

Identification and characterization of QTLs and QTL interactions for Macro- and Micro-elements in rice (*Oryza sativa* L.) grain

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ABSTRACT Improvement of the macro- and micro-elements density of rice (*Oryza sativa* L.) is gradually becoming a new breeding objective. In this study, the genomic regions associated with potassium, calcium, magnesium and iron content in rice grain were identified and characterized by using a doubled haploid (DH) population. Fifty-six simple sequence repeat (SSR) and one hundred and twelve sequence tagged site (STS) markers were selected to construct the genetic linkage map of the DH population with a full length of 1808.3cM scanning 12 rice chromosomes. Quantitative trait loci (QTLs) were detected, and QTL effects and QTL interactions were calculated for five traits related to macro- and micro-elements in the DH population from a cross between 'Samgang' (*Tongil*) and 'Nagdong' (*Japonica*). Twelve QTLs were located on five chromosomes, consisting of two QTLs for potassium, three QTLs for calcium, two QTLs for magnesium, one QTL for iron content and four QTLs for the ratio of magnesium to potassium (Mg/K). Among them, qca1.1 was detected on chromosome 1 with an LOD value of 8.58 for calcium content. It explained 27% of phenotype variations with increasing effects from 'Samgang' allele. Furthermore, fifteen epistatic combinations with significant interactions were observed on ten chromosomes for five traits, which totally accounted for 4.19% to 12.72% of phenotype variations. The screening of relatively accurate QTLs will contribute to increase the efficiency of marker-assisted selection (MAS), and to accelerate the establishment of near-isogenic lines (NILs) and QTL pyramiding.

Introduction

The deficiency of micronutrients will result in adverse metabolic disturbances leading to sickness, poor health, impaired development in children, and large economic costs to society. Most of the micronutrients which humans require come from agricultural products (Graham et al. 1999). Among them, rice is the most important food crop and the staple food for 40 percent of the world population. To increase the density of micronutrients in rice grains, either traditional rice breeding methods or molecular biological techniques is an important

breeding objective in the present and the future.

Previous researches were generally inclined to focus on the physiological mechanism of biosynthesis and transportation of trace elements (Connolly et al. 2002; Hall et al. 2003), but a little genetic exploration had been done in the past few years. In recent years, transgenic strategies have been applied to breed new varieties with enrichment of micronutrients, so as to reduce malnutrition of most women, infants and children. The 'golden rice', genetically modified rice engineered to produce pro-vitamin A, is a good example. It was considered a significant breakthrough in biotechnology as the researchers had engineered an entire biosynthetic pathway (Welch et al. 2004).

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At the same moment, the traditional breeding method was also encouraged to implement and develop micronutrient-enriched rice varieties, even though little progress was made. Genetic mapping of the existing variations will allow marker-assisted breeding to exploit the available natural variation in rice. For this application, ultimately the knowledge of the genes underlying this quantitative variation, called quantitative trait loci (QTL), will be required (Ghandilyan et al. 2006). QTL detection can provide the information on the chromosomal locations of the putative loci and reveal their possible effects leading to the phenotypes of interest.

So far, a typical instance through the traditional breeding to improve the iron and zinc content in brown rice had been administered. As a result, two elite lines with iron-enriched were selected (Gregorio et al. 2000). In addition, fourteen QTLs associated with potassium and magnesium were detected by Joe et al. (2006) using a recombinant inbred population from a cross between 'Milyang23' and 'Giho' in Korea. In the present study, the QTLs and QTL interactions related to potassium, calcium, magnesium and iron content of brown rice using a double-haploid population from a cross between 'Samgang' and 'Nagdong' were identified and characterized, which aimed to isolate the micronutrient-enriched lines and to launch MAS and QTL pyramiding in the future research.

