**RESEARCH NOTE**

# Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains

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## Introduction

The human body requires more than 22 mineral elements that can be supplied by an appropriate diet (Philip and Martin 2005). However, the diets of the populations subsisting largely on cereals, or inhabiting regions where soil mineral imbalances occur, often lack Fe, Zn, Ca, Mg and Mn. These minerals are known to play an essential role in the metabolism and physiological process of human body. Unfortunately, over three billion people in the world are malnourished. Many of those afflicted are dependent on staple crops for their sustenance. Among all the important staple crops, rice has the highest food and food energy yield (Juliano and Villareal 1993). Rice is an indispensable staple food for half of the world's population. In countries where rice is used as staple food, the per capita consumption is very high ranging from 62 to 190 kg/year. Thus, even a small increase in the nutritive value of rice can be highly significant for human nutrition (Grahama *et al*. 1999).

Recent efforts to improve the mineral nutrition in rice grain include selecting for germplasm with greater quantities of essential minerals (such as Fe, Zn etc), breeding mineral-efficient crops that produce high yields and accumulate minerals from infertile soils, and enhancing bio-available minerals in edible portions through increasing the concentrations of metal-binding proteins (Fumiyuki *et al*. 1999; Lucca *et al*. 2001; Holm *et al*. 2002; Zhang *et al*. 2004; Heinemann *et al*. 2005; Philip and Martin 2005). These approaches have met with varied success and many have encountered problems that prevent their widespread acceptance. Recently, many studies demonstrated that quantitative trait loci (QTL) mapping is a powerful approach to study and manipulate complex traits that are important in agriculture, including mineral content. In the present study, with a set of 241 recombinant-inbred lines (RILs) derived from a cross between Zhenshan 97 and Minghui 63, mapping of main-effect QTLs and epistatic QTLs for the Fe, Ca, Zn, Mn and Cu content of rice grain was conducted based on field measurements.

## Materials and methods

### Plant materials and field planting

A population consisting of 241 RILs constructed by a single-seed descendent from a cross between two *indica* cultivars Zhengshan 97 and Minghui 63 were used in this study. The RILs along with both the parents were planted at the Hainan experimental farm, Wuhan University, China, in a randomized block design with two replicates in the year 2003. For each replicate, a three-row-plot for each RIL and a six-row-plot for each parent were planted at 20 cm between rows and 20 cm between plants, each row containing eight plants. The field management followed standard agronomic procedures. Rice grains were collected from four plants in the middle row of each RI line and individually sampled for 16 plants in the middle part of each plot of the parents, respectively.

### Trait measurements

All harvested rice grains including RILs and their parents were dried, cleared, dehusked in an electrical dehusker (model B-76, Huangyan, Zhejiang, China) and milled by sample miller (model JB-20, Huangyan, China). The milled rice samples were ground to 100 mesh with model 3010-019 cyclone grinder (model 3010-019, Fort Collins, Colorado,

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USA), oven-dried at 105°C to get sample powder, and separately filled into 100 ml grinding reagent bottles. The contents of Fe, Zn, Ca, Mn and Cu were measured by Atomic Absorption Spectrophotometry (Hitachi Z-5000 AA Spectrophotometer, Hitachi, Japan) using an atomic absorption method (Huang 1995).

### *Genetic-map construction and QTL detection*

The molecular markers and linkage map were described by (Xing *et al*. 2002), which consisted of 221 marker loci and covered a total of 1796 cM.

The QTLMapper1.0 computer program based on mixed linear models (Wang *et al*. 1999) was used to map main-effect and digenic epistatic QTLs by controlling both main and epistatic effects of important markers. In this study 2.4 LOD value was used as the threshold for claming the presence of main-effect of QTL, while 4.03 LOD value corresponding to $P = 0.005$ was used as the threshold for claiming the presence of epistatic QTLs.

## Results

### *Phenotypic variation*

The phenotypic values of the five traits (Cu, Ca, Zn, Mn and Fe) studied are presented in table 1. The results of *t* statistics indicated that the differences between the parents were significant for Cu, Ca, Fe and Zn content, but not for Mn. On the whole, the mineral element content of 'Minghui 63' was higher than that of 'Zhenshan 97'. Most traits of RIL population segregated continuously and approximately fit normal distributions with absolute values of both skewness and kurtness was not more than 1.0 (except Fe), indicating that these traits were suitable for QTL mapping.

