Abstract  The amino acid composition of rice is a major concern of rice breeders because amino acids are among the most important nutrient components in rice. In this study, a genetic map was constructed with a population of 134 recombinant inbred lines (RILs) from a cross between Dasan-byeo (Tongil-type indica) and TR22183 (temperate japonica), as a means to detect the main and epistatic effect quantitative trait loci (QTLs) for the amino acid content (AAC). Using a linkage map which covered a total of 1458 cM based on 239 molecular marker loci, a total of six main-effect QTLs (M-QTLs) was identified for the amino acid content (AAC). Using a linkage map which covered a total of 1458 cM based on 239 molecular marker loci, a total of six main-effect QTLs (M-QTLs) was identified for the amino acid content (AAC). For all the M-QTLs, TR22183 allele increased the trait values. The QTL cluster (flanked by id3015453 and id3016090) on chromosome 3 was associated with the content of five amino acids. The phenotypic variation, explained by the individual QTLs located in this cluster, ranged from 10.2 to 12.4%. In addition, 26 epistatic QTLs (Ep-QTLs) were detected and the 25 loci involved in this interaction were distributed on all nine chromosomes. Both the M-QTLs and Ep-QTLs detected in this study will be useful in breeding programs which target the development of rice with improved amino acid composition.

Keywords  Quantitative trait loci, Amino acid content, rice, QTL mapping

Introduction

Rice (Oryza sativa L.) is one of the most important staple cereal crops for more than 50% of the world’s population (Mather et al. 2007). A total of 85% of rice production is consumed by humans, and rice provides 21% of the global human per capita energy and 15% of the per capita protein. Amino acids are the principal building blocks of enzymes and other proteins (Panthée et al. 2006), and protein content and amino acid content (AAC) are key factors in the nutritional quality of rice. Thus, improvement of these factors is a major breeding objective in the effort to meet the food demands of an increasing global population. The 20 amino acids have been classified into two groups, essential and non-essential. Essential amino acids, which cannot be synthesized by animals, need to be taken up from plants or other organisms (D’Mello 2003). Non-essential amino acids are also important because they are required for the synthesis of essential amino acids and other biochemical compounds (Wen et al. 2009). Therefore, genetic knowledge regarding non-essential amino acids will also be beneficial to balancing amino acid composition and improving protein quality. Because rice protein content differs greatly among varieties (Maclean et al. 2002), it is important to utilize rice varieties containing high levels of essential amino acids as genetic resources to breed for improved protein content. The average protein level of the 17 587 cultivars of unmilled (brown) rice in the International Rice Research Institute (IRRI) germplasm collection was 9.5%, and ranged from 4.3 to 18.2%. This phenotypic variation in protein content provides a useful genetic basis for breeding.

Protein content and AAC in crop seed have been confirmed to be inherited as quantitative traits with a complex genetic basis that was controlled by multiple genes (Munck et al. 2001). Identification of the genes controlling AAC is important to facilitate breeding for improved amino acid composition. There have been several studies on the mapping of quantitative trait locus (QTL) controlling AAC in soybeans (Panthée et al. 2006), wheat (Jiang et al. 2013), cotton (Quampah et al. 2012; Liu et al. 2012; Liu et al. 2013), and rapeseed (Wen et al. 2014); however, few studies have
been performed to identify QTLs for AAC in rice (Wang et al. 2008; Zheng et al. 2008; Zhao et al. 2009; Lu et al. 2009). These studies have provided useful genetic information for improving the nutritional quality of rice. QTLs underlying AAC have been identified mainly by using the linkage mapping approach with various mapping populations, such as F$_2$, back crosses, doubled haploids, recombinant inbred lines (RIL), and near isogenic lines, whereas linkage disequilibrium (LD)-based association analysis also has been conducted to identify single sequence repeat markers related to marker-trait associations (Zhao et al. 2009).

In this study, we conducted QTL analysis with RILs from a cross between indica and japonica rice varieties and detected the main effect and epistatic effect QTLs for AAC. The main-effect QTLs, including QTL clusters detected on chromosome 3 and various digenic interactions, will provide valuable genetic information for the improvement of amino acid quality by breeders.