Materials and methods

Plant materials and field trial

One hundred and twenty rice DH lines developed by anther culture of the F_1 from a cross between 'Samgang', a *Tongil* variety, and 'Nagdong', a *japonica* variety, were used as a mapping population in this study. The current study was carried out in the rice-growing season on the experimental fields at Kyungpook National University in Gunwie, Korea in 2005. About 30-day-old seedlings of each DH line followed the spacing pattern of 30 cm × 15 cm between rows and plants. For each DH line, three rows with 20 plants per row were planted. The soil fertilities of the planting plots were similar, and two parents were grown for every ten DH lines

as controls. Field management was conducted according to the normal cultivation practices recommended by the Rural Development Administration (RDA) of Korea with an application for fertilizer at the rate of 110 kg N ha⁻¹, 45 kg P₂O₅ ha⁻¹, and 57 kg K₂O ha⁻¹.

Trait measurement

The paddy rice grains were collected from the middle row of three rows of each DH line and parent and dried naturally. The moisture of brown rice was controlled to 13-14%. After hulling the paddy rice, the damaged grains, red grains, green grains and broken grains were removed from the brown rice before the test. The macro- and micro-elements consisting of potassium (K), calcium (Ca), magnesium (Mg) and Iron (Fe) were measured by an inductively coupled plasma atomic emission spectrometer (ICP-AES) instrument. The microwave digestion method and the operational parameters for ICP-AES were followed as described by Phuong et al. (1999) and Jung et al. (2005).

Map construction and QTL detection

A total of 172 DNA markers consisting of 56 simple sequence repeat (SSR) and 112 sequence tagged site (STS) markers were used to create a genetic linkage map using MAPMAKER/EXP, version 3.0 (Lander et al. 1987). The composite interval mapping (CIM) was operated for the QTL detection by WinQTLcart 2.5 at a threshold of *LOD* 2.5 (Zeng 1994, Yang et al. 1999). QTL interactions were identified by a program of QTLMAPPER version 1.6 (Wang et al. 1999) with a threshold of the $P \leq 0.005$.

Data analysis and QTL nomenclature

Correlation coefficient and t-test of macro- and micro-elements in the DH population were calculated by the program of SAS 9.1. QTL nomenclature was as follows: Start each qtl with a lowercase "q". Use a 2-3 letter name in small letters to designate the trait actually measured (the abbreviation of Latin for each trace element). Add a dot (.) and a number to

the end of the chromosome to designate the numerical identifier of QTL for the same trait which is identified on the same chromosome.

Results

Genetic linkage map

The full length of the genetic linkage map is 1808.3 cM spanning the 12 rice chromosomes with an average interval of 10.51 cM between adjacent markers. Distorted segregation was observed on the 46 markers which were determined by a Chi-square test at $P < 0.05$ and $P < 0.01$ level. The 'Samgang' alleles accounted for 41.09% of the whole rice genome.

Statistics for macro- and micro-elements of brown rice in DH population

Comparing to 'Nagdong', 'Samgang' is associated with higher calcium, magnesium, iron content and ratio of magnesium to potassium, and lower potassium content (Table 1). A significant difference between two parents was observed on the ratio of magnesium to potassium by a t-test. The means of DH population for four elements were lower than those of the two parents, suggesting larger transgressive segregations on four traits of brown rice (Figure 1). Wide ranges were concerned with continuous distributions on the DH population, illustrating quantitative inheritance patterns of all elements.

Traits correlation

Potassium content showed significantly positive correlations with calcium ($r = 0.24^{**}$) and magnesium content ($r = 0.60^{**}$). A positive correlation between calcium and magnesium content

($r = 0.18^*$) was observed. There were significantly negative correlations between the ratio of magnesium to potassium and iron content ($r = -0.27^{**}$), or potassium content ($r = -0.46^{**}$).

QTLs identification for macro- and micro-elements of brown rice

QTLs for potassium

Two chromosomal regions were associated with QTLs for the potassium content of brown rice grains. The 'Samgang' parent contributed both QTLs for this trait. The QTL qk6.1 in