### *QTL mapping of the traits*

*Mapping of main-effect QTLs:* A total of 10 QTLs distributed on 10 different intervals of six chromosomes (figure 1) were detected for five mineral elements (Cu, Ca, Zn, Mn and Fe) content traits (table 2).

Only one QTL *qCU-2* for Cu content was resolved, which accounted for 23.57% of phenotypic variation. Its positive allele comes from the parent 'Zhenshan 97', increasing Cu content by 1.59 mg/kg. Three QTLs were detected for Ca content, that were distributed on chromosomes four, five and nine, respectively. The three QTLs collectively explained 48.78% of the total phenotypic variation. Both the additive effect value (~26) of the three QTLs, and the phenotypic variation (16%) individually explained by them, were

**Table 1.** Distribution of phenotypic values for traits of mineral element content among the experimental materials.

| Traits (mg/100g) | Recombinant inbred lines | | | Parents | |
|---|---|---|---|---|---|
| | Mean | Kurtosis | Skewness | Zhenshan 97 | Minghui 63 |
| Cu | 8.26 | 0.7 | 1 | 10.2 | 5.47 |
| Ca | 18.99 | −0.04 | 0.6 | 17.38 | 7.83 |
| Zn | 22.33 | 0.3 | 0.69 | 16.9 | 13.43 |
| Mn | 14.99 | −0.42 | −0.13 | 11.81 | 11.83 |
| Fe | 18.93 | 2.56 | 1.52 | 13.47 | 12.84 |

**Table 2.** QTL mapping for mineral element content QTLs conducted based on QTLmapper 1.0 with a LOD threshold of 2.4.

| Traits | QTL[a] | Chr[b] | Interval | LOD[c] | A[d] | $H^2(\%)$[e] |
|---|---|---|---|---|---|---|
| Cu | *qCU-2* | 2 | G1314a-RM240 | 4.38 | −1.59 | 23.57 |
| Ca | *qCA-5* | 5 | C734b-RZ649 | 5.63 | −2.55 | 14.56 |
| | *qCA-9* | 9 | RG570-RG667 | 6.02 | −2.68 | 16.09 |
| | *qCA-4* | 4 | C820-C933 | 5.53 | 2.84 | 18.13 |
| Zn | *qZN-5* | 5 | R3166-RG360 | 4.27 | −2.37 | 12.34 |
| | *qZN-7* | 7 | RM234-R1789 | 1.8 | −1.55 | 5.3 |
| | *qZN-11* | 11 | C794-RG118 | 5.65 | 2.91 | 18.61 |
| Mn | *qMN-1* | 1 | RM259-RM243 | 2.67 | −1.43 | 10.33 |
| Fe | *qFE-1* | 1 | RG236-C112 | 7.66 | 3.36 | 25.81 |
| | *qFE-9* | 9 | C472-R2638 | 4.25 | −2.2 | 11.11 |