Materials and Methods

Plant materials and field trials

A total of 172 F$_{14}$ RILs, derived from a cross between Dasanbyeo and TR22183 (DT-RILs), were developed using the single seed descent method at the Seoul National University in Korea (Jiang et al. 2011). Of the total number of plants, 134 RILs successfully maintained in Korea were used for QTL analysis associated with amino acids. The plant materials together with the parental lines were seeded at the Seoul National University Experimental Farm in Suwon on plastic tunnel seedbeds during the last week of April 2015. A month after seeding, the plants were transplanted to one seedling per hill at a planting density of 30 × 15 cm. The DT-RILs were cultivated under normal fertilizer conditions (N–P$_2$O$_5$–K$_2$O = 100–80–80 kg/ha) and irrigation. The rice field was regularly irrigated to avoid drought stress to the late-maturing RIL lines. Six plants from the middle row of each RIL were selected for data collection. Phenotyping was performed using 134 RILs grown in Korea.

Measurement of amino acids

Amino acid analysis was performed at the National Instrumentation Center for Environmental Management at the Seoul National University, Korea. AAC was determined using the hydrolysis/high performance liquid chromatography (HPLC) method (Henderson et al. 2000). Approximately 100 mg of each brown rice sample was collected, to which 40 mL of 6 mol/L HCl was added and hydrolysis was conducted for 24 h at 110°C. Samples were analyzed by HPLC (Dionex Ultimate 3000, Thermo Fisher Scientific) after pre-injection derivatization (Henderson et al. 2000). The primary amino acids were reacted first with O-phthalaldehyde (OPA) and the secondary amino acids were derivatized with fluorenylmethyl chloroformate (FMOC) before injection. The content of each amino acid in each hydrolysate was calculated by reference to the standard amino acid and expressed as g/kg rice powder. All values are given on a dry matter basis. The levels of 16 amino acids of rice grains (Ala = Alanine, Arg = Arginine, Asp = Aspartic acid, Glu = Glutamic acid, Gly = Glycine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Pro = Proline, Phe = Phenylalanine, Val = Valine, Tyr = Tyrosine, His = Histidine, Thr = Threonine, and Ser = Serine) were obtained using this method.

Genotyping

Genomic DNA was extracted from the RILs, derived from a cross between Dasanbyeo and TR22183, using the DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer’s protocol (www.qiagen.com) for single nucleotide polymorphism (SNP) genotyping. The Illumina GoldenGate assay (Fan et al. 2003) was performed using VeraCode technology on the BeadXpress Reader, as per the manufacturer’s protocol. Briefly, approximately 100 ng of genomic DNA was used for gDNA biotinylation, which then underwent oligonucleotide hybridization by binding the samples to paramagnetic particles, followed by an allele-specific extension and ligation, PCR, hybridization to the VeraCode Bead Plate, and finally a scan on the BeadXpress Reader. The analysis was employed with the VC0011439-OPA set of 384-SNP markers, which were designed to be informative across indica and japonica germplasm (Thomson et al. 2012), and was run at the Genotyping Services Lab at IRRI (Thomson 2014; http://gsl.irri.org). The raw hybridization intensity data processing was performed using the genotyping module in the BeadStudio package (Illumina, San Diego, CA, USA), and allele calling was conducted using ALCHEMY software (Wright et al. 2010).

Linkage mapping and QTL identification

A total of 239 polymorphic SNP markers from the 384-plex BeadXpress™ indica × japonica assays (Thomson et al. 2012) were used for the marker analyses in DT-RILs. Linkage
map construction was completed using SNP markers polymorphic between parents, Dasanbyeo and TR22183, using ICIM v3.2 (Meng et al. 2015). Markers having a logarithm of odds (LOD) of at least 3.0 based on the Kosambi function were selected for linkage map construction. Inclusive composite interval mapping (ICIM) was applied using the QTL ICIM v3.2 to identify additive QTLs for AAC (Meng et al. 2015). A permutation test was performed with 1 000 iterations to identify putative QTLs. Epistatic QTLs (Ep-QTLs) and digenic interactions were identified by ICIM v3.2 with an LOD value of at least 4.0. Additive effects, as well as percentages of variations explained (PVE) by each QTL and EpQTL, were estimated by ICIM.