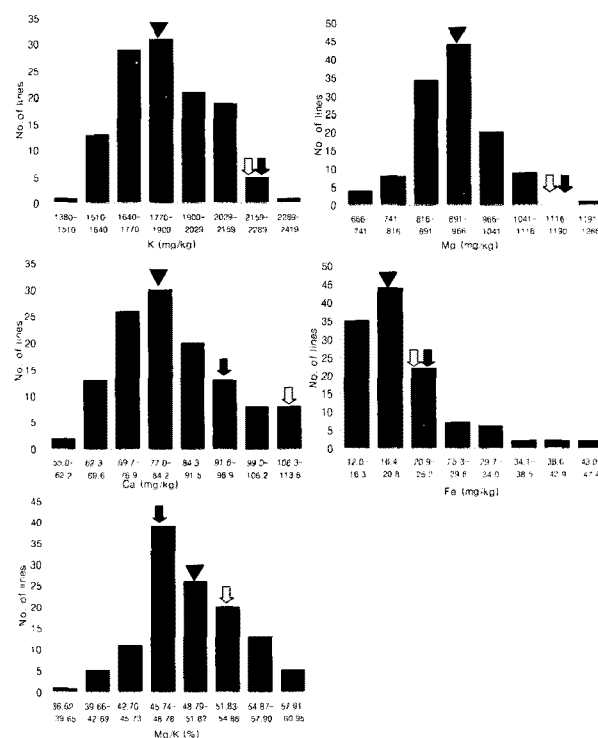


Figure 1. Phenotypic distributions of five traits of a DH population derived from a cross 'Samgang/Nagdong'. White arrows represent 'Samgang' values; Black arrows represent 'Nagdong' values; Black triangles represent the mean values of the DH population.

Table 1 Comparison of mean values for five traits in brown rice of parents and DH lines

Traits	Parents				DH population	
	Samgang	Nagdong	t-value	Mean±SD	Mean±SD	Range
K (mg/kg)	2168.0±65.1	2277.0±60.8	1.68	2222.5±79.9	1853.4±186.2	1380.0-2419.0
Ca (mg/kg)	115.2±0.2	95.0±6.4	3.48	105.0±9.8	83.3±12.6	55.0-113.6
Mg (mg/kg)	1162.0±27.6	1138.0±36.8	0.02	1150.0±26.5	919.9±91.3	666.3-1266.0
Fe (mg/kg)	24.0±4.7	21.9±1.9	1.14	25.0±3.8	20.4±6.7	12.0-47.4
Mg/K (%)	53.1±0.3	48.5±0.3	8.39*	50.8±1.4	49.8±4.4	36.6-60.9

the interval 6013-RM19696 on chromosome 6 accounted for 15% of the variation with an LOD score of 3.97. The qk8.1 flanked by 8013 and RM544 on chromosome 8 with an LOD score of 2.87, which explained 11% of the phenotype variation (Table 2 and Figure 2).

QTLs for calcium

Three QTLs for the calcium content of brown rice were detected on three chromosomes, consisting of qca1.1 on chromosome 1, qca3.1 on chromosome 3 and qca8.1 on chromosome 8. Two of them, qca1.1 and qca8.1 in the

Table 2 QTLs identified for macro- and micro-elements of brown rice in 120 DH lines from the cross 'Samgang/Nagdong'

Traits	QTLs	Chr. ^a	Interval markers	LOD	R ² ^b (%)	Add. ^c	Increase allele
Potassium (K)	qk 6.1	6	6013-RM19696	3.97	15	246.65	Samgang
	qk 8.1	8	8013-RM544	2.87	11	59.88	Samgang
Calcium (Ca)	qca 1.1	1	RM129-1019	8.58	27	6.97	Samgang
	qca 3.1	3	RM282-3023	3.46	9	-4.16	Nagdong
	qca 8.1	8	8013-RM544	3.64	9	3.88	Samgang
Magnesium (Mg)	qmg 1.1	1	1028-1030	4.22	13	32.02	Samgang
	qmg 6.1	6	6013-RM19696	5.08	19	119.64	Samgang
Iron (Fe)	qfe 6.1	6	6022-6023	4.05	13	2.60	Samgang
	qmgk 1.1	1	1024-1026	3.24	6	1.12	Samgang
Ratio of magnesium to potassium (Mg/K)	qmgk 3.1	3	RM186-3038	3.41	6	-1.08	Nagdong
	qmgk 7.1	7	7038-7042	2.52	5	0.96	Samgang
	qmgk 8.1	8	RM544-8017	5.11	13	-1.59	Nagdong

^a Chr. means chromosomes;

^b R² means percentage of phenotype variation explained;

^c Add. means additive effect.

