (1937) Inheritance of inner glume colour in rice. Ind J Agr Sci. 7:126-133

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4326

Nagao S, Takahashi M (1954) Genetical studies on rice plant, XVI. some genes responsible for yellow, brown and black color of glume. Jpn J Breed. 4:25-30

Rao CH, Seetharaman R (1973) Genetic studies in pericarp and hull color in rice. Indian J Genet Pl Br. 33:319-323

Sang T, Ge S (2007) The puzzle of rice domestication. J Integr Plant Biol. 49: 760-768

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. Plant Cell. 18:283-294

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. Genome Res. 11:1441-1452

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet. 100:697-712

Wang L, Xie W, Chen Y, Tang W, Yang J, Ye R, Liu L, Lin Y, Xu C, Xiao J, Zhang Q (2010) A dynamic gene expression atlas covering the entire life cycle of rice. Plant J. 61:752-66

Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 126:485-493

Yu Y, Tang T, Qian Q, Wang Y, Yan M, Zeng D, Han B, Wu CI, Shi S, Li J (2008) Independent losses of function in a polyphenol oxidase in rice: differentiation in grain discoloration between subspecies and the role of positive selection under domestication. Plant Cell. 20:2946-2959

Zhang Q, Shen BZ, Dai XK, Mei MH, Saghai Maroof MA, Li ZB (1994) Using bulked extremes and recessive class to map genes for photo-period-sensitive genic male sterility in rice. P Natl Acad Sci USA. 91:8675-8679

Zhu BF, Si L, Wang Z, Zhou Y, Zhu J, Shangguan Y, Lu D, Fan D, Li C, Lin H, Qian Q, Sang T, Zhou B, Minobe Y, Han B (2011) Genetic control of a transition from black to straw-white seed hull in rice domestication. Plant Physiol. 155:1301-1311