QTLs and analysis of the candidate gene for amylose, protein, and moisture content in rice (Oryza sativa L.)

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Abstract In this study, we determined using NIRS the heritability percentage of amylose, protein, and moisture content in polished and unpolished rice in a CNDH population derived from a cross between Cheongcheong and Nagdong rice varieties. The results revealed a higher heritability percentage for the amylose content and compromised heritability for protein and moisture contents. We also conducted QTL analysis of rice for these major components and identified their chromosomal locations on a physical map. We found a total of four QTLs affecting the amylose, protein, and moisture contents of grain on chromosome 7. We constructed physical maps of seven DNA markers responsible for amylose content, six responsible for protein content, and three responsible for moisture content. Furthermore, we classified these genes according to their functions and found 17 genes (over 77%) to be involved in secondary metabolite synthesis, two genes (about 9%), each related to cell function and abiotic stress, and one gene (about 5%) involved in redox signaling.

Keywords Mapping · Amylose · Protein · Moisture · Rice

Introduction

Rice (Oryza sativa L.) occupies a significant portion of principle human food consumption globally (Juliano 1999). It is an important cereal crop and is considered a model plant because of its relatively small genome size, vast germplasm collection, enormous repertoire of molecular genetic resources, and efficient transformation system (Paterson et al. 2005). Owing to its importance, grain yield potential of rice has been the chief focus of research in the past; however, little attention has been paid to grain quality (Cheng et al. 2014). Quality encompasses the gamut of attributes of a product that have a bearing on the criteria for the satisfaction of consumers [International Standards Organization (ISO) 8402, 1986]. The attributes of rice for consumer satisfaction include appearance, milling, cooking, eating, and nutritional qualities (Bao 2014). To cater to the demand and taste of consumers, the scientists, therefore, seek breeding strategies that can improve the rice grain while keeping the yield potential high (Ni et al. 2011). In recent times, rice breeders have aimed at improving the grain quality aspects while stabilizing the level of production. As a result, publications over the past decade or so have revealed that cooking and eating qualities of rice have a direct relationship with the apparent amylose content (AAC) among other main quality attributes of rice (Amaravathi et al. 2008). The appearance of rice grain is mainly specified by grain shape, as defined by grain length, width, length/width ratio.
Knowledge concerning the inheritance of rice quality is important that breeders acquaint themselves with sound knowledge concerning the inheritance of rice quality traits. Thongbam et al. (2012) demonstrated that the amylose content of rice had high heritability and genetic advance, suggesting that the selection for this trait could improve the genotypic value of the selected plants over those of their parents. The heritability for protein was moderately high, but the genetic advance was very low due to low genotypic variance (GCV) and because of more influence of the environmental factors; therefore, simple phenotypic selection may not bring its genetic improvement. For improvement of crude protein, there is a need for genetic enhancement of variability either through collection of new genetic resources or through hybridization. In a similar work, Ashwini Samak et al. (2011) determined the heritability and genetic advance of F4 generation for total grain protein content and observed a subtle increase in comparison to the F4 generation. The involvement of duplicate gene interaction in determining the total protein content of grain indicates that mild selection can be employed for such a trait in the segregating generation. Nirmaladevi et al. (2015), in their research on genetic variability, heritability, and correlation coefficients of grain quality characters in rice (Oryza sativa L.), discovered highest broad sense heritability and genetic advance for amylose content (93%), indicating that the trait would respond to selection due to its high genetic variability and transmissibility. Such a characteristic needs top priority during selection and can be used as a selection index for improving the grain quality. Shejul et al. (2013) recorded high heritability for water absorption (92.3%) and amylose content (67.3%). However, protein content showed a lower heritability. High heritability signified that the concerned traits were least affected by environmental modifications and selection based on phenotypic performance would, therefore, be reliable. The nutritional features of rice are closely linked to human wellbeing, it being a significant food, globally; such features are often held in high regard by consumers. Protein is the second important component in milled rice after carbohydrate. However, among the amino acids essential for human nutrition, lysine is the most limiting in rice proteins (Juliano 1999). Rice contributes to a sizeable percentage (52%) of the total protein diet in South East Asia. Protein and lysine content are crucial parameters that dictate the nutrient worth of rice. As societies progress, various communities that feed mainly on rice will most likely require rice with discrete nutritional qualities. People living in less developed countries exhibit high micronutrient deficiencies. This calls for biotechnological approaches towards biofortification of rice and other crops with substantial nutrient content, to meet regular nutritional needs of humans (Hirschi 2009). Brown rice is richer in minerals, vitamins, dietary fibers, and phenols compared to milled rice (Bao 2014). Various reports concerning QTL mapping for protein content are available. Bao (2014) identified about 43 QTLs spanning all the 12 chromosomes from nine rice varieties. Chromosomes 1, 2, and 7 harbor a sizeable number of QTLs compared to the other chromosomes. Additionally, Zhang et al. (2005) identified 2, 3, and 4 QTLs for protein fractions, albumin, globulin, prolamin, and glutelin, respectively. The QTLs controlling the composition of various protein fractions could be at the same chromosome locus. Patindol