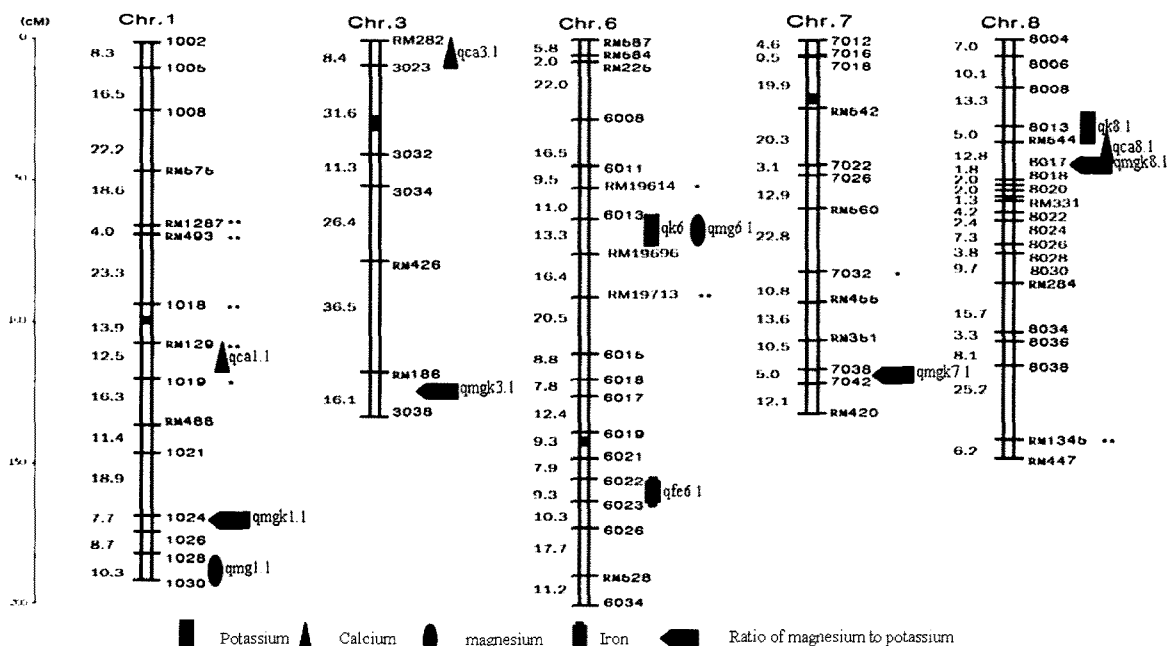


Figure 2. Locations of the QTLs detected for macro- and micro-elements. The values on left side of each chromosome indicate interval distances between markers using cM (centiMorgan) as the unit; but DNA markers were shown on right side of each chromosome. The centromeres are signed by black bar. * and ** indicate that the markers show a distorted segregation at P<0.05 and P<0.01 level of Chi-square test.

intervals RM129-1019 and 8013-RM544 with LOD values of 8.58 and 3.64, explained 27% and 9% of phenotype variations respectively with increasing effects from the 'Samgang' allele. The *qca3.1* was flanked by RM282 and 3023 with an LOD value of 3.46, which explained 9% phenotype variation with an increasing effect from the 'Nagdong' allele.

QTLs for magnesium

The 'Samgang' allele increased the magnesium content of brown rice in the two loci of *qmg1.1* and *qmg6.1*. One of them, *qmg1.1* was detected on chromosome 1 in the interval 1028-1030 with an LOD value of 4.22, explaining 13% of the phenotype variation. Another one, *qmg6.1* was identified on chromosome 6 in the interval 6013-RM19696 with an LOD value of 5.08, explaining 19% of the phenotype variation. In this locus, a QTL of *qk6.1*, affecting potassium content, was also identified, which is consistent with a positive correlation of two traits ($r=0.60^{**}$).

QTLs for iron

Only one QTL *qfe6.1* for iron content of brown rice was detected on chromosome 6. In this locus, the 'Samgang' allele was the donor of increasing effect by 2.60 mg kg⁻¹ on the iron content. It was flanked by 6022 and 6023 with an LOD value of 4.05, explaining 13% of phenotype variation.