Results

Phenotypic variations

The phenotypic values of the 16 traits studied are given in Table 1, including individual AAC. The results of a t-test indicated that, except for the content of Met and Pro, there were significant phenotypic differences between the two parents for the remaining 14 components of AAC. Interestingly, the AAC of TR22183 was higher than that of Dasanbyeo for all amino acids. The RIL population exhibited an almost normal distribution for all amino acids (Fig. 1), indicating a quantitative mode of inheritance of AAC (Zheng et al. 2008). All 16 traits expressed transgressive segregation in both directions.

To determine if correlations existed between amino acids, correlation coefficients were calculated for RILs. Significant positive correlations between amino acid components were detected in the RILs (Table 2). The highest correlation was observed between Val and Leu, whereas Pro exhibited relatively low correlations with the other amino acids.

Mapping of main effect QTLs

A total of six main effect QTLs (M-QTLs) were identified for six components of AAC in brown rice (Table 3), and the phenotypic variation explained by individual QTLs ranged from 10.2 to 12.8%. Among these six M-QTLs, the QTL qVal3 and qLeu3 located in the interval id3015453–id3016090 exhibited the highest heritability, explaining 12.4% of the phenotypic variation for Val and Leu. Alleles of all six QTLs detected with increasing AAC came from TR22183. Of the six QTLs, five (qAla3, qVal3, qPhe3, qIle3, qLeu3, etc.)

Table 1 Variation in amino acid content (g/kg) for DT-RILs and their parents

| Trait | Parents (g/kg) | RIL population (g/kg) |
|-------|----------------|-----------------------|
|       | Dasanbyeo | TR22183 | Mean | Min | Max |
| Val   | 3.64      | 4.43**  | 4.02 | 2.53 | 6.02 |
| Met   | 0.89      | 0.90    | 1.04 | 0.16 | 2.25 |
| Phe   | 3.23      | 4.11**  | 3.75 | 2.31 | 5.71 |
| Ile   | 5.09      | 6.24**  | 5.82 | 3.75 | 8.51 |
| Leu   | 2.50      | 3.07*   | 2.75 | 1.68 | 4.19 |
| Lys   | 1.63      | 1.87*   | 2.12 | 1.28 | 3.49 |
| Thr   | 3.12      | 3.54*   | 3.44 | 1.98 | 4.92 |
| Asp   | 5.55      | 7.13**  | 6.62 | 4.45 | 9.94 |
| Glu   | 12.25     | 15.67** | 14.34| 9.62 | 21.57|
| Ser   | 3.30      | 4.20**  | 3.85 | 1.11 | 5.70 |
| His   | 1.54      | 1.91**  | 1.77 | 1.18 | 2.53 |
| Gly   | 2.63      | 3.15*   | 3.16 | 2.30 | 4.62 |
| Arg   | 4.91      | 6.87**  | 6.04 | 3.71 | 9.15 |
| Ala   | 3.78      | 4.42**  | 4.35 | 3.01 | 6.13 |
| Tyr   | 1.04      | 1.46**  | 1.53 | 0.81 | 3.21 |
| Pro   | 2.37      | 2.42    | 2.63 | 0.73 | 4.78 |

* and ** indicate significant differences of target traits between two parents at $P = 0.05$ and $P = 0.01$ according to the t-test, respectively.

*represents essential amino acid.

*represents non-essential amino acid.
Fig. 1 Distribution of the amino acid content of DT-RILs. D and T represent Dasanbyeo and TR22183, respectively. PVE is the proportion of the total phenotypic variation explained.