and Wang (2003) detected 18 chromosome portions for 19 single amino acids, with that located at the base of chromosome 1 being somewhat a stronger QTL bunch, comprising 19 single quantitative trait loci. There is a vast co-occurrence among the qualitative trait loci and the loci responsible for amino acid metabolic pathways, nitrogen absorption and transfer, and protein biosynthesis (Patindol and Wang 2003). Hu et al. (2004) detected 12 quantitative trait loci for single amino acid and total amino acid content on chromosomes 1, 4, 6, 7, and 11. The quantitative trait loci bunch located on chromosome 1 was linked to the content of eight amino acids. Such results become critical for identification of suitable genes and for marker-assisted procreation with the objective to improve the amino acid content of rice for human consumption. Yun et al. (2014) conducted a QTL analysis for protein content in a population derived from a cross between Cheongcheong and Nagdong. They found three QTLs located on chromosomes 8, 9, and 10. The apparent amylose content (AAC), a critical measure dictating the quality of rice starch, is the main target of rice breeders (Bao 2014). Starch, which is a main constituent in the rice endosperm, comprises amylose and amylopectin. Amylose is comparatively less branched α (1, 4) linked glucose polymer. The quest to improve the amylose content in rice formed the main agenda for recent rice breeding programs. The content of amylose in different rice cultivars is different, with indica and japonica rice containing 18–32 and 10–22% amylose, respectively. High amylose content in rice is often linked to dry, fluffy, and separate cooked rice grain (Juliano et al. 1981). It is, therefore, important to improve the starch quality of rice by reducing the amylose content among the high yielding rice cultivars that are of poor quality. The difference in amylose content between indica and japonica rice is attributed to the existence of two types of waxy alleles, wx a and Wx b (Sano 1984). Wang et al. (1995) pointed out that the amylose content in rice endosperm was closely linked to the post-transcriptional regulation of the Wx gene. Analysis of QTLs associated with amylose content...
conducted using a cross between Cheongcheong and Nagdong rice varieties indicated that the two QTLs were located on chromosomes 1 and 9 (Yun et al. 2014). Another genetic study showed a major gene and QTLs for the amylose content on chromosome 6 and 5 (He et al. 1999). The culinary properties of rice are dependent on rheological features. There is a linear relationship between mobility or flow and moisture content of raw rice. The peak viscosity is a quality attribute of rice, which usually is indicative of the quality aspects of rice (Yasumatsu and Fujita 1962). Patindol and Wang (2003) observed that peak viscosity increased with decreasing harvest moisture content (HMC) despite the rate being different among rice cultivars and under different geographical locations in which they were grown. They further noted that the peak viscosity values were significantly greater for medium grain cultivars as compared to those for long grain type. The former contain more amylose content than the latter and rice flour containing more amylose is usually more viscous (Vandeputtea et al. 2003). In a research dubbed as “gelatinization properties of waxy black rice starch”, Choi et al. (2005) noted deviations in the viscous nature that were largely a result of different molecular weights of amylopectin. The rice grain has a quality of adsorbing and desorbing water that depends on the environmental conditions. Desorption of water is linked to the drying of rice. Various studies have been carried out to investigate the drying methods, which lead to high head rice yield (HRY). Adsorption of water entails reintroduction of moisture into the rice grain. It takes place when the vapor pressure in the grain becomes low compared to that in the local environment. Accelerated water reabsorption leads to the fissuring of rice grain. The cracked grains often disintegrate more with further processing procedures. The main objective of any industrial rice plant is the production of maximum quantity of head rice. Studies have shown that fissuring in rice occurs as a result of the kernels being worn out by tension crevices earlier by accelerated movement of water in and out of the rice grain. Rapid moisture adsorption by rice kernels with low moisture content will most likely have detrimental effects on the milling quality and HRY because of kernel fissuring. This, therefore, means that the critical moisture content below which addition of moisture would cause fissuring becomes important (Bautista et al. 2004). According to the study conducted by Kunze and Hall (1965), rice varieties with high birefringence end-point temperature (BEPT), such as century Patna, tend to be more resistant to fissuring compared to those with low BEPT values. Patindol and Wang (2003) observed that the rice flow positively correlated with amylopectin content \( (p < 0.05) \), and sparkling, a phenomena related to the tendency of amylopectin to promote starch granule bulging. Rice grain supplies 21\% of the world human per capita calorie intakes. Increasing health concerns have compelled the rice breeders to put more emphasis on the nutritional quality of rice. Of utmost concern is starch, whose ratio of amylose to amylopectin directly affects the glycemic index (GI), a measure of sugar in human blood. The GI is an important factor in the control of diabetes, which has recently been a major food-related challenge. It is, therefore, critical to determine the genetic basis for rice amylose so as to provide avenues for producing rice with the right GI numbers to satisfy the nutritional demand of consumers. Rice also supplies 15\% of the world human per capita protein intake although it is nutritional quality is low compared to that of other cereals. From the perspective of nutritional quality, rice is known to be insufficient in some essential amino acids (Sautter et al. 2006). There is, therefore, an urgent need to breed rice for increased quantity as well as for nutritional quality. The tendency of rice for adsorption and desorption of water according to the environmental conditions is directly linked to drying. This accelerates the grain breakage during milling. It is, therefore, important to breed rice with relatively high resistance to fissuring.