[a]QTL nomenclature follows that of McCouch *et al*. (1997). [b]Chromosome on which the QTL is located. [c]Log-likelihood value. [d]Additive effect, the negative value indicates that the allele from *Zhenshan 97* increases phenotypic value. [e]$H^2$ is the percentage variation explained by each QTL.
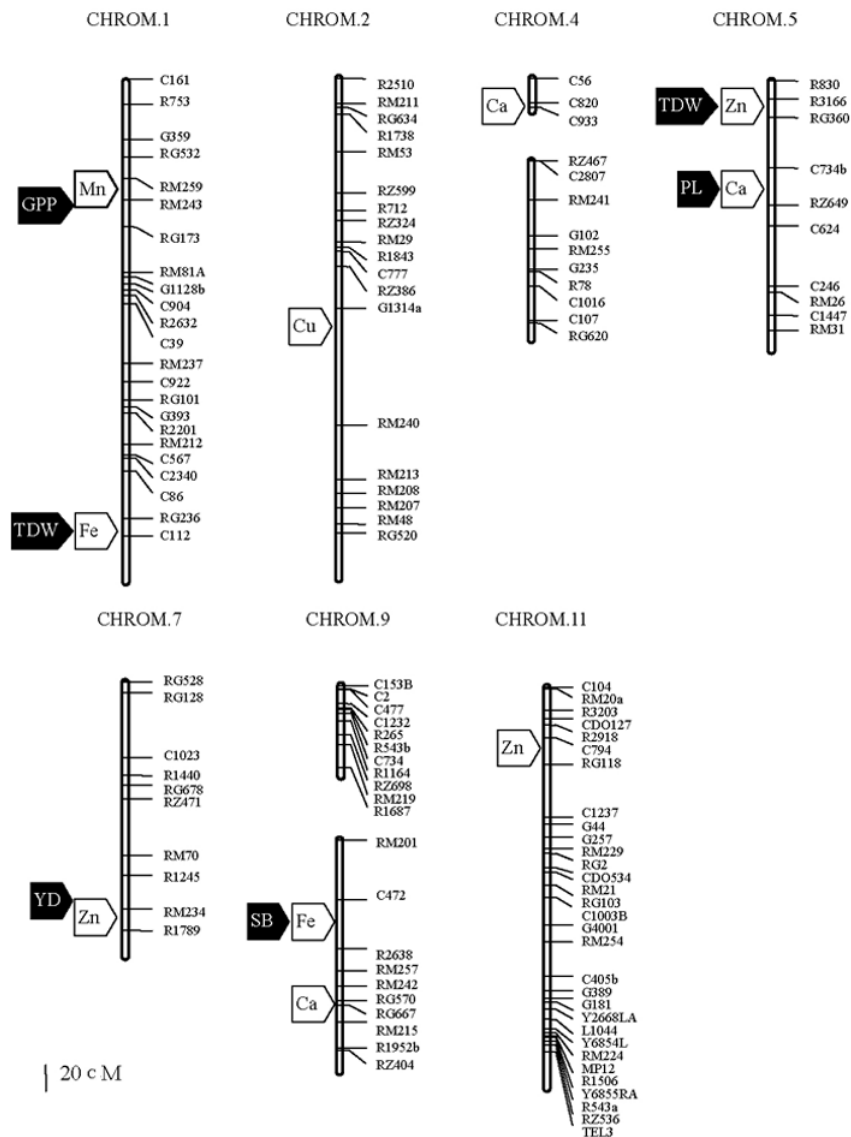
**Figure 1.** Quantitative trait loci (QTLs) for the content of mineral element of rice. With the same population, several QTLs for GPP, grains per panicle; TDW, total dry weight of whole plant; PL, plant length; YD, yield; SB, sheath blight; identified by other researchers located in the same or close interval with those for content of mineral element of rice.

similar. There were also three QTLs investigated for Zn content, that were distributed on chromosomes five, seven and eleven, respectively. Among these QTLs, the major QTL was *qZN-11* with the largest effect accounting for 18.61% of the phenotypic variation The additive effect of the remaining two QTLs were relatively small, and their positive alleles were all inherited from the parent 'Zhenshan 97'. Only one QTL, *qMN-1*, in the interval of RM259-RM243 on chromosome one was identified for Mn content, that accounted for 10.33% for the phenotypic variation. Its positive allele also comes from 'Zhenshan 97'. Two QTLs, *qFE-1* and *qFE-9*, were identified as showing main effects for Fe content, accounting

for 36.92% for the phenotypic variation. '*Minghui 63*' alleles increased the Fe content at the QTL *qFE-1*, while '*Zhenshan 97*' alleles increased the Fe content at QTL *qFE-9*.