Table 2 Correlation coefficients for amino acid content in DT-RILs

|      | Asp | Glu | Ser | His | Gly | Thr | Arg | Ala | Tyr | Val | Met | Phe | Iso | Leu | Lys | Pro |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp  |     | 0.96*** |    |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu  | 0.89*** |     | 0.90*** |    |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser  | 0.94*** | 0.90*** | 0.87*** |    |     |     |     |     |     |     |     |     |     |     |     |     |
| His  |     |     |     | 0.93*** | 0.90*** | 0.88*** | 0.92*** |    |     |     |     |     |     |     |     |     |
| Gly  | 0.83*** | 0.81*** | 0.89*** | 0.76*** | 0.80*** |    |     |     |     |     |     |     |     |     |     |     |
| Thr  | 0.90*** | 0.92*** | 0.83*** | 0.89*** | 0.88*** | 0.83*** | 0.75*** |    |     |     |     |     |     |     |     |     |
| Arg  | 0.93*** | 0.96*** | 0.78*** | 0.86*** | 0.87*** | 0.76*** | 0.91*** |    |     |     |     |     |     |     |     |     |
| Ala  | 0.68*** | 0.70*** | 0.67*** | 0.60*** | 0.59*** | 0.68*** | 0.81*** | 0.66*** |    |     |     |     |     |     |     |     |
| Tyr  | 0.89*** | 0.92*** | 0.76*** | 0.90*** | 0.81*** | 0.64*** | 0.93*** | 0.90*** | 0.61*** |    |     |     |     |     |     |     |
| Val  | 0.57*** | 0.62*** | 0.45*** | 0.62*** | 0.53*** | 0.18* | 0.70*** | 0.60*** | 0.47*** | 0.77*** |    |     |     |     |     |     |
| Met  | 0.94*** | 0.97*** | 0.88*** | 0.92*** | 0.87*** | 0.77*** | 0.97*** | 0.93*** | 0.73*** | 0.96*** | 0.70*** |    |     |     |     |     |
| Phe  | 0.94*** | 0.99*** | 0.87*** | 0.91*** | 0.87*** | 0.78*** | 0.95*** | 0.96*** | 0.71*** | 0.95*** | 0.67*** | 0.99*** |    |     |     |     |
| Iso  | 0.88*** | 0.91*** | 0.76*** | 0.89*** | 0.80*** | 0.62*** | 0.93*** | 0.89*** | 0.61*** | 1.00*** | 0.78*** | 0.96*** | 0.95*** |    |     |     |
| Leu  | 0.74*** | 0.67*** | 0.57*** | 0.76*** | 0.81*** | 0.50*** | 0.59*** | 0.67*** | 0.30*** | 0.64*** | 0.51*** | 0.65*** | 0.65*** | 0.64*** |    |     |
| Lys  | 0.53*** | 0.44*** | 0.32*** | 0.48*** | 0.34*** | 0.29*** | 0.39*** | 0.41*** | 0.31*** | 0.44*** | 0.27*** | 0.42*** | 0.43*** | 0.43*** | 0.52*** |    |

* and *** indicate significant difference in correlation coefficients between two traits at P = 0.05 and P = 0.001 according to the t-test, respectively. Abbreviations are described in the legend for Table 1.
and qLeu3) were co-localized in the interval of id3015453–id3016090, accounting for phenotypic variation in the range of 10.2 to 12.8% for the amino acids. Because these QTLs displayed a similar limit of detection (LOD 3.1–3.6) in this interval (Table 3), it could be assumed that a common QTL exhibited a pleiotropic effect on all five of these amino acids, accounting for 10.2, 12.4, 10.6, 12.4, and 10.8% of the phenotypic variation for Ala, Val, Phe, Ile, and Leu, respectively. The QTL qLys3 identified in the interval id3001422–fd10 explained 10.8% of the phenotypic variation for the essential amino acid Lys.