The objective of the present study was to determine the genetic basis for amylose, protein, and moisture content in rice so as to acquire evidence-based data for marker-assisted selection and for the generation of improved cultivars. The other objectives were to determine the QTLs linked to the amylose, protein, and moisture content in rice, to determine their loss upon milling, to identify the chromosomal location, gene action, and the magnitude of effects of QTLs controlling these traits.

Materials and methods

Cultivation and harvesting of seeds

The ‘Cheongcheong/Nagdong’ doubled haploid (DH) population for constructing a genetic map was developed by another culture of the F1 progeny from a cross between Cheongcheong and Nagdong (Fig. S1). Cheongcheong is a Tongil-type variety showing high yield originated from Oryza nivara, while Nagdong is the leading variety cultivated in the regional area. This population comprised 120 lines, was cultivated on an experimental field at the Kyungpook National University; Gunwi-gun in 2015 and transplanted to the field in May 2015, were spaced 30 × 15 cm apart with one plant taken for each line. All the CNDH lines were planted along with their parents, with only one line per planting row spaced 30 × 15 cm apart.
Sample preparation

Rice samples were prepared following five fundamental operations as suggested by Dela and Khush (2000), who outlined the grain quality evaluation procedures. The steps included cleaning of rough rice to remove leaves, rice stem, and other foreign matters, dehulling of clean rice to remove the husk, cleaning of brown rice to remove the husk remains, milling or polishing of brown rice, and separation of whole grains from broken kernels.

Quality analysis

Dehulling of rice grains to get brown rice and the subsequent measurement of amylose, protein, and moisture content in brown rice, was done using NIRT Grain Tester AN_820 at Gunwi Kyungpook National University Research Center. All the samples were milled to get milled rice and again amylose, protein, and moisture content in brown rice was done using NIRT Grain Tester AN_820.

Genetic linkage map and QTL analysis

Genetic maps consisting of 222 DNA markers were constructed using MAPMAKER/EXP Version 3.0. The distances between the markers were presented in centiMorgans (cM) by Kosambi (1943) junction at a mean interval of 10.6 cM. A complete genetic map prepared at the School of Applied Biosciences, Plant Molecular Breeding Laboratory in Kyungpook National University was utilized. The putative QTLs were identified using composite interval mapping with Win QTL Cartographer 2.5 and an LOD value of 3.0 as the significance threshold to declare a QTL at \( p < 0.05 \). QTL mapper 0.6 was used to detect the main QTL effects, epistatic interactions, and their environmental interactions with a threshold of \( p \leq 0.005 \).