*Detection of epistatic interaction loci*

In total, 28 digenic interactions were detected for five traits of trace element content involving 46 loci which were distributed on all 12 chromosomes (table 3). Six, six, six, three and seven digenic interactions were identified for Cu, Ca, Zn, Mn and Fe, respectively, and explained 47.58%, 40.84%, 50.2%, 31.46% and 47.94% of the total variation for them,

**Table 3.** Epistatic analysis for five mineral elements content of rice in Zhenshan 97 × Minghui 63 RIL population by composite interval mapping at a threshold of LOD 4.0 ($P < 0.005$).

| | QTL(Ai) | | QTL(Aj) | | | | |
|---|---|---|---|---|---|---|---|
| Traits | Ch-Ini[a] | Interval | Ch-Inj[a] | Interval | LOD[b] | AAij[c] | H²(%)[d] |
| Cu | 3–3 | C63-RM232 | 3–16 | R1925-RM148 | 6.93 | 1.71 | 11.14 |
| | 3–14 | RM200-RM227 | 12–8 | R643-C87 | 5.98 | −1.48 | 8.4 |
| | 3–16 | R1925-RM148 | 9–2 | C472-R2638 | 6.69 | 1.47 | 8.29 |
| | 5–5 | RZ649-C624 | 8–7 | C347-RG978 | 4.22 | 1.24 | 5.89 |
| | 5–9 | C1447-RM31 | 9–2 | C472-R2638 | 5.17 | −1.27 | 6.19 |
| | 8–5 | R1629-C483 | 9–1 | RM201-C472 | 4.31 | 1.42 | 7.67 |
| | | | | | | Total | 47.58 |
| Ca | 3–8 | RZ403-R19 | 5–2 | R3166-RG360 | 4.85 | −2.32 | 6.24 |
| | 3–15 | RM227-R1925 | 6–25 | C962-RZ242 | 5.9 | −2.59 | 7.76 |
| | 3–14 | RM200-RM227 | 12–3 | R2672-C996 | 4.1 | −1.97 | 4.49 |
| | 4–6 | RM241-G102 | 11–30 | RZ536-TEL3 | 6.04 | −3.18 | 11.76 |
| | 11–16 | C1003B-G4001 | 4–2 | C820-C933 | 8.02 | −1.92 | 4.27 |
| | 11–27 | R1506-Y6855RA | 12–10 | R496-C909B | 4.01 | 2.33 | 6.32 |
| | | | | | | Total | 40.84 |
| Zn | 1–1 | C161-R753 | 7–2 | RG128-C1023 | 7.52 | 3.69 | 13.55 |
| | 2–11 | C777-RZ386 | 3–1 | C1176-C316 | 7.71 | −3.25 | 10.51 |
| | 5–6 | C624-C246 | 10–14 | C405a-C223 | 5.77 | 2.83 | 7.96 |
| | 6–14 | RZ398-RM204 | 6–24 | R2549-C962 | 4.26 | 2.26 | 5.08 |
| | 9–14 | R2638-RM257 | 11–13 | CDO534-RM21 | 6.04 | 3.13 | 9.77 |
| | 9–19 | RM215-R1952b | 10–6 | RM239-C1633 | 4.29 | 1.83 | 3.33 |
| | | | | | | Total | 50.2 |
| Mn | 1–22 | RG236-C112 | 6–3 | R3139-C952 | 4.42 | 1.65 | 8.62 |
| | 3–15 | RM227-R1925 | 8–4 | RM25-R1629 | 5.42 | 2.05 | 13.27 |
| | 7–8 | R1245-RM234 | 8–4 | RM25-R1629 | 4.69 | −1.74 | 9.57 |
| | | | | | | Total | 31.46 |
| Fe | 1–5 | RM259-RM243 | 7–8 | R1245-RM234 | 7.79 | −3.71 | 12.15 |
| | 1–7 | RG173-RM81A | 8–6 | C483-C347 | 4.1 | 2.51 | 5.57 |
| | 2–3 | RG634-R1738 | 6–5 | Waxy-C1496 | 4.9 | −2.4 | 5.09 |
| | 6–10 | R1962-C764 | 11–30 | RZ536-TEL3 | 4.47 | −2.27 | 4.56 |
| | 6–24 | R2549-C962 | 12–3 | R2672-C996 | 4.6 | −2.35 | 4.87 |
| | 7–1 | RG528-RG128 | 12–1 | RM20b-C732 | 5.36 | −2.84 | 7.14 |
| | 10–10 | RG561-R2625 | 11–6 | C794-RG118 | 6.47 | −3.11 | 8.56 |
| | | | | | | Total | 47.94 |

[a]Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis. [b]Log-likelihood value of AAij. [c]AAij is the effect of additive-by-additive interaction between points i and j; a positive value indicates that the two-locus genotypes are the same as those in the female or the male parent with a positive effect, while the recombinants had negative effects. [d]H² is the percentages of the phenotypic variation explained by AAij.

respectively. Each of the interactions individually accounted for 2.92%–13.55% of the phenotypic variation. Among these interactions, five main-effect QTLs (*qZN-5, qZN-11, qMN-1, qFE-1* and *qFE-9*) involved in digenic interactions were found. However, no interaction between main-effect QTLs was found.