QTLs for the ratio of magnesium to potassium (Mg/K)

Four QTLs affecting the ratio of magnesium to potassium (Mg/K) were detected on four chromosomes, consisting of *qmgk1.1*, *qmgk3.1*, *qmgk7.1* and *qmgk8.1*. The QTLs of *qmgk1.1* and *qmgk7.1* in the intervals 1024-1026 and 7038-7042 were detected on chromosomes 1 and 7 with LOD values of 3.24 and 2.52, explaining 6% and 5% of phenotype variations. They increased the trait by 1.12% and 0.96% with the alleles from the 'Samgang' parent, respectively. The QTLs of *qmgk3.1* and *qmgk8.1* in the intervals RM186-3038 and RM544-8017 were detected on chromosomes 3 and 8 with LOD values of 3.41 and 5.11, explaining 6% and 13% of phenotype variations. They increased the trait by 1.08% and 1.59% with the alleles from the 'Nagdong' parent, respectively. In comparison with *qmgk1.1* and *qmgk7.1*, the QTLs of *qmgk3.1* and *qmgk8.1* showed large LOD values and contributions for phenotype variations, indicating that the increasing alleles from 'Nagdong' should be considered as a relatively high heritability of the traits.

QTL interaction for the traits

In total, fifteen epistatic combinations showed significant interactions for five traits, covering 10 chromosomes except for chromosomes 6 and 10 (Table 3). Two kinds of epistatic QTLs

Table 3 Marker interactions between different loci covering the genome for macro- and micro-elements

Traits	Chr-N ^a	Marker A	Chr-N ^a	Marker B	F value ^b	R ² (%) ^c
K	7-8	7032	11-2	11005	20.88**	12.72
	3-4	3034	4-3	4006	13.09**	7.97
Ca	1-3	1008	5-8	RM289	16.47**	12.25
Mg	8-4	8013	9-4	9012	27.99**	11.80
	1-2	1005	7-7	RM560	22.12**	9.32
	2-3	2009	11-10	11023	22.83**	9.63
	8-18	8038	11-18	11031	13.51**	5.70
	3-6	RM186	5-8	RM289	12.65**	9.68
Fe	2-1	2005	11-18	11031	16.83**	4.19
Mg/K	5-1	5003	7-6	7026	27.47**	6.84
	3-4	3034	11-7	11019	32.37**	8.06
	8-12	8026	11-15	11027	25.86**	6.44
	4-1	4002	12-5	12013	18.08**	4.60
	3-6	RM186	12-2	12008	20.20**	5.03
	1-15	1030	11-2	11005	19.91**	4.96

^a Chr-N represents the chromosome number-peak of the points being tested in the analysis;

^b F value represents F-test for marker A and marker B;

^c R² means contribution of phenotype variation explained by QTL interactions.

could be classified, which include that one has no significant effects on traits detected for both loci, and a significant effect detected on traits for one locus or both loci (including main-effect QTLs). Fourteen pairs of loci were detected for five traits, which involved both loci without significant effects on traits. One combination of RM186 and 12008 for the ratio of magnesium to potassium involved a main-effect QTL. The interactions of two combinations for potassium content collectively explained 20.69% of phenotype variations. One combination for calcium and iron content showed interactions explaining 12.25% and 9.68% of phenotype variations, respectively. Four pairs of loci with high interactions collectively accounted for 36.45% of phenotype variations for magnesium content, but seven pairs of loci totally accounted for 40.12% of phenotype variations for the ratio of magnesium to potassium.

Discussion

In this study, a DH rice population from a cross of 'Samgang (Tongil type) / Nagdong (*Japonica*)' was used to QTL detection for macro- and micro-elements. A total of 12 QTLs were identified for potassium, calcium, magnesium, iron content and ratio of magnesium to potassium of brown rice. The iron content was considered as a polygenic inheritance, and environmental effects were smaller than the genetic effects (Gregorio et al. 2000). The same results for five traits can be all observed via the phenotypic frequency distribution as shown on Figure 1. To need complement, the epistatic interactions also occupied considerable proportions explaining 9.68% to 40.12% of the phenotype variations of five traits in the present study.