Results

In unpolished rice, the amylose content of CNDH population (17.8) was found to be intermediate between that of the parents, Nagdong (17.9) and Cheongcheong (17.4), but was closer to that of the latter. This shows a high percentage of heritability from Cheongcheong compared to that from Nagdong. However, the protein content of CNDH (7.5) was higher compared to that from Nagdong, showing a higher percentage of heritability from Cheongcheong. The moisture content of CNDH population (11.4) was higher than that of Cheongcheong (10.5) and Nagdong (11.0), as shown in Table S1. This shows high percentage of heritability from Cheongcheong compared to that from Nagdong. These results, therefore, suggest a high percentage of heritability for amylose content trait from Cheongcheong parent compared to that from Nagdong. They also suggests a moderate heritability of protein and moisture content traits of CNDH from Cheongcheong and a higher variation in their contents from parents due to environmental interactions. Using polished rice, in CNDH population, the amylose (17.1) and moisture (10.8) contents were found to be same as that of parent Cheongcheong (17.1 and 10.8, respectively). These results suggest a high percentage of heritability from Cheongcheong. The protein content in CNDH population (6.44 ± 1.0) was found to be higher than in Cheongcheong (5.73 ± 0.2) and Nagdong (5.38 ± 0.5), as shown in Table S1. The results, therefore, suggest variations due to environmental factors among the protein and moisture contents of CNDH and that of parents. Using both polished and unpolished rice, the amylose content showed high evidence of heritability from their parents, whereas the protein content did not show high evidence of heritability in both the cases. The moisture content in unpolished rice was found to be higher to that in the parents, suggesting variations due to environmental factors but was found to be among its parents and providing high evidence of heritability. The results, therefore, suggest that the heritability of amylose and protein content are not affected by milling unlike that of moisture content (Fig. 1).

Using both unpolished and milled rice samples from the same population, we identified a total of four QTLs on chromosome 7 (Table 1; Fig. S2), of which three were from unpolished rice and one was from milled rice. A QTL for protein content (qPC7) identified at RM8261 had the highest variance percentage R2 (14%) and highest LOD value (3.97), compared to the QTL for the amylose content (qAC7) at a flanking marker RM8261 that had a variance percentage R2 of 9% and lowest LOD value of 3.05. Two QTLs for moisture content were identified, both between the flanking marker RM1134–RM420, with lowest variance percentage R2 of 7%; one QTL (qWC7) from unpolished rice had an LOD value of 3.12 and the other (qWC7p) from polished rice samples had an LOD value of 3.48.

We identified a total of 552 DNA markers on a genetic map at marker RM420–RM21972 over a distance of 4 Mb at an average interval of 100 kb located on chromosome 7 (Fig. S3). Among these, 13 markers responsible for amylose content, six for protein content, and three for moisture content were identified. The QTL for amylose content was identified on chromosome 7 (qAC7) at flanking marker RM8261 with a variance percentage of R2 being 9% and
the LOD value being 3.05, and was an allele from Cheongcheong. The QTL for protein content was identified on chromosome 7 (qPC7) at flanking marker RM8261 with a variance percentage of R2 being 14% and the LOD value being 3.97, and was an allele from Cheongcheong. The QTL for moisture content qWC7 was identified at flanking marker RM1134–RM420 that had a variance percentage of R2 (7%) and LOD value of 3.12 from Cheongcheong. We further classified these genes according to their functions into four groups. The first group contained 17 genes (over 77%) that function in secondary metabolites synthesis; all the 13 (i.e., 100%) genes responsible for amylose content, three (i.e., 50%) of the identified genes responsible for protein content, and one (i.e., 33%) of the identified genes responsible for moisture content belonged to this group. The second group included genes involved in cell function; it contained two genes (about 9% of all the identified genes) both (100%) of which were responsible for protein content. The third group comprised two genes (9% of all the identified genes), which were involved in abiotic stress and both were responsible for moisture content. The fourth group contained genes that function in redox signaling and comprised about 5% of the identified genes; one gene in this group was responsible for protein content in the grain and made about 33% of the genes related to the protein content (Table 2). In this study, we identified the mechanism by which genes contribute to the amylose content of grains.