## Discussion

The overall genetic basis for mineral concentration in rice as revealed by this study is that each trait is controlled by a few QTLs with considerable epistatic interactions. In the present study, 10 QTLs were identified for five mineral element con-

tents of rice grain distributed on chromosome two, four, five, seven, nine and eleven. The phenotypic variation explained by these QTLs was significant. Except QTL *qZN-7*, that explained 5.3% of phenotypic variation, the variation explained by other QTLs was more than 10% (ranging from 10.33% to 25.81%), indicating that these QTLs detected were major QTLs (Falconer and Mackay 1996).

Several researchers have mapped QTL for content of Fe and Zn in rice grain, which are close to the QTL detected in our study or from the same chromosome. A previous study on Fe reported three QTLs on chromosomes seven, eight and nine, respectively (Gregorio *et al*. 2000). Consisted with his result, we also detected a QTL for Fe content in the interval C472-R2638 on chromosome nine, accounting for 11.11% of the phenotypic variation. For Zn, Avendano (2000) conducted QTL mapping using a RIL population, which detected a QTL for high zinc content in rice grain and a QTL for zinc deficiency tolerance to be located on chromosome five. In our study too, a QTL for Zn content was observed on the same chromosome.

Rice varieties rich in micronutrients often seems to have other desirable qualities, such as high yield, good tolerance to nutrition deficiency stress, and pest resistance. Moreover, Gregorio *et al*. (2000) found that the high-zinc seeds are nutritious not only for humans but also for the next generation of seedlings, which become more vigorous and better able to withstand weed competition, and pathogen and pest attack. Eva (1993) previously reported that mean content of Cd, Cu and Pb, per individual grain was exponentially correlated with grain size and weight. Therefore, it is not surprising to find that many QTLs for mineral content detected in this study were also associated with QTLs for biomass, yield, and disease tolerance in rice investigated by other scientists with the same population. Using composite interval mapping, the QTL *qTDW-1* and *qTDW-5* detected for total dry weight of whole plant (TDW) by Cui *et al*. (2003) was also detected for Fe (*qFE-1*) and Zn (*qZN-5*) content, respectively, in the present study. For yield and yield-component traits (including tillers per panicle (TP), grains per panicle (GPP), panicle length (PL) and 1000 grain weight (KGW) etc), *qCA-5* for Ca was found to be closely coincident with QTL *pl5* for panicle length investigated previously (Ma *et al*. 2004). The QTL *qZn-7* for Zn is located in the adjacent interval to QTL *yd7b* for yield and QTL *tp7a* for TP, resolved previously by Li *et al*. (2000). Similarly, the QTL *qMN-1* for Mn is located close to the interval with QTL *gp1* for GPP identified previously (Xing *et al*. 2002). Moreover, *qFE-9* for Fe is located in the same interval as C472-R2638 with resistance to sheath blight (QTL *qSB-9*) suggested previously by Han *et al*. (2002).

At the same time, the analysis resolved 28 additive-by-additive interactions which influenced the five traits of mineral element content, involving 46 different loci (epistatic QTLs) distributed over the whole genome. Total variation explained by epistatic loci of different traits was significant. All of them explained more than 30% of phenotypic variation, suggesting that epistasis, in the form of additive-by-additive interactions, plays a very important role in controlling the expression of these five traits. In this study, many main-effect QTLs were involved in epistatic interactions, which suggests that the effects of the single-locus QTLs are mostly dependent on the genotypes of other loci. Thus, an attempt for utilization of such QTLs in the breeding programmes has to take such epistatic effects into account.

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