The QTLs of potassium content in rice grain were located on the chromosomes 1, 2, 4, 7, 8, 9, 12 using an RI population from a cross between 'Milyang23 (maternal)/Giho (paternal)' (Joe et al. 2006). Among them, one QTL located on chromosome 7 in the interval RG156-C507 with a high LOD value and contribution of phenotype variations across three years was considered as a putative QTL controlled by the major gene. In this study, two QTLs for potassium content, qk6.1 and qk8.1 were identified on chromosomes 6 and 8. It

is not the same location between qk8.1 and the chromosomal region in RM33-RM210 interval detected by Joe et al. (2006). In addition, Kang et al. (1998) reported that a QTL affecting potassium content was identified on the short arm of chromosome 6, which was near the qk6.1. The chromosomal region of qmg6.1 controlling magnesium content with flanking markers 6013 and RM19696, which was closed to the QTL of mag6.1 with flanking markers RG213-E13M59.270P1 detected on chromosome 6 by Joe et al. (2006). Other QTLs affecting magnesium content were identified on chromosomes 2, 3, 8 and 9 as described by Joe et al. (2006). However, a new locus of qmg1.1 for magnesium was mapped on the long arm of chromosome 1 in this study.

Gregorio et al. (2000) located three QTLs associated with the high-iron traits individual on chromosomes 7, 8 and 9. The QTL of qfe6.1 was mapped on chromosome 6 and explained 13% of phenotype variation for the iron content of brown rice grains in this study. Moreover, qmgk3.1 was likely a similar location to the mag/pot3.1 with flanking markers G200 and RZ575 on chromosome 3. Three QTLs controlling calcium content were mapped on chromosomes 1, 3 and 8, of which qca1.1 was located near the centromere of chromosome 1 with flanking markers RM129 and 1019. This is the first time to locate the chromosome regions associated with calcium content in brown rice grain.

The co-localization of QTLs associated with macro- and micro-elements appeared with each other on chromosomes of rice in this study. The QTLs of qk8.1, qca8.1 and qmgk8.1 were found in the same locus on chromosome 8 associated with potassium, calcium content and the ratio of magnesium to potassium. In the meantime, significantly positive ($r=0.24^{**}$) and negative ($r=-0.46^{**}$) correlations were observed between potassium and calcium, and the ratio of magnesium to potassium, respectively. It is a convincing proof to illuminate that there are some relationships between chromosomal regions controlling specific traits and phenotypic correlation.

QTL affecting the same or similar traits in the different species may have a common origin. Vreugdenhil et al. (2004) reported the QTL mapping for eight trace element characteristics using an RIL population from a cross of 'Ler/Cvi' of

Arabidopsis. With respect to the phenotypic correlations among traits in the *Arabidopsis* population, calcium content was positively correlated to potassium, magnesium, or iron content. These results are mostly similar to the present study except the positive correlation between potassium and magnesium. Four QTLs controlling calcium content which were located on chromosomes 1 and 3 were detected in the *Arabidopsis* genome. The QTL near centromere was located on the long arm of chromosome 1 with a peak marker of GD160, which co-localizes very well with three genes from the *CAX* gene family of vacuolar cation antiporters (Vreugdenhil et al. 2004). Salse et al. (2002) detected a non-random distribution of the syntenic points between chromosome 1 of rice and the five *Arabidopsis* chromosomes, most of which were found on the long arm of the rice chromosome 1. The similar chromosomal region described as qca1.1 was mapped near 18.98Mb position on the rice chromosomes indicated that colinearity between *Arabidopsis* and rice genomes should be a useful and feasible approach to map and clone QTL of rice or other crops (Ghandilyan et al. 2006).

In brief, the target loci concerned with macro- and micro-elements should be dissected into single Mendelian factors and precisely mapped by constructing chromosome segment substitute lines with different QTLs. It is a necessary step not only to the identification and characterization of interest genes, but also to the new cultivar breeding with high macro- and micro-elements by QTL pyramiding and marker assisted selection. Actually, the corresponding work is unfolding in the same lab as this current experiment.

Acknowledgements

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