Table 1 The analysis of QTL-related components in CNDH population

| Rice type | Components | QTL   | LOD value | Additive effect | $R^2$ (%) | Flanking marker | Allele     |
|-----------|------------|-------|-----------|-----------------|-----------|----------------|------------|
| Brown     | Amylose    | qAC7  | 3.05      | 2.09            | 9         | RM8261         | Cheongcheong |
|           | Protein    | qPC7  | 3.97      | 1.14            | 14        | RM8261         | Cheongcheong |
|           | Water      | qWC7  | 3.48      | 1.17            | 7         | RM1134-RM420   | Cheongcheong |
| Milled    | Water      | qWC7  | 3.12      | 1.19            | 7         | RM1134-RM420   | Cheongcheong |

The content of protein, amylose, and moisture were investigated using brown rice (I, II, and III) and milled rice (IV, V, and VI). The white (A) and black (B) arrows represent Cheongcheong and Nagdong, respectively.
and found the involvement of genes of secondary metabolite and pyruvate synthesis pathway, pyruvate being the key substrate for amylose synthesis (Fig. 2).

Table 2 Functional classification of genes related to amylose, protein, and moisture content

| Function                  | Gene                                                                 | No. of genes | Related QTL |
|---------------------------|----------------------------------------------------------------------|--------------|-------------|
| Abiotic stress            | Glycosyltransferase AER61                                           | 1            | qWC7        |
|                           | Glycosyltransferase, family 8 protein                               | 1            | qWC7        |
| Secondary metabolites     | Similar to thioredoxin reductase (chloroplast detoxification)       | 1            | qWC7        |
| synthesis                 | Similar to 60S ribosomal protein L22-2                              | 1            | qPC7        |
|                           | Similar to 60S ribosomal protein L18                                 | 1            | qPC7        |
|                           | Similar to 60S ribosomal protein L19-3                               | 1            | qPC7        |
|                           | Similar to short-chain dehydrogenase/reductase fragment              | 3            | qAC7        |
|                           | Short-chain dehydrogenase/reductase SDR domain-containing protein    | 4            | qAC7        |
|                           | Aldehyde dehydrogenase domain-containing protein                     | 1            | qAC7        |
|                           | Similar to oxidoreductase, short-chain dehydrogenase/reductase family protein, expressed | 5            | qAC7        |
| Redox signaling           | Similar to thioredoxin reductase                                    | 1            | qPC7        |
| Cell function             | Ribosomal protein S8e domain-containing protein                      | 1            | qPC7        |
|                           | Ribosome biogenesis factor, NIP7 domain-containing protein           | 1            | qPC7        |

Fig. 2 Function of genes related to amylose, protein, and moisture content

Discussion

The present study described the heritability analysis of the main constituents in polished and unpolished rice. The frequency of distribution of amylose, protein, and moisture contents in polished and non-polished CNDH population of rice were determined using NIRS as indicated in Fig. 1 and Tables 1 and 2. In unpolished rice, the amylose content in the CNDH population (17.8) was found to be intermediate between that of the parents, Nagdong (17.9) and Cheongcheong (17.4), but was closer to the content in Cheongcheong. This shows a high percentage of heritability from Cheongcheong parent than from Nagdong. However, the protein content of CNDH (7.5) was higher compared to that of the parents, Cheongcheong (6.6) and Nagdong (6.2), but was close to that of Cheongcheong, revealing a high percentage of heritability from the parent Cheongcheong than from Nagdong. The moisture content of CNDH population (11.4) was higher than that of Cheongcheong (10.5) and Nagdong (11.0), as shown in Table 1, indicating a high percentage of heritability from the Cheongcheong parent than from Nagdong. These results, therefore, suggests a high percentage of heritability for the amylose content trait from Cheongcheong parent rather than from Nagdong. It also suggests a moderate heritability of protein and moisture content traits of CNDH from the parent Cheongcheong and also a higher variation in their contents compared to that in the parents due to environmental interaction. The amylose content had high heritability and genetic advance suggesting that selection for this trait would improve the genotypic value of the selected plants over those of their parents. These results lend credence to the work conducted by Thongbam et al. (2012) and later by Nirmaladevi et al. (2015), wherein they noted a high amylose content heritability and genetic advance and also suggested that selection for this trait would improve the genotypic value of the selected plants over those of their parents. They further pointed out that the protein content heritability was moderately high while genetic advance was very low due to low genotypic variation and influence of environmental factors. Similarly, Ashwini Samak et al. (2011) estimated the heritability and genetic advance of F5 generation for total rice grain protein content and observed an insignificant increase compared to the F4 generation. The existence of duplicate gene interaction in the expression of total protein content of grain indicates that mild selection can be
employed for such a trait in the segregating generation. Shejul et al. (2013) recorded high value of heritability for moisture absorption (92.30%) and amylase content (67.33%). However, the protein content showed a lower heritability. The high heritability signified that the concerned traits were least affected by environmental modifications and that a selection based on phenotypic performance would be reliable.