### Digenic interactions

A total of 26 digenic interactions were identified for the content of seven amino acids by composite interval mapping with an LOD value of at least 4.0, but no interaction existed for the other nine amino acids (Table 4). A total of 26 E-QTL pairs were detected for Asp, Ser, His, Gly, Arg, Ala, and Tyr involving 25 loci distributed on the nine

### Table 3 QTLs identified for amino acid content in DT-RILs

| Trait | Chr | QTL | Left Marker | Right Marker | LOD | PVE (%) | Adda |
|-------|-----|-----|-------------|--------------|-----|--------|------|
| Ala   | 3   | qAla3 | id3015453   | id3016090    | 3.0 | 10.2   | -180.8 |
| Val   | 3   | qVal3 | id3015453   | id3016090    | 3.6 | 12.4   | -233.2 |
| Leu   | 3   | qLeu3 | id3015453   | id3016090    | 3.6 | 12.4   | -165.8 |
| Iso   | 3   | qIso3 | id3015453   | id3016090    | 3.3 | 11.2   | -300.6 |
| Phe   | 3   | qPhe3 | id3015453   | id3016090    | 3.1 | 10.6   | -205.9 |

aThe negative sign (-) before additive effect indicates the allele from TR22183 increased the value of the trait. PVE is the proportion of the total phenotypic variation explained.

### Table 4 Digenic interaction for content of the amino acids by composite interval mapping for LOD ≥ 4

| Traits | Chr | Interval i | Chr | Interval j | LOD | PVE (%) | Aia | Aj | AAij |
|--------|-----|------------|-----|------------|-----|--------|-----|----|------|
| Asp    | 3   | id3001422-fd10 | 5   | id5013798-id5000043 | 5.4 | 16.72  | -513.8 | -290.6 | 699.3 |
| Ser    | 10  | id3005194-id3005824 | 10  | id3005824-id3006872 | 4.1 | 4.61   | -627.2 | 558.7 | 800.0 |
| His    | 5   | id3013798-id5000043 | 11  | id11001652-id11008929 | 4.0 | 10.35  | 38.1  | 40.1 | -182.0 |
| Lys    | 7   | id7003591-id7004065 | 12  | id12001043-id12001567 | 4.0 | 10.8   | -70.5 | 7.7  | 107.1 |
| Gly    | 5   | id3001422-fd10 | 9   | id9007328-id9007180 | 4.0 | 9.54   | -144.7 | -136.3 | 259.2 |
| Arg    | 5   | id5013798-id5000043 | 10  | id5000811-id5001423 | 4.1 | 15.92  | -207.3 | -252.4 | 601.9 |
| Ala    | 5   | id3001422-fd10 | 5   | id5013798-id5000043 | 4.2 | 15.44  | -135.0 | -58.7 | 194.8 |
| Tyr    | 4   | id4002032-id4012501 | 7   | id7004870-id7005137 | 4.6 | 0.47   | 150.8  | -150.7 | -480.2 |
| Tyr    | 11  | id1007562-id1001764 | 10  | id4006867-ud4001552 | 4.6 | 0.47   | -213.3 | 160.5 | -196.3 |
| Tyr    | 3   | id3001422-fd10 | 8   | id8000795-id8003383 | 4.3 | 0.54   | -137.0 | -236.1 | 218.6 |
| Tyr    | 10  | id4006867-ud4001552 | 5   | id5000811-id5001423 | 4.7 | 0.49   | -80.7  | 54.1  | -489.0 |
| Tyr    | 6   | id6000073-id6010766 | 6   | id6000073-id6010766 | 4.7 | 0.51   | 260.7  | -230.7 | 227.6 |
| Tyr    | 1   | id1007562-id1001764 | 6   | id6000073-id6010766 | 4.2 | 0.54   | -219.3 | -175.5 | 276.0 |
| Tyr    | 5   | id5013798-id5000043 | 7   | id7004870-id7005137 | 4.6 | 0.47   | 150.8  | -150.7 | -480.2 |

aThe negative sign (-) before additive effect indicates the allele from TR22183 increased the value of the trait. Aia and Aj are additive effects of loci i and j, respectively. AAij is the effect of additive by additive interactions between loci i and j.
chromosomes (Table 4). One digenic interaction was identified for Asp, Ser, Arg, and Ala, explaining 16.72, 4.61, 15.92 and 17.53% respectively. Two and three digenic interactions were identified for Gly and His, explaining 21.27% and 30.49% of the total variation, respectively. Interestingly, 17 digenic interactions were identified for His, explaining 8.76% of the total variation with relatively low individual phenotypic variation values of 0.43-0.61%. Among these interactions, only one M-QTL located in the id3001422-fd10 interval was involved in epistatic interactions, but the other locus (id3015453-id3016090) was not involved in any digenic interaction.