Using polished rice, the amylose (17.1) and moisture (10.8) contents in the CNDH population were found to be same as that in the parent Cheongcheong (17.1 and 10.8 for amylose and moisture, respectively). This suggests a high percentage of heritability from Cheongcheong. The protein content in the CNDH population (6.44 ± 0.96) was found to be higher than that in Cheongcheong (5.73 ± 0.16) and Nagdong (5.38 ± 0.5), as shown in Table 2. The results, therefore, suggest variations among the protein content and moisture contents in CNDH and parents due to environmental factors.

### Analysis of QTLs

Using both unpolished and milled rice samples from the same population, we identified a total of four QTLs on chromosome 7; three QTLs were identified from unpolished rice whereas one was identified from milled rice. A QTL for protein content (qPC7) identified at RM8261 had highest variance percentage R2 (14%) and highest LOD value (3.97), compared to the QTL for amylose content (qAC7) at the same flanking marker RM8261 that had a variance percentage R2 of 9% and lowest LOD value of 3.05. Two QTLs for the moisture content were identified between the flanking marker RM1134–RM420, with the lowest variance percentage R2 of 7%, one (qWC7) using unpolished rice with an LOD value of 3.12 and the other (qWCp7) with and LOD value of 3.48 from the polished rice samples. This result is different from that reported by Yun et al. (2014), who analyzed QTLs associated with the amylose content using a cross between Cheongcheong and Nagdong and found two QTLs located on chromosome 1 and 9, and the one reported by He et al. (1999), who identified important QTLs and genes responsible for amylose content on chromosome 6 and 5. The analysis of QTLs responsible for protein content in unpolished rice indicated a QTL on chromosome 7; this QTL, qPC7 was located at marker RM8261 on chromosome 7 and had an LOD of 3.97. This result is consistent with that of Bao (2014) who used nine rice varieties and found out that chromosomes 1, 2, and 7 harbored more QTLs for protein content. However, it is different from the results of Yun et al. (2014) who used the same variety as described in this study but found three QTLs on chromosomes 10, 9, and 8. Two QTLs linked to the moisture content were identified; qWC7 in unpolished rice located at marker RM1134–RM420 on chromosome 7 with an LOD of 3.12 and qWC7 located at marker RM1134–RM420 on chromosome 7 with an LOD of 3.48 were identified in polished rice as indicated in Table 1. These results, therefore, indicate the existence of differences in the QTLs, markers, and LODs among the polished and unpolished rice grains of the same population.

### Chromosomal location of QTLs on physical map

We identified a total of 552 DNA markers on a genetic map at marker RM420–RM21972 over a distance of 4 megabases at an average interval of 100 kb located on chromosome 7. Among these, 13 markers responsible for amylose content, six for protein content, and three for moisture content were identified.

We further classified these genes according to their functions and found that 17 genes (over 77%) function in secondary metabolites synthesis, two genes (about 9%), each were involved in expression of cell function and abiotic stress, and one gene (about 5%) functions in redox signaling. We also constructed three physical maps for amylose, protein, and moisture content with a total of 22 DNA markers on chromosome 7.

### Conclusion

A CNDH population derived from a cross between Cheongcheong, a Tongil variety, and Nagdong, a japonica variety, was used to map the QTLs associated with rice amylose, protein, and moisture content with the aim of providing reliable information for marker-assisted selection and development of new varieties. We conducted heritability study of CNDH population from their parents using polished and unpolished rice samples and found higher heritability percentage for amylose content and compromised heritability for protein and moisture contents. We also identified a total of 22 DNA markers on a genetic map at marker RM420–RM21972 with a distance of 4 megabases at an average interval of 100 kb located on chromosome 7, among which 13 markers responsible for the amylose content were located at RM 8261, six for protein content were located at RM 8261, and three responsible for moisture content were located at RM1134–RM420. We further classified these genes according to their functions and found that 17 genes (over 77%) function in secondary metabolites synthesis, two genes (about 9%), each, were involved in expression of cell function and abiotic stress, and one gene (about 5%) functions in redox signaling. We also constructed three physical maps for amylose, protein, and moisture content with a total of 22 DNA markers on chromosome 7.
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Compliance with ethical standards

Conflict of interest None declared.

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