Discussion

Genetic improvement of nutritional quality is an important target in crop breeding programs, and protein content and amino acid composition in rice are the most important contributors to rice nutrient quality. Although several studies have been conducted to identify QTLs underlying the amount of each amino acid, the genetic basis for amino acid composition has not been fully elucidated for rice.

In this study, using an interval-mapping approach, we identified six M-QTLs located on chromosome 3 and 26 epistatic QTLs distributed on nine chromosomes, in which 25 loci were involved in digenic interactions. One of the noticeable results of this study was that most M-QTLs detected were co-localized within the genome. QTL analysis using ICIM for AAC identified six M-QTLs in two loci on chromosome 3, but no significant QTLs were identified in other chromosomes. Among them, five out of six QTLs were located in the same interval (id3015453-id3016090). A common QTL for Leu, Ile, and Val was identified in the interval id3015453-id3016090 on chromosome 3, in agreement with the fact that the essential amino acids Val, Leu, and Ile, which are branched-chain amino acids, have a common biosynthetic pathway (Lu et al. 2009). QTLs for Phe, another essential amino acid, and Ala, a non-essential amino acid, were also identified in the same interval (id3015453-id3016090) on chromosome 3. Similar QTL clusters were previously identified. Wang et al. (2008) identified 10 QTL clusters where more than one QTLs were co-localized in the genome, and the two QTL clusters located in chromosome 1 (flanked by RM472-RM104) and chromosome 8 (flanked by MRG2572-RM544) contained Val, Leu, Ile, Ala, and Phe. Additionally, we also identified qLys3 for Lys in a different interval (id3001422-fd10) from the other QTLs on the same chromosome. Lysine was identified as the first limiting essential amino acid of milled rice protein, in terms of human requirements, by the World Health Organization (1973). Thus, qLys3 identified for Lys and the QTL cluster for several essential amino acids, such as Val, Leu, Ile, and Phe will be very valuable to breeders for the improvement of the nutrient value of rice.

A QTL cluster is a valuable genetic resource to simultaneously introduce useful gene(s) underlying several promising traits into a recipient variety. However, the overall performance of the variety must be considered for successful plant breeding. Thus, we must ensure that the target traits are not accompanied by negative effects from other traits. This requires information on the QTL regions controlling other agronomical traits in the variety under study. We previously performed QTL analysis for yield-related traits for the same DT-RILs (Navea et al. 2017) and found qGN3.1 (flanked by fd10-id3015453) for grain number, which is located near the QTL cluster (id3015453-id3016090) identified in this study. However, Dasanbyeo contributed to the increase of grain number, whereas the improvement of AACs was contributed by TR22183, indicating that the genomic region underlying AACs in TR22183 is negatively linked to grain number. Thus, these two QTLs must be separated if they are closely linked, and if the effects are not pleiotropic, when this QTL is used in breeding. Further analysis needs to be performed to elucidate the genetic basis for the regulation of both AACs and grain number.

In addition to the six M-QTLs, we identified a total of 26 additive × additive interactions for AACs on the entire genome with LOD values of at least 4.0. Obviously, the number of E-QTLs and phenotypic variation explained by epistatic QTLs for traits was greater than that of the M-QTLs, indicating that epistasis also plays an important role in controlling the expression of AAC. Thus, these epistatic effects need to be considered when these QTLs are used in breeding programs. Furthermore, validation of the putative QTL effects detected in the present study is necessary in various environments and populations before applying these QTLs as molecular markers.

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